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Frontiers of Neuroscience

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Volume 118 | Number 4 | July/August 2021 **www.msma.org/missouri-medicine**

EDITOR'S DESK
Missouri Medicine Again Indexed by Scopus
by **John C. Hagan, III, MD** 308

PRESIDENT'S FORUM
MSMA Helped Pass SB 514 Mitigating Onerous Requirements of Prior Authorization
by **Alexander R. Hover, MD**.....310

AMA REPORT
AMA House of Delegates Holds Virtual Annual Meeting
by **Charles W. Van Way, III, MD** & **the Missouri Delegation to the AMA**.....311

ALLIANCE
Reflections on My AMA Alliance Presidential Year
by Sue Ann Greco 314

MEDICAL | LEGAL
Be Careful What You Write: Employment, Disability, and Healthcare Law Considerations for Doctor's Notes
by Julianne Story, JD & Erica Ash, JD.....318

SCIENCE OF MEDICINE | PRO/CON - THE LAST WORD
The Flaws of Recent Omega-3 Clinical Trials Should Not Prevent Their Use
by James DiNicolantonio, PharmD & **James H. O'Keefe, MD** 322

Fish Oil Supplements for Prevention of Cardiovascular Disease: Response to Opponents
by Hekmat B. Khoukaz, MD, Mojgan Golzy, PhD & **William P. Fay, MD**.....323

SCIENCE OF MEDICINE | FEATURE SERIES
Frontiers of Neuroscience Presented by Henry and Amelia Nasrallah Center for Neuroscience, Saint Louis University

Center for Neurosciences Responsive to Jesuit Catholic Mission of Saint Louis University
by Daniela Salvemini, PhD 325

Novel Non-Opioid Based Therapeutics for Chronic Neuropathic Pain
by Timothy M. Doyle, PhD, Kathryn Braden, BS, Caron M. Harada, BS, Fatma Mufti, MS, Rachel M. Schafer, BS & Daniela Salvemini, PhD 327

Current and Future Immunotherapies for Multiple Sclerosis
by Jessica Bourque, BS & Daniel Hawiger, MD, PhD.....334

RNA-Based Therapies for Neurodegenerative Diseases
by Yuna M. Ayala, PhD & Andrew D. Nguyen, PhD.....340

Hydrogel-Based Spheroid Models of Glioblastoma for Drug Screening Applications
by Joey Bruns, MS & Silviya Petrova Zustiak, PhD 346

The Deductive Reasoning Strategy Enables Biomedical Breakthroughs
by Gina L. C. Yosten, PhD, Grant R. Kolar, MD, PhD, Daniela Salvemini, PhD & Willis K. Samson, PhD 352

Novel, Neuroscience-Informed Approaches to Trauma Care in Community Clinical Settings
by Andrew Oberle, MHA, Whitney Postman, PhD & Carissa van den Berk-Clark, PhD, LMSW.....358

SCIENCE OF MEDICINE
Plastic Bronchitis in Adult and Pediatric Patients: A Review of its Presentation, Diagnosis, and Treatment
by **Niel Patel, BS**, **Mayur Patel, BS**, Ravali Inja, BS, **Armin Krvavac, MD** & Andrew J. Lechner, PhD 363

What Are the Risk Factors for an Upper Extremity Deep Venous Thrombosis After Orthopaedic Irrigation and Debridement and Peripherally Inserted Central Catheter Placement?
by **Mark J. Lambrechts, MD**, Bradley S. Spence, MD, **Shelby M. Harris, BS**, Andrea Gilmore, BS, Jasraj Marjara, BA, Zhengye Si, MS, Blaine T. Manning, MD, James L. Cook, DVM, PhD, Emily V. Leary, PhD & **Matthew J. Smith, MD**.....374

Current Hybrid Interventions in Vascular Surgery: Merging Past and Present
by Jennifer Huffman, MD, **W. Kirt Nichols, MD** & **Jonathan Bath, MD**.....381

Home-Based Arm Cardiac Rehabilitation in Disabled Veterans: A Pilot Study
by **Anand Chockalingam, MD**, Albert Chan, MD, Jill A. Kanaley, PhD & Kul Aggarwal, MD 387

FROM YOUR ASSOCIATION
One Picture = 1,000 Words.....313
Correspondence.....315
New Members 316
Missouri Medicine in the News.....317

momedical @momedicalassn mostatemedicalassociation momedical momedical

MSMA member names in bold

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About Missouri Medicine

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With a large and respected editorial board and regular participation by Missouri's six medical schools on eight campuses, *Missouri Medicine* is among the nation's foremost state medical journals.

Articles are frequently cited in impact factor journals and regularly featured in the national media including: *Wall Street Journal*, CSPAN-2, KevinMD, *Outside Magazine*, *Runner's World*, *NBC News*, *General Surgery News, Ophthalmology Times*, and many others. Issues of *Missouri Medicine* are printed on acid-free paper with open access to digital issues available at www.msma.org/missouri-medicine-library.

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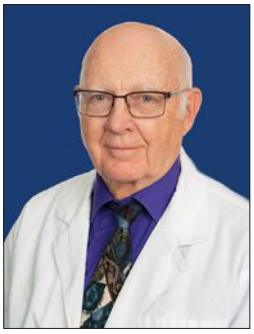
by John C. Hagan, III, MD

Twenty *Missouri Medicine* articles were in the top 10% most viewed publications in 2012-2016.

Missouri Medicine is pleased to announce that indexing of manuscripts from the Journal will resume in Elsevier Scopus for works published in 2021 and thereafter. Publications for *Missouri Medicine* works dating from 1945 to 2016 are currently indexed in Scopus.

Scopus indexes from more than 25,000 multi-disciplinary sources including journals, book series, and conference papers that have an International Standard Serial Number (ISSN).

There are nearly 82 million records in Scopus dating from 1788 and over 17 million author profiles. This ensures that the meticulous work of published authors will be retrievable and appear in internet searches world-wide. Access to Scopus is by subscription only. Please check with your campus librarian to confirm access.



John C. Hagan, III, MD, FACS, FAAO, is a Kansas City, Missouri, ophthalmologist and *Missouri Medicine* Editor since 2000 and member since 1975. He is a multi-year Diamond Contributor to MMPAC.

The more observant among you will notice the changes on the masthead page which now includes the Scopus icon proudly displayed along with our other major indexing and archiving services: MEDLINE, PubMed, PubMed Central and EBSCOhost databases. This ensures that the meticulous work of our published authors will be retrievable and appear in internet searches world-wide.

We wish to thank and acknowledge Cathy C. Sarli, Senior Librarian at the world-famous Bernard Becker Medical Library of Washington University School of Medicine in St. Louis, Missouri, for assistance in completing the re-enrollment process in SCOPUS. Additionally, Cathy is working with *Missouri Medicine* and Elsevier to retrospectively index our content at Scopus published from 2017 to 2020.

In publication for 117 years, *Missouri Medicine* has become one of the top three state medical journals. The Journal's reputation is based on being peer-reviewed, published six times/year, indexed by the world's most important data bases, regular superlative content by six medical schools on eight Show-Me state campuses, and a distinguished Editorial Board of experts and Contributing Editors. Our medical school theme issues, the mainstay of our quality content, are subscribed through January/February 2023. Our articles are regularly cited by journals listed in Clarivate Analytics Journal Citation Reports and the media. This past year scientific articles were published

Publication Summary of *Missouri Medicine* articles published from 2012 to 2016 in Scopus:

- 495 articles
- 687 authors
- 23 affiliations represented by the authors
- 4,333 views by users in Scopus
- 20 articles were in the top 10% most viewed publications

Citation Summary for *Missouri Medicine* articles published from 2012 to 2016 worldwide in Scopus:

- 1,130 citations
- 2.3 citations per article
- 86 countries represented by affiliations of citing authors
- 11 non-English languages represented by citing articles

from South Africa and a first literature report from Morocco and non-published manuscripts received from three other countries. *Missouri Medicine* has an international readership. The number of unsolicited scientific manuscripts has risen steadily over the years, precipitously during the COVID-19 pandemic, and our publication queue now exceeds eight months. Our acceptance rate for unsolicited manuscripts is about 40-50%.

This issue's membership pages also include profiles of four new Editorial Board members: Beth E. Rosemergey, DO, (Family Medicine), Leonid Shunyakov, MD, (Hematology & Oncology), David J. Haustein, MD, MBA, (Physical Medicine & Rehabilitation), and Andrew J. Bierhals, MD, MPH, (Radiology). We also thank these previous Editorial Board members for their time and expertise: Michael L. O'Dell, MD, Amy K. Patel, MD, Gregory M. Worsowicz, MD, and Stephanie L. Graff, MD. I never miss an opportunity to thank Liz Fleenor our highly capable managing editor. Liz has worked with *Missouri Medicine* for almost two decades and deserves much credit for its reputation and its artistic composition. Coming on board in 2000, I thank the members of MSMA for the honor and privilege of being the Journal's second-longest serving Editor.

Missouri Medicine is supported by MSMA members. I urge all Missouri physicians to join MSMA. I ask all current MSMA members, especially those in leadership positions, to help recruit new members. MSMA dues are among the lowest in the nation; the accomplishments of MSMA for Missouri physicians and their patients at the very top of all state medical associations. MSMA needs you and you and your patients need MSMA. Contact Cassie Williams at cwilliams@msma.org for renewals or prorated new member enrollment.

MM



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MSMA Helped Pass SB 514 Mitigating Onerous Requirements of Prior Authorization

by Alexander R. Hover, MD

A young woman was referred to me with progressive bloody diarrhea through the day and night, abdominal pain, nausea, arthralgias, and profound fatigue. Colonoscopy confirmed severe left-sided distal ulcerative colitis. She was referred as she did not have a complete resolution with oral mesalamine preparations.

I told her that the current recommendation based on clinical trials and specialty guideline recommendations was to add rectal mesalamine. The rectal mesalamine would cost $400 per month without insurance coverage. I changed her to an oral mesalamine preparation approved by her new insurance company and prescribed rectal mesalamine enemas. Initially, the insurance denied the rectal mesalamine prescription despite a call and letter. The insurance company's decision was appealed and finally approved. Unfortunately, during this time she had missed more work and had difficulty even affording even the co-pay. Her evaluation, colonoscopy, and decision on treatment was complete in the month I saw her, so she could have begun effective therapy almost two months earlier absent the prior authorization difficulty. This is a common story in my practice. I will bet it is in your practice as well.

In 2019 MSMA helped pass SB 514 that mitigated some of the most onerous requirements of prior authorization. SB 514 provided multiple improvements to smooth the prior authorization process for physicians. Those improvements were to have been in place no later than January 1, 2021.

Among the improvements included in the bill were: 1) an adverse determination notice must include a written statement of the clinical rationale rather than the physician having to request that; 2) each health insurance carrier or utilization review entity must post their current prior authorization requirements; 3) that restrictions including written clinical criteria, and requirements for step therapy must be described in detail; and 4) that an enrollee's grievance involving an adverse utilization review or prior authorization determination would require the carrier to submit the grievance for review to two independent clinical peers in the same or similar specialty as would typically manage the case being reviewed. I have not experienced a consistent application of the 2019 law when I deal with health carriers. A December 2020 AMA survey of physicians confirmed that little progress has been made in reducing prior authorization regulatory burden for physicians over the past few years. The lack of progress is despite a 2018 multi-organizational consensus agreement on principles for reform that included the AMA, AHIP, AHA, BCBS, MGMA, and APhA. Clearly, MSMA and the AMA have work yet to do. You can review all 21 principles and concurring organizations in the "Prior Authorization and Utilization Management Reform Principles" on the AMA website.

Well, this is where you should play the video clip of actor Peter Finch in Network exclaiming, "I'm mad as hell and I'm not going to take it anymore."

Take heart, progress is being made through the work of MSMA in Missouri and the AMA nationally. I have seen some improvement since the 2019 Missouri Senate bill 514. This is also a reminder, however, that progress is only made by our tireless advocacy with legislators for medical practice that benefits our patients and not the health carriers. That requires MSMA members paying dues, but more is needed. We must all be advocates for the MSMA to gain more members and, thereby, more clout with legislators. We must also participate in the White Coat Day and Friday virtual lunches with legislators when they continue next year. Sometimes we do have to stand up, go to the window, and shout on behalf of our patients. **MM**



Alexander R. Hover, Jr., MD, FACP, AGAF, is President of the Missouri State Medical Association for 2021-2022. He is a Gastroenterologist from Ozark, Missouri.

AMA House of Delegates Holds Virtual Annual Meeting

by Charles W. Van Way, III, MD & the Missouri Delegation to the AMA

The 2021 Annual Meeting of the AMA House of Delegates (HOD), normally several days in Chicago, was held as a virtual "special" meeting from June 11 through June 16. Normally, the annual meeting considers many issues, and sets new policy for the AMA. This year, the HOD was able to consider the greatest part of its normal business. The numbers of resolutions and other items was somewhat limited, because of the requirements of the virtual platforms. This meeting also included inauguration of the President and elections of officers and leaders. Jack Resnick, Jr, MD, was selected as President-Elect. Bruce Scott, MD, and Lisa Egbert, MD, were re-elected as Speaker and Vice-Speaker. Madeline Butler, MD, and S. Bobby Mukkamala, MD, were elected to the Board of Trustees (BOT).

Outgoing President, Sue Bailey, MD, spoke of the strength and resilience of the physician community. During the COVID pandemic, we have all lived through a "hero's journey." She said we have inspired many young people to become physicians, following our examples. How we have come through this pandemic will shape the AMA and medicine itself, the health care system, and our entire society.



Charles W. Van Way, III, MD, FACS, FCCP, FCCM, Missouri/AMA Delegate, and *Missouri Medicine* Contributing Editor, is Emeritus Professor of Surgery, University of Missouri - Kansas City, Kansas City, Missouri.

James Madiera, MD, Executive Director, praised physicians for "standing tall," advocating for physicians and patients and supporting good science and our public health system. AMA membership has grown during the last year. The increase in 2020 was the greatest year-to-year increase in the last 70 years. The AMA was the leading non-partisan voice in the national response to the pandemic. Visits to the AMA website in 2020 were more than the previous two years combined. Innovation, health equity, and advocacy have been the accelerators of our public exposure. Our policy is to define the "right thing" with increasing strength and deeper granularity. We continue to be dedicated to the house of medicine.

Gerald "Gerry" Harmon, MD, was inaugurated as the 176th president of the AMA. A physician from South Carolina, he is active in medical education and childhood health. He has been a strong advocate for physician autonomy. He was a Major General in the South Carolina National Guard. He has served the AMA on the Council of Medical Services (CMS) and the BOT, serving as Chair in 2017-18. His inaugural address spoke of battles for freedom in the American Revolution and the Civil War. Dr. Harmon echoed the memory of Dr. Joseph Warren, another Major General, who died in the Revolution. We are still in battle to care for our patients during the pandemic, he noted, to provide justice for our fellow citizens, to provide freedom for oppressed peoples throughout the world, to work for health equity, to support public health. As physicians, we struggle with bureaucracy, with regulators, with ignorance. But amid the tribulations of the pandemic, many of us have discovered renewal of our dedication to our profession. We don't have to be elected to the AMA to lead. We can lead by simply being physicians, as clinicians, researchers, academics,

teachers. As Dr. Warren said over two centuries ago, "Act worthy of ourselves and of our cause."

The House of Delegates does more than simply listening to speeches and electing the physicians who will give next year's speeches. The real business of the HOD is to set policy or recommend action to be taken by the AMA. This is done in two ways. First, the BOT or one of the Councils may submit a report, with recommendations for policy changes or actions. Second, resolutions may be submitted to the HOD proposing policy or action.

Health equity, racial discrepancies in health outcomes, and systemic racism were consistent themes throughout the meeting. Existing AMA policy promotes health equity and opposes racial discrimination in any form. Such policies were re-stated and strengthened in a number of areas. There were initiatives to reduce racism and discrimination in medicine, to enhance medical education in order to increase diversity, and to address equity in telehealth. A resolution was passed to establish a task force to guide the AMA further into health equity and racial justice.

The HOD considered a number of BOT and Council reports. A special task force on elections at the AMA made 41 separate recommendations, which were vigorously discussed. All but three were adopted. The overall effect will be to modernize and streamline the election process and to increase participation in leadership offices and activities.

Law enforcement works closely with emergency medical services (EMS). With increased emphasis on avoiding force has come a renewed demand for using sedatives to assist arrests of agitated individuals. The AMA wants to ensure that any such intervention is under medical control, with collaboration among EMS, law enforcement and behavioral health professionals.

New policy was adopted by the HOD to encourage work with specialty societies and state and local medical societies to develop practices for notification of abnormal test results. A Council on Science and Public Health report analyzed youth suicide, recommending a broad set of new policies to address the problem. A resolution from the Senior Physician Section called increased use of advanced directives among physicians, and promoting their use for patients.

The Council on Medical Education (CME) and others had several concerns relating to increasing the physician supply. Of particular interest to Missouri, CME report #2 called for setting up alternate licensing procedures for international medical graduates. This would involve alternatives to graduate medical programs, as long as they were not called "assistant physicians." CME report #4 called for streamlining the entry of qualified international graduates into the health care system. CME report #5 called for pathways to increase diversity in medicine. And CME report #3 addressed optimizing outcomes from the residency match (NIRMP). A related resolution called for rescinding USMLE Step 2 CS and COMLEX Level 2 PE examinations, removing one more barrier to physician training. Resolutions called for forgiving student loans, providing childcare and other benefits to residents, and decreasing examination fees charged by the American Board of Internal Medicine. Others called for increased salaries for residents and for debt cancellation for medical education.

On the perennial problem of opioid use, a resolution called for increased availability of opioid use disorder treatment in correctional facilities, and better health care in general for prisoners. The ill health of prisoners has concerned the AMA for years. Another resolution called for making naloxone more widely available as an over-the-counter drug.

The COVID pandemic winds down in the US, but spreads across the world. Several resolutions called for sharing COVID-19 resources with other countries where the pandemic is still accelerating. Temporary waiver of patent protections for vaccines was considered and was referred to the BOT. A BOT report called for the development of Federal standards for vaccination certificates. Resolutions recognized post-acute sequelae of COVID (PASC or "Long COVID") as a diagnostic entity, calling for research and funding. Resolutions called for improvements in personal protective equipment, for strengthening public health services, for universal access to public health services for modernization of reporting data systems.

The Council on Medical Service (CMS) reports addressed telehealth, calling for greater broadband access and for expansion of license recognition to facilitate the use of telehealth. Other resolutions called for a study of reimbursement for telehealth visits and for universal access to broadband services.

Prior authorization requirements often allow insurance companies to delay or deny services needed by patients, and are a perennial concern of the AMA. A CMS report discussed prior authorization, making a total of 11 recommendations to improve processes and accountability. Another report proposed a number of policy changes to improve regulation of urgent care centers.

The continuing effort of non-physician practitioners to increase their scope of practice continues to concern the AMA. Resolutions called for restricting such terms as "residency" and "fellowship" to physician training programs and for opposition to the recent effort by the Academy of Physician Assistants to call themselves "Physician Associates."

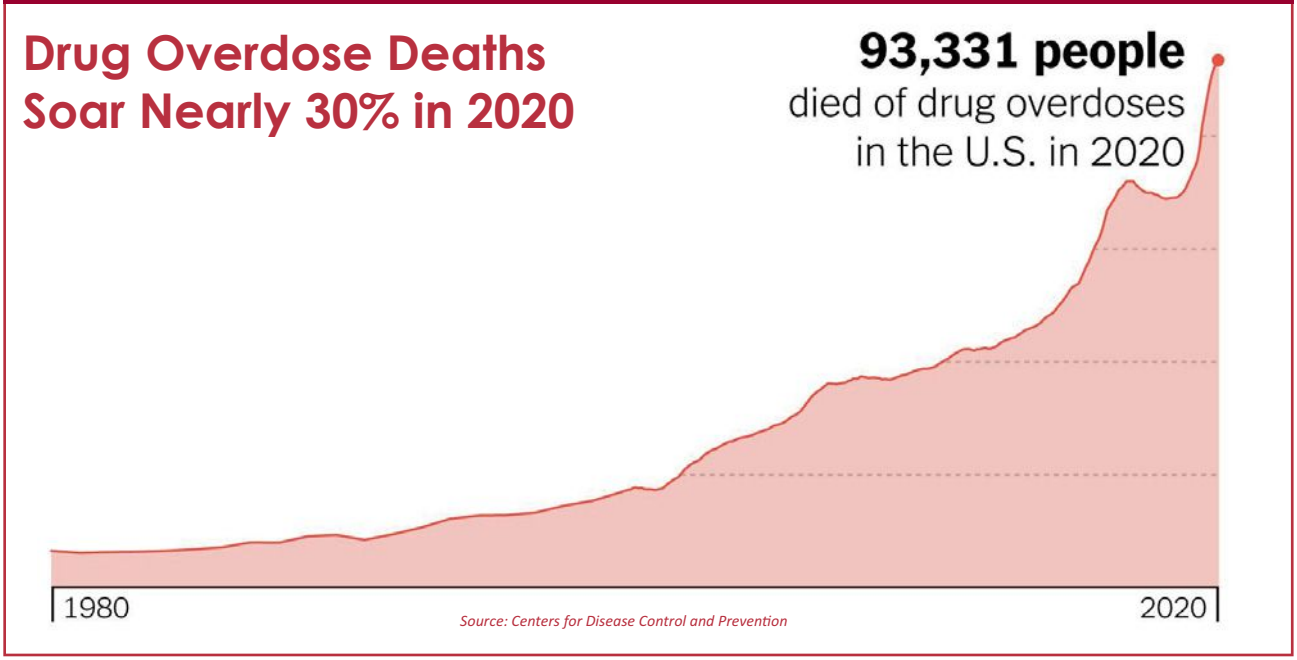
Concerns about social media were expressed, calling for studies of the impact of social media on adolescent mental health and for means to deal with medical "misinformation" on social media. This last amounted to a demand for censorship of medical information on social media, as noted by an editorial in the *Wall Street Journal* (Doctors for Progressive Conformity, WSJ, Saturday, June 19, 2021).

As noted earlier, one of the great strengths of the AMA is its public respect. It has gained respect from both sides of the political spectrum precisely because it is non-partisan. There was an increasing tendency this year to inject partisan politics into the debates. On issues ranging from banning "ghost guns" to waiving patent protection for vaccines, the HOD advocated policies of the current Federal administration, even where those policies were only marginally related to medicine and public health. There is a danger in failing to maintain impartiality. Perhaps this was attributable to the virtual conference format, which definitely repressed debate. Both Twitter bullying and political "groupthink" were visible. We can hope the next meeting will be in person.

Several physicians from Missouri serve in leadership roles. David Barbe, MD, represents the AMA on the world stage as President of the World Medical Association. Edmond Cabbabe, MD, serves as Vice-Chair of the Council for Long Range Planning and Development. Elie Azrak, MD, serves on the board of the AMA Political Action Committee. Jerry Kennett, MD, serves on the AMA Foundation Board. Marc Mendelsohn, MD, is chair of the Young Physicians Section. Sue Ann Greco closed her year as the AMA Alliance President. Charles Van Way, III, MD, was re-elected to the Steering Committee of OSMAP, the Organization of State Medical Association Presidents.

All of the business of the meeting, as well as summaries of the addresses by Drs. Harmon and Bailey, are available on the AMA website. Highlights from the meeting are at https://www.ama-assn.org/house-delegates/special-meeting/top-10-stories-june-2021-ama-special-meeting and https://www.ama-assn.org/house-delegates/special-meeting. The Interim Meeting of the HOD is scheduled to take place in Orlando, Florida, November 13-16, 2021. We all hope that it will proceed in person, and that the worst of the pandemic will be behind us. **MM**

ONE PICTURE = 1,000 WORDS



Reflections on My AMA Alliance Presidential Year

by Sue Ann Greco, Immediate Past AMA Alliance President

Now that I finally have a chance to sit and catch my breath, I can seriously say my tenure as president of the AMA Alliance this past year was challenging but exhilarating. We started out the year saying we would make lemonade out of lemons, and we did! Our bi-monthly Zoom calls on various subjects throughout the year helped us stay in touch and get to know each other in a new way. As physician spouses, we experienced COVID from a truly unique perspective. Many of us had spouses who were on the front line, or at least never missed a day of going into the office.

This past year was challenging to all types of member organizations. The AMA Alliance found that the key to keeping our members engaged was to present content meaningful to them at this point in their lives.

One of our first presentations was on dealing with the challenge of homeschooling children. Long time AMA Alliance member Debbi Ricks, suggested her daughter, an education specialist, as a presenter. Members on the call, both young and old, offered advice. It was truly a chance to share an experience that was new to everyone.

As the year went on, we reached out to AMA members and staff to present content on COVID and related topics to keep our members informed. This gave us a chance to really strengthen our partnership with AMA. Throughout the year we used social media to get AMA's messages out to our members and to support their MaskUp program.

We also encouraged state and county Alliance affiliates to stay engaged with their local members through Zoom and small social distancing projects. It was amazing to see what some counties were able to accomplish, including our own counties here in Missouri. Greene county won the Health Awareness Promotion award for their billboard thanking essential workers. St. Louis County used a Health Policy Promotion Grant to thank essential frontline workers at two local hospitals with treat bags.



Correspondence

Cannabis Harmful & Unhealthy: Oppose Recreational Use in Missouri

I want to comment on the article by Eric A. Voth, MD, "Physicians Warn Cannabis Can Cause Serious Health Hazards," *Missouri Medicine* 2021 May-June 118 (3):203-205. Dr. Voth stressed and documented the negative and serious health hazards of the use of marijuana such as psychosis and paranoia resulting in violent behavior. Also noted was increased depression with a threefold risk of suicide in young people. Low body weight and future cognitive and emotional problems are negative effects associated with neonatal exposure. Dr. Voth discusses the negative neurological consequences on brain structure and function that increase the "likelihood" of addiction.

States that have marijuana programs have 23% higher opioid overdose death than states without (ref.19 in the Voth paper). More visits to emergency department, more hospitalizations, traffic fatalities and the Cannabis Hyperemesis Syndrome, potentially fatal, have been observed. This alone should call the attention of physicians, parents, and teachers. Drug abuse should be treated medically, patients should not be treated as criminals and drugs should remain controlled until evidence shows that they are not harmful. References supporting these claims are in the paper.

Popular culture and the cannabis industry describe the effects of marijuana as producing relaxing, peaceful, and nonviolent behavior, ignoring Dr. Voth's cited data. Lack of motivation and low energy levels have devastating effects on the lives of young people affecting their present performance and their future development and maturity. I always remember one young patient who started marijuana during his military wartime service, he could not tell what he did with his life during the past ten years previous to the interview. He said: "I was empty, ten years were lost, I don't know what happened."

We commend International Academy on the Science & Impact of Cannabis (IASIC) physicians for their effort researching and utilizing data to slow and reverse the proliferation of recreational/medicinal cannabis. I ask MSMA members to act to keep high THC recreational marijuana from legalization in our state of Missouri. Our population needs motivation, ideals, work, and physical activity to face reality and to live a full and productive life. As the Roman poet Juvenal told us, we should strive for a "Mens sana in corpore sano" (A healthy mind in a healthy body). To give access to another mind-altering drug that increasingly reaches younger and younger people is dangerous and irresponsible.

Juan C. Corvalan, MD
Distinguished Life Fellow
American Psychiatric Association
St. Louis, Missouri

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AMA Alliance, continued

One thing we learned this past year is that physician families are very resilient. Physician spouses have always had to adapt to their spouses' long hours and career paths, but during COVID, we had to adapt to the fact that our spouses were putting themselves in harm's way on a daily basis. That is why keeping our connection to our members this past year was so important. We all supported each other and helped keep each other informed about the latest news about the virus and vaccinations. We became role models for our communities. I hope Alliance members can always be seen as positive influencers when it comes to public health.

I would like to take this opportunity to thank the members of the Missouri State Medical Association and the MSMA Alliance for all the support you have given me this past year. Many of you stepped up to work at the national level, and I truly appreciated having you by my side.

I want to especially thank MSMAA and MSMA for honoring me at the end of my term. Knowing that I had my state and county Alliances and medical societies behind me meant more than I can possibly express.

I wasn't born in Missouri, but it has been my home for the past 40 years, and I am so proud of how Missouri has represented itself through the both the AMA Alliance and the AMA. Both organizations truly make the health of our citizens their number one priority.

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New *Missouri Medicine* Editorial Board Members



Andrew Bierhals, MD, MPH, is the new Editorial Board member for Radiology. Dr. Bierhals is an Associate Professor at Washington University School of Medicine in St. Louis. He replaces Amy Patel, MD, who is stepping down due to a conflict of interest policy with another medical journal.

Missouri Medicine welcomes **David J. Haustein, MD, MBA**, who is our new Physical Medicine and Rehabilitation Editorial Board member. Dr. Haustein is an Associate Professor, PMR, University of Missouri-Columbia School of Medicine and Associate Dean of MU's Springfield Clinical Campus. He replaces **Gregory Worsowicz, MD**, who has joined the Mayo Clinic in Florida.



Beth Rosemergey, DO, is the new Editorial Board member for Family Medicine. Dr. Rosemergey practices Community and Family Medicine, Pediatrics, and Family Care at the Bess Truman Family Medicine Center and Maternal Care Clinic, and the Truman Medical Center Lakewood, Kansas City, Missouri. She replaces **Michael O'Dell, MD**, who is retiring from practice. We thank Dr. O'Dell for his service to *Missouri Medicine*.

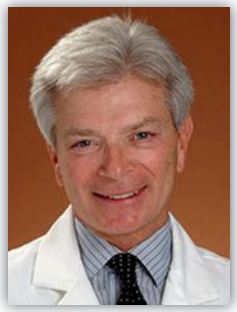




Leonid Shunyakov, MD, is the new Editorial Board member for Hematology/Oncology. He is board certified in Hematology, Internal Medicine, and Oncology. He practices at Citizens Memorial Healthcare in Bolivar, Missouri. He replaces **Stephanie Graff, MD**, who has accepted a faculty position at Brown University School of Medicine in Providence, Rhode Island.

Past *Missouri Medicine* Editor is Installed as President of the Illinois State Medical Society

J. Regan Thomas, MD, Past Editor of *Missouri Medicine* (1992-2000) was installed as the Illinois State Medical Society President for 2021-2022. In addition to otolaryngology, he is board-certified in facial plastic and reconstructive surgery. Dr. Thomas practices at Northwestern Medicine, Chicago, where he is a professor of otolaryngology – head and neck surgery at the Northwestern Feinberg School of Medicine. He earned his medical degree from University of Missouri School of Medicine.



Seeking Pain Management Member for *Missouri Medicine* Editorial Board

Missouri Medicine has an opening on the editorial board in Pain Management. Applicants must be active MSMA members, board certified, and capable of high quality peer-reviews of pain management manuscripts. Send CV to the Editor at drjhagan3@gmail.com. All applications must be submitted by September 1, 2021.



Letters to the Editor

Submissions reflect readers' opinions and may be edited for length. Use in subject line: "Letter to Editor."

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Be Careful What You Write: Employment, Disability, and Healthcare Law Considerations for Doctor's Notes

by Julianne Story, JD & Erica Ash, JD

Introduction

In a perfect world, employees would start the interactive process of asking for employment accommodations, including extended leave or a remote work arrangement, for a health condition before the condition affected their work. However, often times employees do not disclose such issues to their employers until after a performance problem arises, and the employee believes their position is in jeopardy. In some of those instances, a healthcare providers' certification stating that the employee has a health condition requiring accommodation can seem like a convenient excuse–especially when medical certifications can be easily forged or simply downloaded from a suspect website without ever having been examined by a healthcare provider. These issues are further complicated when the employer is a medical practice or other covered entity.





Julianne Story, JD, (left), a labor and employment partner, and Erica Ash, JD, (right), a healthcare regulatory associate, from the Kansas City office of Husch Blackwell, wrote this article. Husch Blackwell represents a full spectrum of healthcare providers and other businesses in regulatory compliance and litigation matters. The information contained in this article should not be construed as legal advice or a legal opinion on any specific facts or circumstances. The contents are intended for general information purposes only, and readers are encouraged to consult their own attorney concerning their specific situation and specific legal questions.

When facing these types of issues, employers and healthcare providers must keep in mind certain legal considerations, including 1) whether an employer can require a certification from a healthcare provider substantiating both the existence of a health condition and the need for accommodation; 2) the extent to which an employer can push back on suspect certifications without violating employees' rights; 3) the consequences of providing a false certification to a patient, either purposefully or because the patient misrepresented their symptoms; and 4) patient privacy.

Employers–Considerations for Requiring a Medical Examination and Certification

The Americans with Disabilities Act ("ADA") and the Missouri Human Rights Act ("MHRA") prohibit employers from discriminating against individuals with disabilities.[1] Furthermore, in situations where an employee's disability is also considered a "serious health condition" under the Family and Medical Leave Act ("FMLA"), an employer may be subject to the requirements of both FMLA and the ADA.

The prohibitions in the ADA and MHRA against discrimination apply to people with qualifying physical and mental impairments and/or people who have a record of such impairments. These laws also require employers to make reasonable accommodations for known limitations (disabilities) of otherwise qualified applicants and employees, unless doing so poses an undue hardship.[2] The ability of an employer to inquire about health conditions varies depending on the stage of the employment relationship. For pre-employment applicants, the ADA generally restricts medical inquiries and examinations by employers and generally does not allow employers to make inquiries likely to illicit information about potential disabilities during the hiring process. Once the applicant is hired, however, these laws allow employers to request medical information in certain circumstances.

The U.S. Equal Employment Opportunity

Commission ("EEOC") has stated that one specific circumstance in which employers may request medical information is when an employee requests an accommodation for a medical condition, which may occur as the first step in an "informal, interactive process between the individual and the employer."[3] In such a situation, under both the ADA and FMLA, the employer may request information sufficient to substantiate the limitation or serious health condition, but may not ask for information unrelated to the specific disability or serious health condition.[4] In other words, under the ADA and/or MHRA, the employer's inquiry must be limited to the impairment and the job at issue. It must be job-related and consistent with business necessity.[5] Under the FMLA, the employer "requests" needed information through the Health Care Provider Certification form.

Documentation is sufficient if it substantiates that the individual has a disability and needs the accommodation requested. Specifically, sufficient medical documentation should describe the nature, severity, and duration of the impairment, the activity or activities that the impairment limits, the extent to which the impairment limits the employee's ability to perform the activity or activities of the employee's position, and should also substantiate why the requested reasonable accommodation is needed.[6] If the documentation is not sufficient, the employer should explain to the employee why it is insufficient and give the employee time to get the needed information. Absent express written authorization from the employee, the employer should not contact the healthcare provider directly to obtain the additional information needed, and, for reasons more fully explained below, the healthcare provider should not provide such information unless the patient has specifically authorized him/her in writing to do so.[7]

Employers–Considerations for Suspect Certifications

Suspected Employee Falsification

Employers may contact a medical provider who has supposedly provided medical certification in order to authenticate that document under FMLA; however, the employer should first give the employee an opportunity to clarify or cure any deficiencies with the documentation.[8] If the employee fails to satisfactorily cure the deficiencies, then the employer's "authentication" must be limited to the most basic, factual inquiry. Specifically, the employer can give the health care provider a copy of the certification and request the health care provider to confirm that the document was, in fact, provided and signed by that office/professional for the date(s) in question. This employer contact should be made by someone who is not in a direct supervisory role of the employee in order to protect confidentiality and minimize the possibility of improper use of the information learned.

Suspected Healthcare Provider Falsification

A more difficult instance is when an employer receives a certification that it believes is not accurate or is suspect for some reason. Because healthcare providers often have to rely on what a patient tells them about their alleged symptoms, it can be difficult for an employer to know whether the provider knowingly falsified a medical certification or simply documented the subjective reports of an employee patient concerning the patient's condition.

One simple way for an employer to check whether the certification was provided by a legitimate provider, as opposed to an online service that sells fake certifications, is to ensure the provider listed on the certification is licensed in the state where the examination was performed. Typically, states require that physicians are licensed in the state where the patient is located if they are performing medical examinations for treatment or diagnostic purposes. Whether a provider holds a license to practice in a particular state is usually readily available to the public through the state's licensing body's website (e.g. the Board of Healing Arts). If the physician is not licensed in this state, it could mean the certification was bought through a website or that the provider should not be examining patients in the state.

Applicability of Non-Medical Opinions

More often than false certifications, providers will sometimes include non-medical opinions in certifications. For example, a certification may explain an illness and state that "this employee should not be terminated" or, commonly in the era of COVID-19, "this employee must continue

in work from home status." The former statement is not a medical opinion regarding the employee's health condition. The latter could be related to a possible accommodation of a medical condition but does not link the possible accommodation to any medical need. In short, both comments are intended to assist the employee patient in maintaining employment, which is not the provider's role. An employer may consider but is not obligated to abide by such statements. Further, attempting to step outside the provider's role by offering non-medical opinions, detracts from the provider's credibility.

Healthcare Providers–Licensing Considerations

Providing False Certifications

Many conditions require healthcare providers to rely on the patient's own report of their symptoms (e.g. migraines). Thus, it can be difficult for providers to ascertain the severity of the condition and whether the patient is being truthful in all instances. That said, it is important that physicians and other healthcare providers take care in ensuring that they are not misrepresenting a condition or committing fraud.

Under Missouri Revised Statute § 334.100, the Missouri Board of Healing Arts may revoke a physician's license to practice medicine for "[m]isconduct, fraud, misrepresentation, dishonesty, unethical conduct or unprofessional conduct in the performance of the functions or duties." This can include "[k]nowingly making, or causing to be made, or aiding, or abetting in the making of, a false statement in any . . . certificate or document executed in connection with the practice of the person's profession[.]" Thus, if a physician were to knowingly certify to an employer that a patient had a certain condition, the physician could lose their license to practice medicine in Missouri. Furthermore, if the physician were licensed in more than one state, such actions would likely cause all the states of licensure to investigate the physician.

Patient Abandonment Considerations when Terminating the Relationship

In extreme cases, physicians may consider terminating the physician/patient relationship with a patient who they believe is not being honest with them or patients who ask them to misrepresent their condition to an employer. In such cases, if a physician does not properly terminate the relationship to ensure the patient has continuity of care, it could be considered patient abandonment and the physician could face discipline by the Missouri Board of Healing Arts.

Prior to taking such extreme action; however, physicians should: 1) counsel the patient and explain why the provider does not think he/she has a condition that would interfere with his/her job; 2) clearly document these interactions and the patient's response; 3) send a warning letter explaining the health consequences of the patient's continued non-compliance and explain that it could lead to termination of their relationship if not corrected immediately; and 4) if none of these options get through to the patient, the provider may terminate his/her relationship with the patient but should first review the network agreement with the patient's insurance to determine if there are any obligations to give the payor notice or if any prohibitions exist.

In addition to contractual obligations in terminating a physician/patient relationship, there are state law requirements physicians must adhere to in order to avoid patient abandonment. In *Weiss v. Rojanasathit*, the Missouri Supreme Court explained that:

The duty to attend the patient continues so long as required unless the physician-patient relationship is ended by 1) the mutual consent of the parties; 2) the physician's withdrawal after reasonable notice; 3) the dismissal of the physician by the patient; or 4) the cessation of the necessity that gave rise to the relationship. Absent good cause to the contrary, where the doctor knows or should know that a condition exists that requires further medical attention to prevent injurious consequences, the doctor must render such attention or must see to it that some other competent person. . .does so until the termination of the physician-patient relationship.[9]

Generally speaking, reasonable notice when terminating a physician/patient relationship should be 1) provided in writing; 2) allow the patient sufficient notice to find a new physician (at least thirty days); and 3) documentation of the notice should be retained, keeping the return receipt and a copy of the letter in the patient's file should be standard practice in such instances.

Health Care Providers–Privacy Considerations

Healthcare Providers Disclosures to Employers

The information a provider may release to an employer is governed by the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") Privacy Rule and state confidentiality laws. Generally, this rule applies to disclosures made by a health care provider and not the questions an employer may ask. If an employer asks a health care provider directly for information about an employee/patient, the provider cannot give the employer the information without patient authorization unless other laws require them to release such information.[10] Healthcare organizations have been sanctioned for improperly disclosing protected health information ("PHI") to an employer without patient authorization. Thus, healthcare providers must be careful not to disclose PHI. That said, if a health care provider receives a picture or fax of a certification or note purporting to be from the provider, they can verify whether the provider did, in fact, provide such information.

Healthcare Providers as Employers

Because the Privacy Rule protects PHI and not employment records, it can be confusing when the employer is a healthcare provider. In those instances, the employer may have access to both employment records and PHI, and it is important to maintain these records separately to ensure that health records containing PHI are adequately protected. This issue may arise when fellow employees or supervisors look at other employees' health records without authorization or a valid reason to do so.

For example, suppose a nurse at a hospital system presented a doctors' note stating that the nurse had tested positive for COVID and, thus, needed to isolate for fourteen days. The nurse's supervisor remembers that this nurse presented a similar note six months ago and is suspicious of its validity. It would not be permissible for the supervisor to look at the nurse's medical record and check the results of the nurse's lab work without the nurse's express authorization to do so. If the supervisor took such actions, it could be considered an improper access of the employee's medical record and would be impermissible under HIPAA. In a similar case, the Department of Health and Human Services Office of Civil Rights ("OCR") imposed sanctions on a clinic when a supervisor accessed an employee's medical record. OCR explained that, while the employee record was not protected by HIPAA, the medical record was protected, and the supervisor accessed PHI in the employee's medical record and shared it impermissibly. Thus, OCR imposed sanctions and required the clinic to take corrective action and retrain its staff regarding these issues. [11]

The correct course of action in the example described above would be for an employee who was not the nurse's direct supervisor to contact the physician and ask whether the note was authentic and from the time stated. Alternatively, the nurse could authorize the physician to give the supervisor or the human resources department such information.

Conclusion

While the internet makes life much more convenient, it can also make a false medical certification much easier to obtain. However, due to patient confidentiality requirements and employment laws prohibiting discrimination, employers and healthcare providers must use caution in their communications about employee/patients' health. As such, having robust policies in place and following such policies when these issues arise is imperative for compliance.

References

1. *See* 42 U.S.C. § 12112, Mo. Stat. Rev. § 213.055.
2. 42 U.S.C. §§ 12101–12117.
3. EEOC Enforcement Guidance: Reasonable Accommodation and Undue Hardship Under the Americans with Disabilities Act (Oct. 2002).
4. *Id.*
5. EEOC Enforcement Guidance: Disability-Related Inquiries and Medical Examinations of Employees Under the Americans With Disabilities Act (ADA) (June 2017).
6. *Id.*
7. The FMLA does provide limited circumstances in which an employer may contact a healthcare provider directly: to authenticate or clarify illegible handwriting on the FMLA forms. 29 C.F.R. §825.307.
8. 29 C.F.R. §825.307.
9. (emphasis added) 975 S.W.2d 113, 119–120 (Mo. 1998).
10. *See* 45 C.F.R. §§ 160.103 and 164.512(b)(1)(v).
11. *See* OIG, All Case Examples: Clinic Sanctions Supervisor for Accessing Employee Medical Record (Rev. Jun.2017), available at https://www.hhs.gov/hipaa/for-professionals/compliance-enforcement/examples/all-cases/index.html.

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The Last Word

The Flaws of Recent Omega-3 Clinical Trials Should Not Prevent Their Use

by James DiNicolantonio, PharmD & James H. O'Keefe, MD

We appreciate the article by Khoukaz and Fay suggesting that "The Jury is Still Out" regarding fish oil supplements for prevention of cardiovascular disease.[1] However, we wanted to take this opportunity to reply to some of their arguments in their article. The failure of the OMEGA trial was not surprising as the rate of sudden cardiac death was unexpectedly low.[2] The ASCEND trial[3] failed likely due to the low dose of Lovaza that was used (1 gram/day), whereas the REDUCE-IT[4] utilized 4 grams of Vascepa and found a significant 25% reduction in the primary endpoint. STRENGTH cannot be considered a complete failure, as there was a significant 15% reduction in coronary events in patients with established cardiovascular disease at baseline.[5] If anything, STRENGTH teaches us the difficulties for showing benefits with the use of marine omega-3s when given to patients without cardiovascular disease and in a short-term clinical study (2.5 years from end of study recruitment to study termination). OMEMI waited 2-8 weeks after an acute MI had taken place before giving omega-3s, a length of time that would have missed many of the fatal or ischemic events that occur early after a MI.[6]

We believe there is sufficient evidence for the use of marine omega-3s, especially for the secondary prevention of cardiovascular disease. This is based on decades worth of clinical studies indicating reductions in all-cause mortality, sudden cardiac death, and myocardial infarction in this clinical setting.[7] Considering the safety of omega-3s, as well as their benefits for reducing blood pressure, heart rate and stabilizing atherosclerotic plaque, there should be little reason to not prescribe them for the secondary prevention of cardiovascular disease.[7-9]

We appreciate the opportunity to have an open scientific debate with our esteemed colleagues and thank them for their contribution to the scientific literature.





James J. DiNicolantonio, PharmD, (left), and **James H. O'Keefe, MD**, (right), are at Saint Luke's Mid America Heart Institute, Kansas City, Missouri.

References

1. Khoukaz HB and Fay WP. Fish Oil Supplements for Prevention of Cardiovascular Disease:The Jury Is Still Out. Missouri Med. 2021.118:3:215-221.
2. Rauch B, Schiele R, Schneider S, et al. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. Circulation. 2010 Nov 23;122(21):2152–2159.
3. Bowman L, Mafham M, Wallendszus K, et al. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus. The New England journal of medicine 2018;379:1540-50.
4. Bhatt DL, Steg PG, Miller M, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. The New England journal of medicine 2019;380:11-22.
5. Nicholls SJ, Lincoff AM, Garcia M, et al. Effect of High-Dose Omega-3 Fatty Acids vs Corn Oil on Major Adverse Cardiovascular Events in Patients at High Cardiovascular Risk: The STRENGTH Randomized Clinical Trial. JAMA : the journal of the American Medical Association 2020;324:2268-80.
6. Kalstad AA, Myhre PL, Laake K, et al. Effects of n-3 Fatty Acid Supplements in Elderly Patients after Myocardial Infarction: A Randomized Controlled Trial. Circulation 2020.
7. DiNicolantonio JJ, Niazi AK, McCarty MF, et al. Omega-3s and cardiovascular health. The Ochsner journal 2014;14:399-412.
8. DiNicolantonio JJ, Meier P, O'Keefe JH. Omega-3 polyunsaturated fatty acids for the prevention of cardiovascular disease: do formulation, dosage & comparator matter? Missouri medicine 2013;110:495-8.
9. DiNicolantonio JJ, O'Keefe JH. The Benefits of Omega-3 Fats for Stabilizing and Remodeling Atherosclerosis. Missouri medicine 2020;117:65-9.

Disclosure

JJD is Director of Scientific Affairs at Advanced Ingredients for Dietary Products. JOK is an owner of a nutraceutical company that sells omega-3 supplements.

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THE LAST WORD

Fish Oil Supplements for Prevention of Cardiovascular Disease: Response to Opponents

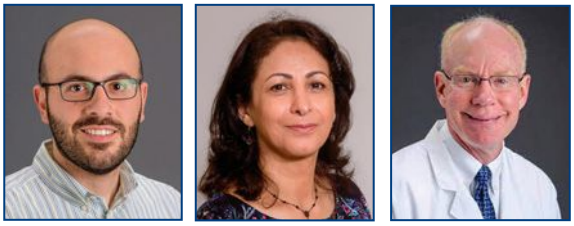
by Hekmat B. Khoukaz, MD, Mojgan Golzy, PhD & William P. Fay, MD

It was a pleasure to read the "pro" article by DiNicolantonio and O'Keefe,[1] and we commend them for their numerous contributions in the field of preventive cardiology. Nevertheless, we disagree with several of their comments and conclusions regarding the use of fish oil supplements to prevent cardiovascular diseases.

Foremost, we object to the heavy use of secondary endpoints to support their argument that "most 'negative' fish oil trials can actually be considered positive." DiNicolantonio and O'Keefe rely heavily on secondary endpoints in making this statement because many clinical trials have failed to find a beneficial effect of EPA/DHA supplementation on primary endpoints, which are the most consequential output of any clinical trial. Secondary endpoints are important, as they provide key information about an intervention, such as its mechanism of action or associated toxicity. The utility of secondary endpoints, particularly if they are prespecified at the time of study design, is to help interpret the study's primary results and guide future research.[2] However, secondary endpoints often lack the statistical power of primary endpoints. As such, they should not be used to interpret a clinical trial as "positive" if there was no statistically significant effect of the intervention on the study's primary endpoint.[3] Put another way, secondary endpoints are at best suggestive, but they are not conclusive, and they should not be used to override negative primary endpoint results and adopt an intervention into clinical practice.[2, 4-9]

Similarly, an individual component (e.g., myocardial infarction, MI) of a study's composite primary endpoint (e.g., MI, stroke, or cardiovascular death) should not be teased-out to interpret the study as positive when the primary composite endpoint was negative,[7] though such data can be used to support hypotheses and inform future prospective studies.

Let's discuss some specifics. Regarding the VITAL study,[10] the conclusion by DiNicolantonio and O'Keefe that fish oil supplementation produced a "statistically significant 17% reduction in death from coronary heart disease" is incorrect, as Table 2 of this study shows that the difference in coronary deaths between treatment groups was statistically insignificant. Furthermore, this parameter was neither a primary nor secondary endpoint, which was specifically mentioned by VITAL's authors. In the case of ASCEND,[11] vascular death was an individual component of the composite endpoint; as such, it does not provide rigorous evidence of benefit. We are intrigued that our Kansas City colleagues disapprove of including "soft events such as hospitalization" in composite primary endpoints, yet they do not criticize the JELIS[12] or REDUCE-IT[13] trials, positive fish oil studies, for including hospitalization endpoints such as unstable angina in the composite endpoint. Similarly, we find it surprising that DiNicolantonio and O'Keefe refer to ASCEND



Hekmat B. Khoukaz, MD, (left), is in the Department of Medicine; Mojgan Golzy, PhD, (center) is in the Department of Health Management and Informatics and the Biostatistics & Research Design Unit, and **William P. Fay, MD**, (right), is in both the Departments of Medicine and Medical Pharmacology and Physiology, University of Missouri-Columbia School of Medicine, Columbia, Missouri. Dr. Fay is also in Research Service, Harry S Truman Memorial Veterans Hospital, Columbia, Missouri.

as a "failure" because it found no significant effect of n-3 fatty acid (FA) supplementation on the primary composite endpoint of non-fatal MI, ischemic stroke, transient ischemic attack, or vascular death. Negative clinical trials are not failures, rather important findings that help physicians and patients avoid the cost and potential side-effects of treatments that do not provide rigorously determined benefits. Our opponents inaccurately state that "most deaths occur early on after a myocardial infarction." While the risk of death is higher shortly after an MI than later, this does not mean that most deaths occur within this time window.

DiNicolantonio and O'Keefe cite the important role of meta-analyses in formulating clinical practice guidelines, with which we agree. In citing the meta-analysis by Bernasconi et al.,[14, 15] our opponents refer to "the totality of this evidence regarding the cardiac effects of omega-3 supplementation." However, the Bernasconi meta-analysis included trials that were unblinded and lacked a placebo control.[12, 16] Furthermore, a closer look at the 40 included trials reveals that 9 of them (adding to 7,220 participants) studied populations and/or diseases lacking any cardiovascular connection.

For example, the FOSTAR trial studied the effects of fish oil on knee osteoarthritis without any assessment of cardiovascular outcomes.[17] Also misleading is the statement by DiNicolantonio and O'Keefe that "fish oil supplementation has decades worth of evidence including a recent pooled analysis of 17 prospective studies in 42,466 individuals," as these 17 studies were not randomized trials of n-3 FA supplementation! Rather, they were epidemiologic studies in which plasma n-3 FA levels (among many other parameters) were measured and correlated with risk of death.

In conclusion, we believe that currently available evidence is still not sufficiently convincing to endorse broad use of n-3 FAs to lower cardiovascular risk. We hold our position of restrictive use pending new data from refined clinical trials, as discussed in our "con" article. Until then, uncontested benefit may lie in the simplicity of recommending two meals of fish weekly to our patients. We appreciate the opportunity to participate in this scientific debate and commend our colleagues for their thoughtful argumentation and faithful disclosure of financial conflicts of interests.

References

1. DiNicolantonio J, O'Keefe JH. Does Fish Oil Reduce the Risk of Cardiovascular Events and Death? Recent Level 1 Evidence Says Yes: PRO: Fish Oil is Useful to Prevent or Treat Cardiovascular Disease. Mo Med. May-Jun 2021;118(3):214-218.
2. Endpoints in asthma drug trials--what do they mean? Drug Ther Bull. Mar 2006;44(3):21-4. doi:10.1136/dtb.2006.44321
3. Brown-Tuttle M. The Regulatory Strategist Toolbox: Clinical Endpoint Analysis Tools. Regulatory Affairs Professionals Society. Accessed 06/23, 2021. https://www.raps.org/news-and-articles/news-articles/2018/9/the-regulatory-strategist-toolbox-clinical-endpoi
4. Dmitrienko A, Tamhane AC, Bretz F. Multiple Testing Problems in Pharmaceutical Statistics Preface. Ch Crc Biostat Ser. 2010;33:1-33.
5. O'Neill RT. Secondary endpoints cannot be validly analyzed if the primary endpoint does not demonstrate clear statistical significance. Control Clin Trials. Dec 1997;18(6):550-6; discussion 561-7. doi:10.1016/s0197-2456(97)00075-5
6. EMA/CHMP/44762/2017 EMA. Guideline on multiplicity issues in clinical trials. Accessed May 2021, https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-multiplicity-issues-clinical-trials_en.pdf
7. GUIDANCE D. Multiple endpoints in clinical trials guidance for industry. Center for Biologics Evaluation and Research (CBER). 2017;
8. Freemantle N. Interpreting the results of secondary end points and subgroup analyses in clinical trials: should we lock the crazy aunt in the attic? BMJ. Apr 21 2001;322(7292):989-91. doi:10.1136/bmj.322.7292.989
9. Moye LA. End-point interpretation in clinical trials: the case for discipline. Control Clin Trials. Feb 1999;20(1):40-9; discussion 50-1. doi:10.1016/s0197-2456(98)00051-8
10. Manson JE, Cook NR, Lee IM, et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. N Engl J Med. Jan 3 2019;380(1):33-44. doi:10.1056/NEJMoa1809944
11. Group ASC, Bowman L, Mafham M, et al. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus. N Engl J Med. Oct 18 2018;379(16):1540-1550. doi:10.1056/NEJMoa1804989
12. Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet. Mar 31 2007;369(9567):1090-8. doi:10.1016/S0140-6736(07)60527-3
13. Bhatt DL, Steg PG, Miller M, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med. Jan 3 2019;380(1):11-22. doi:10.1056/NEJMoa1812792
14. Bernasconi AA, Lavie CJ, Milani RV, Laukkanen JA. Omega-3 Benefits Remain Strong Post-STRENGTH. Mayo Clin Proc. May 2021;96(5):1371-1372. doi:10.1016/j.mayocp.2021.03.004
15. Bernasconi AA, Wiest MM, Lavie CJ, Milani RV, Laukkanen JA. Effect of Omega-3 Dosage on Cardiovascular Outcomes: An Updated Meta-Analysis and Meta-Regression of Interventional Trials. Mayo Clin Proc. Feb 2021;96(2):304-313. doi:10.1016/j.mayocp.2020.08.034
16. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet. Aug 7 1999;354(9177):447-55.
17. Hill CL, March LM, Aitken D, et al. Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. Ann Rheum Dis. Jan 2016;75(1):23-9. doi:10.1136/annrheumdis-2014-207169

Disclosure

None reported.

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SAINT LOUIS UNIVERSITY
HENRY AND AMELIA NASRALLAH CENTER FOR NEUROSCIENCE

Center for Neurosciences Responsive to Jesuit Catholic Mission of Saint Louis University

by Daniela Salvemini, PhD

As director of the Henry and Amelia Nasrallah Center for Neuroscience, I welcome this opportunity in *Missouri Medicine, the Journal of the Missouri State Medical Association,* to introduce you to the Center, its mission and scope, and a small sample of the diverse and transformative research of its members.

The Henry and Amelia Nasrallah Center for Neuroscience, endowed in 2018, brings together researchers and clinicians throughout Saint Louis University who share a common interest in the neurosciences. The center is comprised of more than a 100 members across six schools and colleges in 23 departments, institutes, and centers.

Center scientists and clinicians focus on fundamental questions relevant to the continued health of our society:



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- Understand how we sense and perceive pain, to develop safer and more effective non-opioid pain medications;
- Understand how age-related dementias, genetic disorders of the nervous system, and injuries due to trauma or stroke cause pathology, to develop new drugs to delay or prevent dementia and promote recovery from injury;
- Develop new ways to translate basic research discoveries into clinical practice, such as drugs, drug delivery systems, and other medical devices;
- Identify new approaches to identify and mitigate the effects of psychological stress, depression, aging, and other factors on cognitive function, to improved neuro-psychiatric conditions for underserved communities.

Together, these objectives are highly responsive to the Jesuit Catholic mission of Saint Louis University to serve the least fortunate and most vulnerable among us.

The articles in this issue highlight some of the work being advanced by research labs in the Center, funded by the National Institutes of Health and various foundations. The Salvemini lab is pioneering research to fill the need for non-opioid analgesics to treat chronic pain. The Hawiger lab is pursuing novel immunotherapeutic approaches to Multiple

Collectively, such advances will continue to revolutionize our understanding of the nervous system under physiological and pathological settings and accelerate discovery of new approaches to treat and prevent disease and impact patient care.

Sclerosis. The Ayala and Nguyen labs are seeking to develop innovative RNA-based therapies for neurodegenerative diseases like Alzheimer's and amyotrophic lateral sclerosis. The Zustiak lab is developing new models for brain tumors using hydrogel technology to provide better drug screening tools. Drs. Yosten, Kolar, Salvemini, and Samson describe a strategy to drive breakthroughs in drug development targeting pathways controlling pain, diabetes, appetite behaviors, infertility and obesity. And Drs. Oberle, Postman, and van den Berk-Clark describe recent advances in clinical interventions for trauma in hospital, primary care, and community settings.

Center faculty have a strong record of publication, are recognized worldwide for their research and clinical expertise, train the next generation of clinical and research neuroscientists, and are actively engaged in outreach activities to improve the lives of individuals experiencing the burden of neurological disorders and injuries. Moreover, we are entrepreneurial, creating novel SLU-based start-ups, recognizing that private sector investment is key to moving new medicines forward to the marketplace to impact patient care.

Building on the tremendous progress made over the last decades, the future of neuroscience continues to be highly exciting. Advances are expected to be many and in multiple areas. For example: single cell transcriptomics/proteomics to expand our understanding of the huge diversity of cell types within the brain; high-resolution microscopy to allow us to visualize subcellular compartments; use of artificial intelligence for disease diagnosis; development of new technologies to dissect and understand specific neuronal circuits; integration of brain-computer interfaces to improve patient health; refined use of organoid systems to expand our understanding of neuronal plasticity or use in drug discovery screening efforts; development of animal models that more closely mimic disease in humans to further mechanistic understanding and aid in neurotherapeutic development.

Collectively, such advances will continue to revolutionize our understanding of the nervous system under physiological and pathological settings and accelerate discovery of new approaches to treat and prevent disease and impact patient care. Additional areas within the neuroscience field are also anticipated to grow as we move forward. For example, we are already witnessing how systems neuroscience is contributing to improved educational strategies aimed at students with neurorelated disorders.

Interdisciplinary collaborative approaches are key to the continued advancement of neuroscience. Members of Henry and Amelia Nasrallah Center for Neuroscience are poised to make extraordinary contributions in the field touching on multiple components from research and patient care to education, outreach and social justice.

Finally, I would like to express my deepest gratitude to Joel Eissenberg, PhD, for all the help and support he has given us while putting together this issue and his dedication to our efforts.

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Novel Non-Opioid Based Therapeutics for Chronic Neuropathic Pain

by Timothy M. Doyle, PhD, Kathryn Braden, BS, Caron M. Harada, BS, Fatma Mufti, MS, Rachel M. Schafer, BS & Daniela Salvemini, PhD

SAINT LOUIS UNIVERSITY
HENRY AND AMELIA NASRALLAH CENTER FOR NEUROSCIENCE

Since there will never be that "silver bullet" to treat pain, we need to continue to find multiple avenues to tailor our treatment of chronic pain to the patient.



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Abstract

Chronic neuropathic pain is currently a major health issue in U.S. complicated by the lack of non-opioid analgesic alternatives. Our investigations led to the discovery of major signaling pathways involved in the transition of acute to chronic neuropathic pain and the identification of several targets for therapeutic intervention. Our translational approach has facilitated the advancement of novel medicines for chronic neuropathic pain that are in advanced clinical development and clinical trials.

Introduction

Neuropathic pain arises from injury to peripheral nerves following trauma, disease, or toxin exposure. These conditions are severe and can be chronic and debilitating.[1] They are also often associated with many co-morbidities, such as depression and anxiety.[2] Chronic neuropathic pain is currently a major health issue in the U.S. that affects an estimated 50 million people, with over 19.6 million suffering debilitating chronic pain (high-impact chronic pain)[3] and an economic burden exceeding $600 billion.[4] Treatment of neuropathic pain is complicated by the lack of effective analgesics, leaving patients and physicians to rely on opioids that are themselves limited by loss of efficacy during long-term use, severe side effects and strong abuse liability.[5, 6] The mission of our investigative group has been to define the underlying mechanisms of chronic pain and to find novel pharmacological strategies to maintain opioid efficacy and lower its adverse effects while developing essential novel non-narcotic therapeutics. Our work has significantly impacted the understanding of the role of nitro-oxidative stress and several G protein-coupled pathways in pain (Figure 1). In this review, we will encapsulate our work, highlighting how it is leading to the next generation of pain therapies.

Nitroxidative Stress

Beginning in the mid 1990s and continuing over the last 25 years, we have identified a role for nitro-oxidative stress in the development and maintenance of chronic pain. Nitro-oxidative stress is an imbalance in the production of reactive oxygen (e.g., superoxide) and nitrogen (e.g., peroxynitrite) species and their removal by cellular antioxidant mechanisms (e.g., superoxide dismutases).[7] Injection of superoxide and peroxynitrite into the hind paw of a rodent produced painful thermal hypersensitivity by activating inflammation and cyclooxygenase-dependent pro-nociceptive PGE2 synthesis in animal paws.[8] We

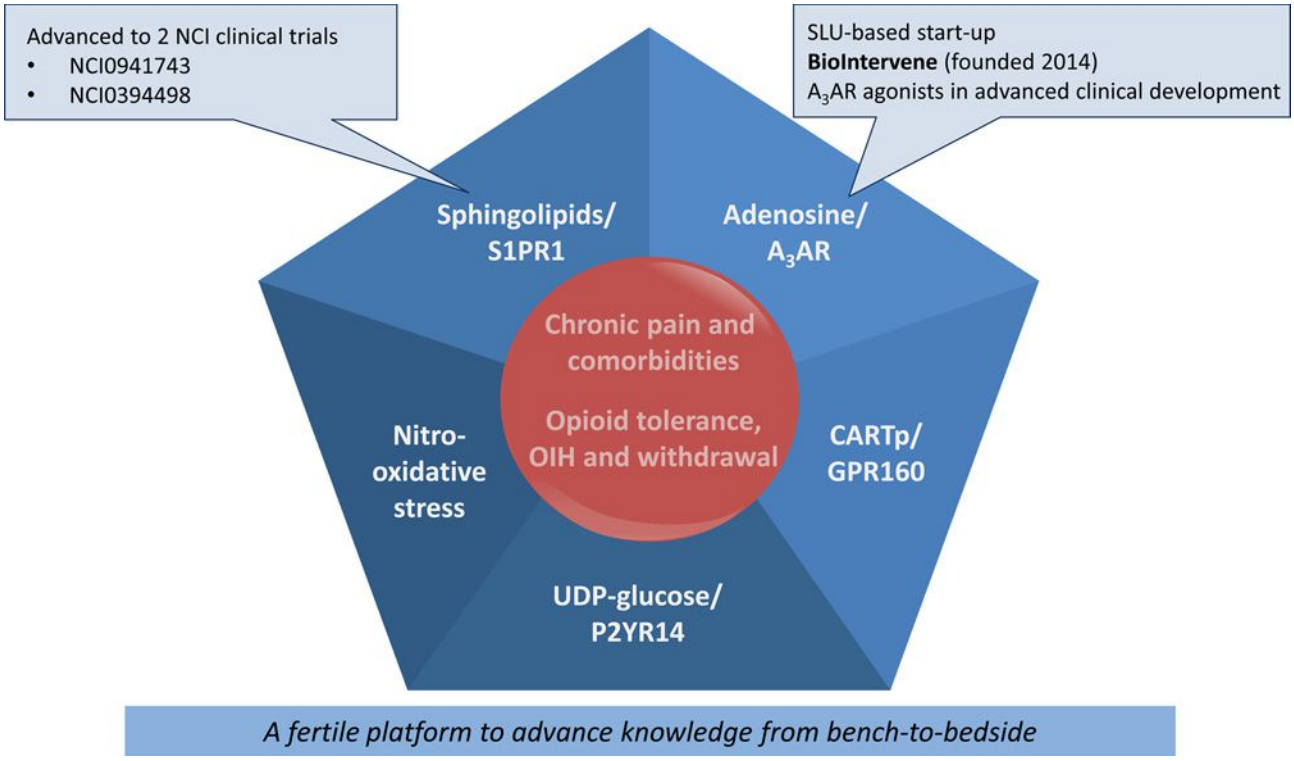


Figure 1. Avenues our group has investigated to identify underlying mechanisms of pain and adverse opioid effects in order to develop novel strategies to treat pain.

found that if levels of superoxide and peroxynitrite are reduced using drugs that catalytically remove superoxide and/or peroxynitrite (collectively referred to catalysts), we could block the development of painful hypersensitivities to thermal or mechanical (e.g., mechano-allodynia and mechano-hyperalgesia) stimuli in response to peripheral inflammation,[9,10] traumatic nerve injury,[11] chemotherapy-induced neuropathy (CIPN)[12, 13] or bone cancer.[14] These catalysts also reverse established neuropathic pain.[12] In addition to their anti-nociceptive effects, superoxide/peroxynitrite catalysts also protected opioid utility to treat pain. Long-term morphine-induced tolerance to its antinociceptive effects and a paradoxical opioid-induced hypersensitivity (OIH) in animals; mimicking adverse opioid effects in patients.[5, 6] We found these phenomena can be prevented or reversed by catalysts in animal models.[9, 10, 15-18]

Superoxide and peroxynitrite cause oxidative and nitrative modifications to biomolecules that regulate a variety of cellular pathways.[7, 9] Nitro-oxidative stress drove inflammation in peripheral tissues that led to inflammatory pain[9] and axonal mitochondrial dysfunction in sensory neurons, resulting in CIPN.[13] In the spinal cord, nitro-oxidative stress can sensitize neurons by enhancing excitatory glutamatergic neurotransmission via the N-methyl-d-aspartate glutamate receptor.[7, 9] This occurs through nitration and activation of glutamate receptors and nitration and deactivation of glutamate channels and metabolic enzymes that remove synaptic glutamate.[7, 9] We found increased nitration of these proteins in the spinal cord concurrent with pain that is attenuated when painful hypersensitivities were blocked or reversed by superoxide/peroxynitrite catalysts. Neuroinflammation associated with opioid tolerance,[9, 17, 18] OIH,[17] or neuropathic pain[12] was also blocked by superoxide/peroxynitrite catalysts in rodents. We also discovered oxidation and nitration of supraspinal met-enkephalin (an endogenous opioid) disrupted its ability to inhibit pain signaling at the spinal cord level.[19] Noteworthy, local injections of a peroxynitrite decomposition catalyst into supraspinal sites prevented met-enkephalin modifications and restored descending inhibitory signaling.[19]

Superoxide and peroxynitrite maintain nitro-oxidative stress in pain states by nitrating and inactivating endogenous mitochondrial manganese

superoxide dismutase (MnSOD) that constitutes a large part of a cell's antioxidant capacity. In several pain models, nitration and inactivation of this enzyme occured in the spinal cord of animals with peripheral inflammation[20, 21] and neuropathic pain[12, 13] and in the axons of peripheral nerves from animals with neuropathic pain.[13] Nitration and inactivation of MnSOD were reduced at these sites in animals when pain was attenuated with various catalysts.[12, 13, 20, 21]

We are now seeing clinical trials of compounds targeting nitro-oxidative stress in pain, such as the Phase II CIPN trials of the SODms mangafodipir and calmangafodipir (PLEDOx; PledPharma AB) and Phase II trials of a potential peroxynitrite-targeting therapeutic, ACP-044 (CERSCI Therapeutics, a subsidiary of ACADIA Pharmaceuticals) for surgical, osteoarthritic, and diabetic pain.

G Protein-Coupled Receptors

A major focus of our investigative group is now on using or targeting G protein-coupled receptors (GPCRs). GPCRs are membrane-bound receptors containing seven transmembrane domains; their genes make up about 4% of the human genome.[22] Their signaling is transduced through guanine nucleotide-binding proteins (G proteins) classified by their alpha subunit and the ability to stimulate (Gαs) or inhibit (Gαi) cyclic AMP formation, activate protein kinase C beta (Gαq/11) or transduce Rho signaling (Gα12/13).[23-25] With as much as 34% of our current FDA-approved drugs acting at GPCRs,[26] these receptors are highly druggable. Our investigations have now shed light on new roles in pain for four GPCRs: S1PR1, A_3AR, GPR160, and P2YR14.

S1PR1

S1PR1 is one of the five receptors (S1PR1-5) for the sphingolipid, sphingosine-1-phosphate (S1P), and couples to Gαi.[27] S1P is formed by sphingosine kinase phosphorylation of sphingosine generated from ceramide produced by the enzymatic hydrolysis of sphingomyelin or by de novo synthesis starting from serine and palmitoyl CoA.[27] Fingolimod (FTY720) is the first FDA-approved treatment for multiple sclerosis (MS) and targets S1PR1 signaling by reducing lymphocyte trafficking to the CNS and/or attenuating astrocyte-specific neuroinflammation in MS.[27]

The role of sphingolipids in painful hypersensitivities was not known until the early 2000s. Early studies identified a gain-of-function mutation in serine palmitoyltransferase (first step reaction of the de novo synthesis) as the cause of hereditary sensory neuropathy in humans[28] and ceramide-induced increased painful hypersensitivity in rodents.[27] Studies in peripheral nerves showed ceramide and S1P enhance sensory neuron excitability.[27] Then, a group of studies found that S1PR1 activation produced antinociceptive effects, leading to the notion that S1PR1 agonism could be used to treat pain.[29-31] These findings were based in part on the use of FTY720. As a S1P analog, FTY720 acts as an agonist of S1PR1, 3, 4 and 5; however, it also prevents S1PR1 from recycling back to the plasma membrane, resulting in long-lasting downregulation of S1PR1 and thus, FTY720 will act as a functional S1PR1 antagonist.[27]

In contrast to analgesia conferred through S1PR1 agonism, our investigations of sphingolipids and S1PR1 found that intraplantar injections of ceramide induced painful hypersensitivity in rats by activating PGE_2 and TNF signaling, which was readily attenuated by sphingosine kinase inhibitors, anti-S1P antibodies, or S1PR1-specific antagonists.[27] These pro-nociceptive effects of ceramide could be recapitulated with intraplantar injections of S1P or the S1PR1-specific agonist, SEW2871, which also induced nitro-oxidative stress in the paw.[27] In animal models of inflammatory pain, FTY720 and selective S1PR1 antagonists attenuated painful hypersensitivity and neutrophil infiltration into the paw.[27] Collectively, these were transformative findings, as they were the first to show S1PR1 antagonism, not S1PR1 agonism, was anti-nociceptive. This was further supported in animal models of neuropathic pain associated with CIPN, bone cancer,[32] and traumatic nerve injury.[27] Moreover, S1PR1 antagonists prevented opioid-induced antinociceptive tolerance, OIH, and withdrawal in animals to preserve the utility of opioids for pain treatment. In cancer bone pain,[32] bortezomib-induced neuropathic pain,[33] and morphine withdrawal,[34] de novo sphingolipid metabolism was active in the spinal cord.

The effects of S1PR1 antagonists revealed that S1PR1 signaling is a major contributor to neuroinflammation in the spinal cord following chemotherapy,[33, 35] traumatic nerve injury[36] or morphine use.[34, 37] We now think this is due in part to direct activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome

and IL-1β signaling in the spinal cord.[38] NLRP3 is one of the inflammasome mechanisms that activate caspase-1 for post-translational maturation of IL-1β.[39] We found that intrathecal administration of SEW2871 increased NLRP3 expression, caspase 1 activation and IL-1β maturation in the spinal cord.[38] Blocking NLRP3 or IL-1β signaling prevented SEW2871-induced painful hypersensitivities as did S1PR1 antagonist (selective or functional) and genetic deletion of S1PR1 in astrocytes.[38]

Over the last decade, several orally bioavailable and CNS penetrant S1PR1 functional and competitive antagonists have been developed.[27] Two S1PR1 functional antagonists are now FDA-approved for the treatment of MS: the pro-drug FTY720 (Gilenya®, Novartis) approved in 2010 and ozanimod (RPC1063, Zeposia®, Celgene) approved in 2020.[27] S1PR1 competitive antagonists, such as NIBR-15 and TASP0277308, are in advanced preclinical development for a variety of disease states.[27] Our work in this field has now led to the two NCI clinical trials (NCI0941743 and NCI0394498) to test the efficacy of S1PR1 antagonists in the prevention and treatment of CIPN in breast cancer patients.

A_3AR

Adenosine is released in response to physiological stress and inflammation. It can act at four GPCRs (A_1, A_{2A}, A_{2B} and A_3).[40] Adenosine has been known for over 30 years to possess potent and long-lasting analgesic effects in both preclinical and human subjects.[40] Pain studies have focused solely on the role of A_1AR and A_2AR in these analgesic effects.[40] However, these targets have not achieved clinical relevance due to adverse cardiovascular side effects.[40] With the development of more specific A_3AR agonists, such as IB-MECA (50-fold selective for A_3AR over A_1 or $A_{2A}AR$), our group began investigating adenosine A_3 receptor (A_3AR) as a potential target for treating chronic pain.[40] A_3AR is expressed throughout the nervous system, including neurons and glia.[40] A_3AR couples to Gαi to inhibit cyclic AMP signaling and regulate MAPK pathways.[40] In 2012, our investigations demonstrated that IB-MECA and Cl-IB-MECA reverse traumatic nerve injury-induced neuropathic pain in rodents without affecting normal protective nociception.[41] This was further confirmed with the development of even more highly selective A_3AR agonists, MRS1898 and MRS5698 in models of traumatic nerve injury- and chemotherapy-induced neuropathic pain.[40] The antinociceptive effects of MRS5698 are abolished in $A_3AR^{-/-}$ mice or following administration of the A_3AR antagonist, MRS1523.[42] Importantly, A_3AR agonists were effective at reversing traumatic nerve injury-induced neuropathic pain whether administered intradermally (paw ipsilateral to injury), intrathecally, or directly into the RVM; indicating that A_3AR is functionally involved in the maintenance of neuropathic pain at all levels of the pain pathway.[42] Recently, we also found that A_3AR agonists prevented the development of opioid-induced antinociceptive tolerance, OIH and withdrawal.[43]

We found that the analgesic effects of A_3AR agonists were mediated by several mechanisms that contribute to their potency and efficacy as therapeutics. A_3AR was able to inhibit neuropathic pain and adverse morphine effects by suppressing glial activation and pro-inflammatory cytokine production, such as IL-1β and TNF, while increasing anti-inflammatory IL-10.[40] A_3AR agonists also inhibited NADPH oxidase activity and activation of MAPK and NFκB signaling in response to nerve injury,[40] which contributes to inflammation and neuronal hyperexcitability.[44] Following nerve injury, A_3AR agonists were able to prevent the inactivation of GLT-1 and glutamate synthetase in the spinal cord and triggered GABAergic signaling through direct modulation of GAD65 and GAT-1 or indirectly through KCC2.[40]

The multiple molecular mechanisms underlying A_3AR analgesics mirror those provided by opioid therapies, but A_3AR agonists avoid the tolerance and abuse potential associated with opioids.[42] Importantly, A_3AR agonists are currently in clinical trials for inflammatory diseases and cancer with good safety profiles,[45-47] suggesting that the cardiovascular side effects that have limited clinical use of adenosine receptor therapy in the past can be overcome by targeting the A_3AR for the treatment of neuropathic pain. Our investigations have led to the founding of BioIntervene, a Saint Louis University-based biotech start-up, to identify potent and efficacious A_3AR agonists for clinical studies in pain.

CARTp/GPR160 in Pain

Recently, we discovered a role of GPR160 in neuropathic pain.[48] Investigation of GPR160 came after unbiased receptomic canvassing for orphaned GPCRs in pain suggested a role of GPR160 in pain.[48]

Follow-up PCR and RNA-Seq studies revealed up-regulation of GPR160 in the dorsal spinal cord on the side of nerve injury relative to the non-injured side.[48] When we blocked GPR160 signaling by gene silencing or antibody neutralization, nerve injury-induced neuropathic pain was reversed or prevented.[48] This suggested for the first time that GPR160 contributed to neuropathic pain.

However, as an orphaned GPCR, the ligand for GPR160 was not known. We were able to deorphanize GPR160 by identifying the cocaine- and amphetamine-regulated transcript peptide (CARTp) as its ligand.[48] CARTp is an anorexigenic neuropeptide and involved in many biological processes.[49-52] CARTp is processed into at least two bioactive peptides, which are CARTp (55-102) and CARTp (62-102) in animals.[53, 54] CARTp is expressed in unmyelinated peptidergic sensory neurons that terminate mainly in pain processing centers of the spinal cord.[55] In our studies, CARTp not only colocalized with GPR160 in cells, but immunopreciptated with GPR160, suggesting GPR160 was a receptor for CARTp.[48] Early studies in pain produced contradictory evidence for pro-[56] and anti-[57-60] nociceptive effects of CARTp in animals. In our studies in mice, a single intrathecal injection of CARTp induced painful hypersensitivities within 1 h after injection; suggesting CARTp was a pro-nociceptive agent.[48] Moreover, the effects of CARTp were blocked when it or GPR160 were neutralized by antibodies.[48] This provides conclusive evidence that CARTp signaling is transduced by GPR160 to produce a pain state in animals.

We are still investigating the mechanisms by which CARTp/GPR160 signaling promotes chronic pain. CARTp potentiates hyperalgesia mediated by the excitatory glutamatergic receptor, N-methyl-d-aspartate receptor (NMDAR).[61, 62] CARTp also induces phosphorylation of extracellular signal-regulated kinase (ERK) and cAMP-response element binding protein (CREB) in cell cultures[48] that contribute to the development and maintenance of neuropathic pain states.[63-66] In our CARTp-induced pain models, ERK and CREB phosphorylation and signaling increased in the spinal cords of mice, which was blocked by attenuating GPR160 signaling.[48]

A major focus now for our group is to identify and develop small molecule GPR160 inhibitors for which there are currently none. This will provide the tools to define GPR160 function in pain, but more importantly, to develop molecules as potential non-opioid drug candidates for clinical studies. We have no reason to believe that interrupting GPR160 signaling to treat pain would have adverse effects, as GPR160 knockout animals are do not exhibit adverse health effects.[67] Identifying small molecule GPR160 inhibitors and linking CARTp to GPR160 affects not only pain, but other diseases where CARTp plays a roles, such as feeding disorders,[68] substance abuse,[68] and depression.[69]

P2Y14 Receptor

The P2Y14 receptor (P2RY14) is a purinergic Gαi-coupled GPCR that binds nucleotide-sugars, such as UDP-glucose.[70] P2RY14 protein is found in nervous and immune tissues[70-72] and its transcript is found in glial cells within the rat dorsal root ganglia and spinal cord,[73] and in murine microglial cell lines.[74]

There is evidence of the involvement of P2RY14 in pain. In rat traumatic nerve injury-induced[75] and post-surgical[76] pain models, P2RY14 transcript levels were found to increase on the same side of the spinal cord as the nerve injury. P2RY14 elevation was also observed in the rat trigeminal ganglia in an inflammatory pain model after a complete Freund's adjuvant (CFA) injection.[72] Attenuating P2RY14 signaling with an antisense-locked nucleic acid also reduced traumatic nerve injury-induced neuropathic pain.[75] In our studies, we found that administration of P2RY14 antagonists reversed traumatic nerve injury-induced neuropathic pain in a dose-dependent manner.[77]

Apart from inhibiting cAMP signaling,[78, 79] the signaling pathways and effects of P2RY14 activation are not well characterized. Some studies observed increased P2RY14-dependent ERK,[78, 80] p38,[80] and JNK[80] phosphorylation in cell culture. UDP-glucose induced RhoA activation and chemotaxis in human neutrophils.[81, 82] However, not much is known about the role of P2RY14 in the nervous system. Current evidence suggests its involvement in neuroimmune responses. For example, P2RY14 transcripts increased in various areas of rat brain after an acute bacterial endotoxin challenge.[83,71] In CFA pain models, the P2Y14R antagonist, PPTN, attenuated the production of proinflammatory cytokines IL-1β, CCL2, and TNFα in the trigeminal ganglia.[84]

Several P2RY14 antagonists, with varying potencies and bioavailabilities, have been developed. One main group is naphthoic acid derivatives, which includes aforementioned PPTN and its analogues.[85]

However, this is an area ripe for developing novel small molecules that can be advanced as candidate drugs for clinical studies for pain. Our current focus is identifying such molecules and assessing their effectiveness in pre-clinical studies.

Conclusion

In light of the current opioid epidemic, an increasing aging population, and the lack of adequate therapies to combat pain, we will continue to be faced with the social and economic tolls that chronic neuropathic pain exacts on our society. We anticipate that our work will produce much-needed clinical solutions to the unmet medical need. Since there will never be that "silver bullet" to treat pain, we need to continue to find multiple avenues to tailor our treatment of chronic pain to the patient.

References

1. Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet neurology. Feb 2015;14(2):162-73. doi:10.1016/S1474-4422(14)70251-0
2. Nicholson B, Verma S. Comorbidities in chronic neuropathic pain. Pain medicine. Mar 2004;5 Suppl 1:S9-S27.
3. 67 Prevalence of Chronic Pain and High-Impact Chronic Pain Among Adults — United States, 2016. 1001–1006 (2018).
4. Institute of Medicine. Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research. https://www.ncbi.nlm.nih.gov/books/NBK92525/
5. Angst MS, Clark JD. Opioid-induced hyperalgesia: a qualitative systematic review. Anesthesiology. Mar 2006;104(3):570-87. doi:10.1097/00000542-200603000-00025
6. Collett BJ. Opioid tolerance: the clinical perspective. British journal of anaesthesia. Jul 1998;81(1):58-68. doi:10.1093/bja/81.1.58
7. Grace PM, Gaudet AD, Staikopoulos V, et al. Nitroxidative Signaling Mechanisms in Pathological Pain. Trends in neurosciences. Dec 2016;39(12):862-879. doi:10.1016/j.tins.2016.10.003
8. Ndengele MM, Muscoli C, Wang ZQ, Doyle TM, Matuschak GM, Salvemini D. Superoxide potentiates NF-kappaB activation and modulates endotoxin-induced cytokine production in alveolar macrophages. Shock. Feb 2005;23(2):186-93.
9. Janes K, Neumann WL, Salvemini D. Anti-superoxide and anti-peroxynitrite strategies in pain suppression. Biochim Biophys Acta. May 2012;1822(5):815-21. doi:10.1016/j.bbadis.2011.12.008
10. Salvemini D, Little JW, Doyle T, Neumann WL. Roles of reactive oxygen and nitrogen species in pain. Free radical biology & medicine. Sep 1 2011;51(5):951-66. doi:10.1016/j.freeradbiomed.2011.01.026
11. Rausaria S, Ghaffari MM, Kamadulski A, et al. Retooling manganese(III) porphyrin-based peroxynitrite decomposition catalysts for selectivity and oral activity: a potential new strategy for treating chronic pain. Journal of medicinal chemistry. Dec 22 2011;54(24):8658-69. doi:10.1021/jm201233r
12. Doyle T, Chen Z, Muscoli C, et al. Targeting the overproduction of peroxynitrite for the prevention and reversal of paclitaxel-induced neuropathic pain. Research Support, N.I.H., Extramural. The Journal of neuroscience : the official journal of the Society for Neuroscience. May 2 2012;32(18):6149-60. doi:10.1523/JNEUROSCI.6343-11.2012
13. Janes K, Doyle T, Bryant L, et al. Bioenergetic deficits in peripheral nerve sensory axons during chemotherapy-induced neuropathic pain resulting from peroxynitrite-mediated post-translational nitration of mitochondrial superoxide dismutase. Pain. Nov 2013;154(11):2432-40. doi:10.1016/j.pain.2013.07.032
14. Slosky LM, BassiriRad NM, Symons AM, et al. The cystine/glutamate antiporter system xc- drives breast tumor cell glutamate release and cancer-induced bone pain. Pain. Nov 2016;157(11):2605-2616. doi:10.1097/j.pain.0000000000000681
15. Doyle T, Bryant L, Batinic-Haberle I, et al. Supraspinal inactivation of mitochondrial superoxide dismutase is a source of peroxynitrite in the development of morphine antinociceptive tolerance. Neuroscience. Dec 1 2009;164(2):702-10. doi:10.1016/j.neuroscience.2009.07.019
16. Batinic-Haberle I, Ndengele MM, Cuzzocrea S, Reboucas JS, Spasojevic I, Salvemini D. Lipophilicity is a critical parameter that dominates the efficacy of metalloporphyrins in blocking the development of morphine antinociceptive tolerance through peroxynitrite-mediated pathways. Free radical biology & medicine. Jan 15 2009;46(2):212-9. doi:10.1016/j.freeradbiomed.2008.09.037
17. Little JW, Cuzzocrea S, Bryant L, et al. Spinal mitochondrial-derived peroxynitrite enhances neuroimmune activation during morphine hyperalgesia and antinociceptive tolerance. Pain. Jul 2013;154(7):978-86. doi:10.1016/j.pain.2013.02.018
18. Doyle T, Esposito E, Bryant L, Cuzzocrea S, Salvemini D. NADPH-oxidase 2 activation promotes opioid-induced antinociceptive tolerance in mice. Research Support, N.I.H., Extramural. Neuroscience. Jun 25 2013;241:1-9. doi:10.1016/j.neuroscience.2013.02.042
19. Little JW, Chen Z, Doyle T, et al. Supraspinal peroxynitrite modulates pain signaling by suppressing the endogenous opioid pathway. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. The Journal of neuroscience : the official journal of the Society for Neuroscience. Aug 8 2012;32(32):10797-808. doi:10.1523/JNEUROSCI.6345-11.2012
20. Wang ZQ, Porreca F, Cuzzocrea S, et al. A newly identified role for superoxide in inflammatory pain. J Pharmacol Exp Ther. Jun 2004;309(3):869-78. doi:10.1124/jpet.103.064154
21. Muscoli C, Mollace V, Wheatley J, et al. Superoxide-mediated nitration of spinal manganese superoxide dismutase: a novel pathway in N-methyl-D-aspartate-mediated hyperalgesia. Pain. Sep 2004;111(1-2):96-103. doi:10.1016/j.pain.2004.06.004
22. Wu V, Yeerna H, Nohata N, et al. Illuminating the Onco-GPCRome: Novel G protein-coupled receptor-driven oncocrine networks and targets for cancer immunotherapy. The Journal of biological chemistry. Jul 19 2019;294(29):11062-11086. doi:10.1074/jbc.REV119.005601
23. Neves SR, Ram PT, Iyengar R. G protein pathways. Science. May 31 2002;296(5573):1636-9. doi:10.1126/science.1071550
24. Kamato D, Thach L, Bernard R, et al. Structure, Function, Pharmacology, and Therapeutic Potential of the G Protein, G⍰/q,11. Front Cardiovasc Med. 2015;2:14-14. doi:10.3389/fcvm.2015.00014
25. Stone LS, Molliver DC. In search of analgesia: emerging roles of GPCRs in pain. Molecular interventions. 2009;9(5):234-251. doi:10.1124/mi.9.5.7
26. Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. Nature reviews Drug discovery. 2017;16(12):829-842. doi:10.1038/nrd.2017.178
27. Squillace S, Spiegel S, Salvemini D. Targeting the Sphingosine-1-Phosphate Axis for Developing Non-narcotic Pain Therapeutics. Trends in pharmacological sciences. Nov 2020;41(11):851-867. doi:10.1016/j.tips.2020.09.006
28. Dawkins JL, Hulme DJ, Brahmbhatt SB, Auer-Grumbach M, Nicholson GA. Mutations in SPTLC1, encoding serine palmitoyltransferase, long chain base subunit-1, cause hereditary sensory neuropathy type I. Nat Genet. Mar 2001;27(3):309-12. doi:10.1038/85879
29. Coste O, Brenneis C, Linke B, et al. Sphingosine 1-phosphate modulates spinal nociceptive processing. The Journal of biological chemistry. Nov 21 2008;283(47):32442-51.
30. Coste O, Pierre S, Marian C, et al. Antinociceptive activity of the S1P-receptor agonist FTY720. J Cell Mol Med. Jun 2008;12(3):995-1004.
31. Sim-Selley LJ, Goforth PB, Mba MU, et al. Sphingosine-1-phosphate receptors mediate neuromodulatory functions in the CNS. J Neurochem. Aug 2009;110(4):1191-202. doi:10.1111/j.1471-4159.2009.06202.x
32. Grenald SA, Doyle TM, Zhang H, et al. Targeting the S1P/S1PR1 axis mitigates cancer-induced bone pain and neuroinflammation. Pain. Sep 2017;158(9):1733-1742. doi:10.1097/j.pain.0000000000000965
33. Stockstill K, Doyle TM, Yan X, et al. Dysregulation of sphingolipid metabolism contributes to bortezomib-induced neuropathic pain. J Exp Med. May 7 2018;215(5):1301-1313. doi:10.1084/jem.20170584
34. Doyle TM, Hutchinson MR, Braden K, et al. Sphingosine-1-phosphate receptor subtype 1 activation in the central nervous system contributes to morphine withdrawal in rodents. J Neuroinflammation. Oct 22 2020;17(1):314. doi:10.1186/s12974-020-01975-2
35. Janes K, Little JW, Li C, et al. The development and maintenance of paclitaxel-induced neuropathic pain require activation of the sphingosine 1-phosphate receptor subtype 1. The Journal of biological chemistry. Jul 25 2014;289(30):21082-97. doi:10.1074/jbc.M114.569574
36. Chen Z, Doyle TM, Luongo L, et al. Sphingosine-1-phosphate receptor 1 activation in astrocytes contributes to neuropathic pain. Proceedings of the National Academy of Sciences of the United States of America. May 21 2019;116(21):10557-10562. doi:10.1073/pnas.1820466116
37. Doyle TM, Janes K, Chen Z, et al. Activation of sphingosine-1-phosphate receptor subtype 1 in the central nervous system contributes to morphine-induced hyperalgesia and antinociceptive tolerance in rodents. Pain. Apr 13 2020;doi:10.1097/j.pain.0000000000001888
38. Doyle TM, Chen Z, Durante M, Salvemini D. Activation of Sphingosine-1-Phosphate Receptor 1 in the Spinal Cord Produces Mechanohypersensitivity Through the Activation of Inflammasome and IL-1beta Pathway. The journal of pain : official journal of the American Pain Society. Aug 2019;20(8):956-964. doi:10.1016/j.jpain.2019.02.007
39. Tsuchiya K, Hara H. The inflammasome and its regulation. Critical reviews in immunology. 2014;34(1):41-80. doi:10.1615/critrevimmunol.2013008686
40. D. S, T.M. D, T.M. L-M, T.W. V. The Adenosine-Receptor Axis in Chronic Pain. . In: P. B, K. V, S. G, S. M, F. V, eds. The Adenosine Receptors The Receptors, vol 34 Humana Press, Cham.; 2018: 413-437.
41. Chen Z, Janes K, Chen C, et al. Controlling murine and rat chronic pain through A3 adenosine receptor activation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. May 2012;26(5):1855-65. doi:10.1096/fj.11-201541
42. Little JW, Ford A, Symons-Liguori AM, et al. Endogenous adenosine A3 receptor activation selectively alleviates persistent pain states. Brain. Jan 2015;138(Pt 1):28-35. doi:10.1093/brain/awu330
43. Doyle TM, Largent-Milnes TM, Chen Z, et al. Chronic Morphine-Induced Changes

in Signaling at the A3 Adenosine Receptor Contribute to Morphine-Induced Hyperalgesia, Tolerance, and Withdrawal. J Pharmacol Exp Ther. Aug 2020;374(2):331-341. doi:10.1124/jpet.120.000004
44. Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci. Jan 2009;10(1):23-36.
45. Stemmer SM, Benjaminov O, Medalia G, et al. CF102 for the treatment of hepatocellular carcinoma: a phase I/II, open-label, dose-escalation study. The oncologist. 2013;18(1):25-6. doi:10.1634/theoncologist.2012-0211
46. Stemmer SM, Manojlovic NS, Marinca MV, et al. A phase II, randomized, double-blind, placebo-controlled trial evaluating efficacy and safety of namodenoson (CF102), an A3 adenosine receptor agonist (A3AR), as a second-line treatment in patients with Child-Pugh B (CPB) advanced hepatocellular carcinoma (HCC). Journal of Clinical Oncology. 2019;37(15_suppl):2503-2503. doi:10.1200/JCO.2019.37.15_suppl.2503
47. David M, Akerman L, Ziv M, et al. Treatment of plaque-type psoriasis with oral CF101: data from an exploratory randomized phase 2 clinical trial. J Eur Acad Dermatol Venereol. Mar 2012;26(3):361-7. doi:10.1111/j.1468-3083.2011.04078.x
48. Yosten GL, Harada CM, Haddock C, et al. GPR160 de-orphanization reveals critical roles in neuropathic pain in rodents. J Clin Invest. May 1 2020;130(5):2587-2592. doi:10.1172/JCI133270
49. Ahmadian-Moghadam H, Sadat-Shirazi MS, Zarrindast MR. Cocaine- and amphetamine-regulated transcript (CART): A multifaceted neuropeptide. Peptides. Dec 2018;110:56-77. doi:10.1016/j.peptides.2018.10.008
50. Bik W, Skwarlo-Sonta K, Szelagiewicz J, et al. Involvement of the cocaine-amphetamine regulated transcript peptide (CART 55-102) in the modulation of rat immune cell activity. Neuro Endocrinol Lett. Jun 2008;29(3):359-65.
51. Lau J, Herzog H. CART in the regulation of appetite and energy homeostasis. Review. Frontiers in Neuroscience. 2014-October-13 2014;8(313)doi:10.3389/fnins.2014.00313
52. Koylu EO, Balkan B, Kuhar MJ, Pogun S. Cocaine and amphetamine regulated transcript (CART) and the stress response. Peptides. 2006/08/01/ 2006;27(8):1956-1969. doi:https://doi.org/10.1016/j.peptides.2006.03.032
53. Dey A, Xhu X, Carroll R, Turck CW, Stein J, Steiner DF. Biological Processing of the Cocaine and Amphetamine-regulated Transcript Precursors by Prohormone Convertases, PC2 and PC1/3. Journal of Biological Chemistry. April 25, 2003 2003;278(17):15007-15014. doi:10.1074/jbc.M212128200
54. Stein J, Steiner DF, Dey A. Processing of cocaine- and amphetamine-regulated transcript (CART) precursor proteins by prohormone convertases (PCs) and its implications. Peptides. 2006/08/01/ 2006;27(8):1919-1925. doi:https://doi.org/10.1016/j.peptides.2005.10.028
55. Kozsurek M, Lukacsi E, Fekete C, Wittmann G, Rethelyi M, Puskar Z. Cocaine- and amphetamine-regulated transcript peptide (CART) is present in peptidergic C primary afferents and axons of excitatory interneurons with a possible role in nociception in the superficial laminae of the rat spinal cord. The European journal of neuroscience. Sep 2007;26(6):1624-31. doi:10.1111/j.1460-9568.2007.05789.x
56. Ohsawa M, Dun SL, Tseng LF, Chang J, Dun NJ. Decrease of hindpaw withdrawal latency by cocaine- and amphetamine-regulated transcript peptide to the mouse spinal cord. European journal of pharmacology. Jul 7 2000;399(2-3):165-9. doi:10.1016/s0014-2999(00)00374-5
57. Damaj MI, Hunter RG, Martin BR, Kuhar MJ. Intrathecal CART (55-102) enhances the spinal analgesic actions of morphine in mice. Brain research. Oct 22 2004;1024(1-2):146-9. doi:10.1016/j.brainres.2004.07.058
58. Damaj MI, Martin BR, Kuhar MJ. Antinociceptive effects of supraspinal rat cart (55–102) peptide in mice. Brain Research. 2003/09/05/ 2003;983(1):233-236. doi:https://doi.org/10.1016/S0006-8993(03)03094-4
59. Damaj MI, Zheng J, Martin BR, Kuhar MJ. Intrathecal CART (55-102) attenuates hyperlagesia and allodynia in a mouse model of neuropathic but not inflammatory pain. Peptides. Aug 2006;27(8):2019-23. doi:10.1016/j.peptides.2005.09.019
60. Upadhya MA, Dandekar MP, Kokare DM, Singru PS, Subhedar NK. Evidence for the participation of cocaine- and amphetamine-regulated transcript peptide (CART) in the fluoxetine-induced anti-hyperalgesia in neuropathic rats. Peptides. Feb 2011;32(2):317-26. doi:10.1016/j.peptides.2010.09.030
61. Hsun Lin H, Chiu HY, Lai CC. Potentiation of spinal N-methyl-D-aspartate-mediated nociceptive transmission by cocaine-regulated and amphetamine-regulated transcript peptide in rats. Neuroreport. Feb 28 2005;16(3):253-7. doi:10.1097/00001756-200502280-00010
62. Chiu HY, Lin HH, Lai CC. Potentiation of spinal NMDA-mediated nociception by cocaine- and amphetamine-regulated transcript peptide via PKA and PKC signaling pathways in rats. Regul Pept. Nov 27 2009;158(1-3):77-85. doi:10.1016/j.regpep.2009.07.012
63. Han M, Huang R-Y, Du Y-M, Zhao Z-Q, Zhang Y-Q. Early intervention of ERK activation in the spinal cord can block initiation of peripheral nerve injury-induced neuropathic pain in rats. Sheng li xue bao : [Acta physiologica Sinica]. 2011/04// 2011;63(2):106-114.
64. Song XS, Cao JL, Xu YB, He JH, Zhang LC, Zeng YM. Activation of ERK/CREB pathway in spinal cord contributes to chronic constrictive injury-induced neuropathic pain in rats. Acta Pharmacol Sin. Jul 2005;26(7):789-98. doi:10.1111/j.1745-7254.2005.00123.x
65. Ma W, Quirion R. The ERK/MAPK pathway, as a target for the treatment of neuropathic pain. Expert Opin Ther Targets. Aug 2005;9(4):699-713. doi:10.1517/14728222.9.4.699
66. Gu X, Bo J, Zhang W, et al. Intrathecal administration of cyclic AMP response element-binding protein-antisense oligonucleotide attenuates neuropathic pain after peripheral nerve injury and decreases the expression of N-Methyl-D-Aspartic receptors in mice. Oncol Rep. 2013/07/01 2013;30(1):391-398. doi:10.3892/or.2013.2437
67. Yang H, Liu H, Chen H, et al. G protein-coupled receptor160 regulates mycobacteria entry into macrophages by activating ERK. Cellular signalling. Sep 2016;28(9):1145-51. doi:10.1016/j.cellsig.2016.05.022
68. Kuhar MJ, Dall Vechia SE. CART peptides: novel addiction- and feeding-related neuropeptides. Trends in neurosciences. Jul 1999;22(7):316-20. doi:10.1016/s0166-2236(98)01377-0
69. Dandekar MP, Singru PS, Kokare DM, Subhedar NK. Cocaine- and amphetamine-regulated transcript peptide plays a role in the manifestation of depression: social isolation and olfactory bulbectomy models reveal unifying principles. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. Apr 2009;34(5):1288-300. doi:10.1038/npp.2008.201
70. Chambers JK, Macdonald LE, Sarau HM, et al. A G protein-coupled receptor for UDP-glucose. J Biol Chem. Apr 14 2000;275(15):10767-71. doi:10.1074/jbc.275.15.10767
71. Moore DJ, Murdock PR, Watson JM, et al. GPR105, a novel Gi/o-coupled UDP-glucose receptor expressed on brain glia and peripheral immune cells, is regulated by immunologic challenge: possible role in neuroimmune function. Brain Res Mol Brain Res. Oct 21 2003;118(1-2):10-23. doi:10.1016/s0169-328x(03)00330-9
72. Lin J, Zhang YY, Liu F, et al. The P2Y14 receptor in the trigeminal ganglion contributes to the maintenance of inflammatory pain. Neurochem Int. Oct 3 2019;131:104567. doi:10.1016/j.neuint.2019.104567
73. Kobayashi K, Fukuoka T, Yamanaka H, et al. Neurons and glial cells differentially express P2Y receptor mRNAs in the rat dorsal root ganglion and spinal cord. J Comp Neurol. Oct 1 2006;498(4):443-54. doi:10.1002/cne.21066
74. Bianco F, Fumagalli M, Pravettoni E, et al. Pathophysiological roles of extracellular nucleotides in glial cells: differential expression of purinergic receptors in resting and activated microglia. Brain Res Brain Res Rev. Apr 2005;48(2):144-56. doi:10.1016/j.brainresrev.2004.12.004
75. Kobayashi K, Yamanaka H, Yanamoto F, Okubo M, Noguchi K. Multiple P2Y subtypes in spinal microglia are involved in neuropathic pain after peripheral nerve injury. Glia. Oct 2012;60(10):1529-39. doi:10.1002/glia.22373
76. Raithel SJ, Sapio MR, LaPaglia DM, Iadarola MJ, Mannes AJ. Transcriptional Changes in Dorsal Spinal Cord Persist after Surgical Incision Despite Preemptive Analgesia with Peripheral Resiniferatoxin. Anesthesiology. Mar 2018;128(3):620-635. doi:10.1097/ALN.0000000000002006
77. Mufti F, Jung YH, Giancotti LA, et al. P2Y14 Receptor Antagonists Reverse Chronic Neuropathic Pain in a Mouse Model. ACS Med Chem Lett. Jun 11 2020;11(6):1281-1286. doi:10.1021/acsmedchemlett.0c00115
78. Fricks IP, Carter RL, Lazarowski ER, Harden TK. Gi-dependent cell signaling responses of the human P2Y14 receptor in model cell systems. J Pharmacol Exp Ther. Jul 2009;330(1):162-8. doi:10.1124/jpet.109.150730
79. Krzeminski P, Pomorski P, Baranska J. The P2Y14 receptor activity in glioma C6 cells. Eur J Pharmacol. Oct 10 2008;594(1-3):49-54. doi:10.1016/j.ejphar.2008.06.092
80. Lin J, Liu F, Zhang YY, et al. P2Y14 receptor is functionally expressed in satellite glial cells and mediates interleukin-1beta and chemokine CCL2 secretion. J Cell Physiol. Nov 2019;234(11):21199-21210. doi:10.1002/jcp.28726
81. Barrett MO, Sesma JI, Ball CB, et al. A selective high-affinity antagonist of the P2Y14 receptor inhibits UDP-glucose-stimulated chemotaxis of human neutrophils. Molecular pharmacology. Jul 2013;84(1):41-9. doi:10.1124/mol.113.085654
82. Sesma JI, Kreda SM, Steinckwich-Besancon N, et al. The UDP-sugar-sensing P2Y(14) receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils. American journal of physiology Cell physiology. Sep 1 2012;303(5):C490-8. doi:10.1152/ajpcell.00138.2012
83. Charlton ME, Williams AS, Fogliano M, Sweetnam PM, Duman RS. The isolation and characterization of a novel G protein-coupled receptor regulated by immunologic challenge. Brain research. Aug 1 1997;764(1-2):141-8. doi:10.1016/s0006-8993(97)00438-1
84. Lin J, Zhang YY, Liu F, et al. The P2Y(14) receptor in the trigeminal ganglion contributes to the maintenance of inflammatory pain. Neurochemistry international. Dec 2019;131:104567. doi:10.1016/j.neuint.2019.104567
85. Lu R, Zhang Z, Jiang C. Recent progress on the discovery of P2Y14 receptor antagonists. Eur J Med Chem. Aug 1 2019;175:34-39. doi:10.1016/j.ejmech.2019.04.068

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Current and Future Immunotherapies for Multiple Sclerosis

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The available treatments for MS aim to achieve at least one of the two main goals: to restore functions that were lost as a result of specific disease processes and to prevent further tissue damage by blocking the underlying aberrant activity of the immune system.





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Abstract

Despite substantial progress in developing new immunotherapies against multiple sclerosis (MS), currently available immunotherapies are only partially effective for this debilitating neurological disease, thus necessitating new therapeutic approaches. Here, we review the immunotherapies already approved for MS as well as relevant clinical trials. Further, we present some experimental approaches that are currently being developed and are focused on modulating the functions of dendritic cells and regulatory T cells.

Introduction

Multiple sclerosis (MS) is a complex autoimmune disease characterized by inflammation of the central nervous system (CNS). It is caused by immune cells that attack components of the myelin sheath surrounding the neuronal axons of the nerves, resulting in demyelination and neurologic dysfunction. Although some patients with MS suffer from the primary-progressive form characterized by its steady progression, most cases of MS are characterized by a relapsing-remitting disease course. However, many MS patients eventually develop secondary-progressive MS, resulting in a steady progression of symptoms.[1,2] While the causes of MS remain elusive, both genetic and environmental factors contribute. It is clear, however, that the key processes leading to MS depend on an aberrant immune response directed against specific antigens derived from components of the CNS.[3-6] This autoimmune process is initiated when specialized antigen presenting cells (APCs) such as conventional dendritic cells (cDCs) activate autoreactive $CD4^+$ T lymphocytes (cells). Such neural antigen-specific (encephalitogenic) $CD4^+$ T cells can enter the CNS and become additionally re-activated and also recruit other effector cells, such as $CD8^+$ T cells, establishing an inflammatory lesion. In addition, $CD4^+$ T cells orchestrate functions of autoreactive B cells that, along with natural killer (NK) cells and macrophages, also contribute to MS immunopathology.[1,2] Given the multi-faceted autoimmune nature of MS, multiple FDA-approved disease-modifying therapies (DMTs) that reduce relapses and slow disease progression of the relapsing-remitting and secondary-progressive MS have been developed to modulate the inflammatory immune response that drives MS pathogenesis.

Further, intense research involving experimental animal models of MS may result in improved future therapy options for patients. In this article, we discuss the immunological mechanisms of current FDA-approved DMTs and some ongoing clinical trials, as well as provide an overview of the relevant pre-clinical research focusing on work by our own group affiliated with the Saint Louis University Center for Neuroscience.

FDA-Approved Disease-Modifying Therapies

The available treatments for MS aim to achieve at least one of the two main goals: to restore functions that were lost as a result of specific disease processes and to prevent further tissue damage by blocking the underlying aberrant activity of the immune system. Immunotherapies are crucial for any treatment options because long-term success of MS therapy ultimately depends on limiting the underlying autoimmune process. Beta-inteferon (currently available in three forms: IFN-β-1b, IFN-β-1a, and pegylated-IFN-β-1b) was approved by the FDA in 1993 as the first DMT for MS and remains a first-line treatment.[7] Although not completely understood in the context of MS, IFN-β exerts broad anti-inflammatory and immunomodulatory effects. By altering several aspects of dendritic cell (DC) biology, a type of APC crucial for antigen presentation and expression of costimulatory molecules and cytokines, IFN-β treatment limits effector $CD4^+$ and $CD8^+$ T cell responses. It may also enhance development of regulatory T cells (Tregs) that are key to restraining effector T cell functions.[7-9] Further, IFN-β dampens B cell responses by maintaining a population of regulatory B cells and inducing apoptosis of pathogenic memory B cells.[10,11]

Another first-line therapy is glatiramer acetate (GA), a mixture of synthetic polypeptides consisting of four amino acids found in myelin basic protein: glutamic acid, lysine, alanine and tyrosine.[8] Like IFN-β, GA exerts broad immunomodulatory effects that are incompletely understood. GA binds indiscriminately to major histocompatibility complex (MHC) class II molecules on DCs and other APCs, thus competing for binding with myelin-derived peptides.[8] Although it is unclear how, GA treatment results in the skewing of autoreactive T cells away from the pathogenic effector T cell responses and towards regulatory functions.[12,13] Other broadly immunomodulatory DMTs include dimethyl fumarate (DMF) and its bioequivalent diroximel fumarate, which activate the antioxidant nuclear erythroid 2-related factor 2 (Nrf2) transcriptional pathway.[8,14] The effects of these fumarates include changes in the differentiation of various effector $CD4^+$ T cells (specifically a shift from T helper Th1/Th17 to Th2 profile), an expansion of Tregs, and additional inhibition of B and NK cell responses.[8]

There are multiple DMTs that prevent lymphocyte migration, thus preventing effector T cells from crossing the blood-brain barrier. Four of these are sphingosine-1-phosphate (S1P) receptor modulators: fingolimod, siponimod, ozanimod, and ponesimod. S1P forms a gradient in the blood to promote lymphocyte egress from secondary lymphoid tissues through S1P receptor 1 (S1PR1) signaling.[15] Fingolimod (FTY720) is structurally similar to naturally occurring S1P and can be phosphorylated, allowing it to bind to S1PRs. This results in receptor internalization and the eventual proteasomal degradation of S1PR1.[16,17] This inhibits lymphocytes from leaving lymph nodes and entering the central nervous system, thus preventing relapses. Fingolimod was approved as the first oral medication for MS by the FDA in 2010.[8] Potential side effects of fingolimod include bradycardia due to S1PR3 signaling, so more specific S1P receptor modulators were developed, including siponimod (approved in 2019), ozanimod (approved in 2020), and ponesimod (approved in 2021).[8,18-20] S1P receptor modulators may also have additional therapeutic benefits by reducing neuropathic pain.[21]

Another DMT that prevents the entry of immune cells into the CNS is natalizumab, which was approved in 2006.[8] Natalizumab is a monoclonal antibody that binds to α_4-integrin. In MS, this prevents interactions between vascular cell adhesion molecule-1 (VCAM-1) and $\alpha_4\beta_1$-integrin (very late antigen-4). VCAM-1 is expressed by blood vessel endothelial cells at sites of inflammation and binds to $\alpha_4\beta_1$-integrin-expressing leukocytes, allowing for the trans-migration of immune cells across the blood-brain barrier. Natalizumab disrupts this interaction, thus preventing the transmigration of leukocytes, including activated auto-reactive T cells, into the CNS.[22]

Another family of DMTs works by depleting lymphocytes that are involved in MS pathology. Ocrelizumab, which was approved in 2017 as the first DMT for primary progressive MS, is a monoclonal antibody that binds to CD20 expressed on immature and mature B cells, thus leading to their depletion.[8] Alemtuzumab is a monoclonal antibody specific for CD52, which is expressed at high levels on activated B and T cells. Alemtuzumab binding leads to antibody-dependent, cell-mediated cytolysis and complement-dependent cytolysis of the targeted cells.[23]

Several DMTs work by suppressing the proliferation of activated T and B cells. Mitoxantrone, a synthetic anthracenedione derivate, was approved for the treatment of MS in 2000 and acts as a type II topoisomerase inhibitor.[8] By disrupting DNA synthesis and DNA repair, mitoxantrone promotes the death of proliferating T and B cells.[24] Cladribine is a purine analogue that is taken up into rapidly proliferating cells, including activated lymphocytes, and incorporated into DNA during synthesis, resulting in DNA strand breakage and apoptosis.[8] Teriflunomide inhibits de novo pyrimidine synthesis by blocking the enzyme dihydroorotate dehydrogenase, which also leads to the apoptosis of proliferating T and B cells.[8]

Clinical Trials of Interest

While there are several ongoing clinical trials investigating therapies similar to already approved DMTs, there are quite a few therapies in development with novel mechanisms of action. Like many of the approved DMTs, some of these therapies have broad immunomodulatory effects. A phase III clinical trial (NCT03896217) is currently investigating the use of simvastatin in secondary progressive MS (SPMS). Simvastatin is an HMG-CoA reductase inhibitor (statin) commonly used for hyperlipidemia, but statins also exert anti-inflammatory effects such as reducing MHC class II antigen presentation, altering DC maturation and reducing effector $CD4^+$ T cell responses. Statins also prevent leukocyte migration across the blood-brain barrier, further making them a promising treatment for MS.[25] A phase II trial showed treatment with high-dose simvastatin reduced SPMS progression, thus advancing it to phase III testing.[26] Another ongoing phase III trial (NCT04291456) is investigating the use of minocycline which reduces the conversion from clinically isolated syndrome to multiple sclerosis.[27] Minocycline is a tetracycline antibiotic with broad immunomodulatory effects that include reducing microglial activation and preventing leukocytes from crossing the blood-brain barrier by inhibiting matrix metalloproteinases (MMPs).[28] The recently completed phase II trial of ibudilast, a small molecule phosphodiesterase inhibitor, in progressive MS slowed progression of brain atrophy.[29] Ibudilast inhibits macrophage migration inhibitor factor (MIF), reduces IL-1β, TNF-a and IL-6 production, and functions as a TLR4 antagonist. Although it is not completely understood, it is thought that these functions reduce neuroinflammation.[29]

Other clinical trials are using more targeted antigen-specific approaches to reduce neuroinflammation. Two phase I trials (NCT02618902 and NCT02903537) are investigating autologous myelin peptide-pulsed monocyte-derived dendritic cells (moDCs) tolerized with 1α,25 dihydroxyvitamin D_3 (tolDC-VitD3) in multiple sclerosis patients.[30] This approach, which has also been used for rheumatoid arthritis, type 1 diabetes, and Crohn's disease, aims to induce antigen-specific T cell tolerance that may modulate the MS disease course. A similar approach using autologous moDCs tolerized with dexamethasone for patients with neuromyelitis optica spectrum disorders and MS showed altered immune profiles following treatment, although better moDC-tolerizing conditions may need to be developed.[31] A different approach to achieve antigen-specific tolerance is being used in the Phase II trial of the TCR peptide vaccine NeuroVax™ (NCT02057159). This trivalent vaccine consists of three peptides (BV5S2, BV6S5, and BV13S1) emulsified in incomplete Freund's adjuvant and induces IL-10-secreting T regulatory 1 (Tr1) cells and $FoxP3^+$ Tregs.[32]

Experimental Approaches at the Pre-Clinical Stage

Despite substantial progress in developing new immunotherapies against MS, currently available immunotherapy protocols are only partially effective, and most of these approaches are burdened by side effects. This necessitates new approaches for immunomodulation of MS to specifically alter the functions of offending immune cells without impairing other immune responses such as those against pathogenic microorganisms and tumors.[33,34]

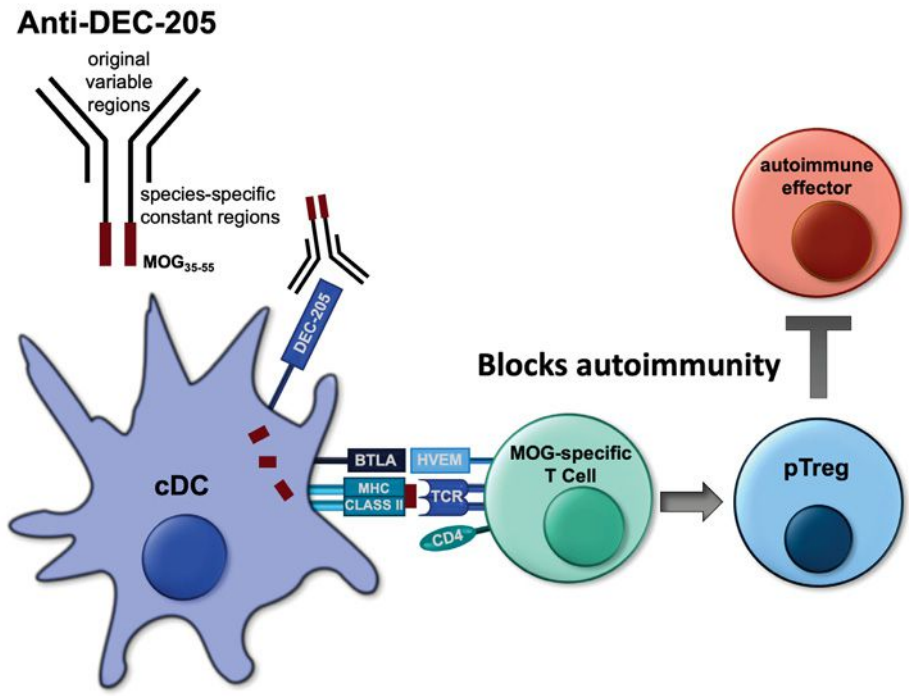


Figure 1. Tolerogenic BTLA^{hi} conventional dendritic cells (cDCs) express the lectin receptor DEC-205, which can serve as a target for specifically delivering antigens for their presentation to T cells with engineered recombinant chimeric antibodies. The constant regions of such DEC-205-specific chimeric antibodies are fused with a defined antigen of choice, such as myelin oligodendrocyte glycoprotein (MOG), that is then processed by BTLA^{hi}DEC-205^{+} cDCs and presented on major histocompatibility molecules (MHCs) for their subsequent recognition by encephalitogenic T cells. Such specific targeting of encephalitogenic antigens to tolerogenic BTLA^{hi} cDCs results in the BTLA-dependent induction of peripheral regulatory T cells (pTregs) that block autoimmune responses mediated by encephalitogenic autoimmune effectors.

There are two main considerations guiding these approaches. First, the neural-derived antigens are specifically recognized only by corresponding encephalitogenic T cells but no other T cells. Second, such specific antigens need to be delivered in a "tolerogenic" context that eventually leads to an elimination or functional inactivation of antigen-activated encephalitogenic T cells. Previous research from various groups showed the efficacy of specific neural antigens in an animal model of MS, experimental autoimmune encephalomyelitis (EAE).[35] The characteristic neural inflammation resulting from infiltration of nervous tissues by perivascular CD4^{+} T and other immune cells, eventually leading to clinical symptoms of ascending paralysis, makes EAE a relevant model for MS and a powerful model of immune regulation of Th1 and Th17-dependent autoimmunity.[35-37]

Immunization with neural antigens such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP) or proteolipid protein (PLP) leads to different variants of EAE resembling the human progressive and relapsing–remitting forms of MS.[35] In contrast, the introduction of MOG, MBP and PLP in a non-immunogenic context blocks the corresponding forms of EAE.[35,38,39] However, administration of such antigens without control over which APCs will present them to T cells in vivo may not guarantee the ultimate therapeutic results. Alternatively, some early experimental approaches relied on transfers of autologous DC with tolerogenic functions induced in vitro, and similar approaches are now in clinical trials as discussed above.[33,40] These therapeutic outcomes can be improved by the specific delivery of antigens to APCs with specialized tolerogenic functions in vivo, thus allowing for better control of the ensuing immunological processes such as the de novo induction of antigen-specific peripheral regulatory T cells (pTregs).[34,40] In contrast to the earlier described and well-established roles of Tregs of a thymic origin (tTregs), the functions of pTregs, although crucial, still remain less characterized. In general, Tregs play a pivotal role in protection and recovery from EAE and MS by suppressing autoreactive T cells, and functional defects of Tregs have been implicated in MS pathogenesis.[35] However, specific pro-inflammatory autoimmune activation can overwhelm tTreg functions. Resembling the sudden onset of MS in patients, the robust

triggering of MS-like EAE in healthy animals indicates that the functions of tTregs are unable to block encephalitogenic T cell activation. In contrast, neuroinflammation can be ameliorated or even completely prevented by antigen-specific pTregs that are formed extrathymically in the peripheral immune system during specific tolerogenic responses to relevant neuronal antigens. Research from our group established that such neural antigen-specific pTregs are induced by a specialized type of cDC and confer a long-lasting tolerance that prevents EAE.[41,42] Although cDCs were initially described as having key roles in priming of effector T cells during immune and autoimmune responses, some cDCs have natural tolerogenic properties that help regulate such effector T cells. These cDCs have been referred to as natural tolerogenic cDCs (ntDCs) to distinguish them from cDCs whose tolerogenic functions can be induced by specific treatments including those administered ex vivo as discussed above and as we reviewed previously.[33] The ntDCs with tolerogenic functions express B and T lymphocyte associated (BTLA) protein, and we defined the specific mechanisms engaged by BTLA that result in enhanced formation of pTregs with anti-autoimmune functions in EAE.[35,41-43] Therefore, further manipulation of these specific BTLA-mediated mechanisms could open new avenues for immunomodulation via manipulating specific formation and functions of relevant pTregs.

Tolerogenic BTLA^{hi} cDCs also express a lectin receptor DEC-205 that can serve as a specific target in strategies for delivering antigens in vivo for their presentation to T cells. We previously pioneered an approach that depends on engineered recombinant chimeric antibodies specific for DEC-205 that also carry peptides for their subsequent recognition by encephalitogenic T cells (discussed in[33,34,44]). This anti-DEC-205 chimeric antibody is comprised of variable (V) regions derived from monoclonal antibodies specific for DEC-205 that are fused with the species-specific heavy and light constant (C) regions derived from separate immunoglobulins. A similar design is found in antibodies used for therapeutic purposes such as the anti-tumor checkpoint blockades.[34,44] Importantly, the constant regions of the DEC-205-specific chimeric antibody are also genetically fused with a defined antigen of choice that is then processed by cDCs and presented on major histocompatibility molecules for its recognition by T cells.[34,44] Such specifically targeted encephalitogenic antigens delivered to tolerogenic BTLA^{hi}DEC-205^{+} cDCs induce pTregs that block encephalitogenic autoimmune effectors (Figure 1).[34,44] Importantly, pTregs that are induced systemically in the secondary immune organs can suppress local effector responses in the CNS.[35,41] Overall, these approaches allow for highly specific immunomodulation that is focused only on autoreactive encephalitogenic T cells without impairing other immune responses. The importance of well-controlled immunomodulation is further underscored by our most recent results that uncovered a concomitant induction of T cells with regulatory and also effector autoimmune properties under normal homeostatic conditions.[45] These findings further highlight the importance of therapeutic interventions aimed at APCs with specific tolerogenic properties for novel efficient immunotherapies that can minimize potential risks of overstimulating potentially harmful T cells. Overall, results of this basic research are likely to inform future therapeutic approaches that will be more selective and have fewer side effects.

References

1. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol. 2015;15(9):545-558.
2. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple Sclerosis: Mechanisms and Immunotherapy. Neuron. 2018;97(4):742-768.
3. International Multiple Sclerosis Genetics C, Beecham AH, Patsopoulos NA, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet. 2013;45(11):1353-1360.
4. International Multiple Sclerosis Genetics C, Hafler DA, Compston A, et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med. 2007;357(9):851-862.
5. International Multiple Sclerosis Genetics C, Lill CM, Schjeide BM, et al. MANBA, CXCR5, SOX8, RPS6KB1 and ZBTB46 are genetic risk loci for multiple sclerosis. Brain. 2013;136(Pt 6):1778-1782.
6. Patsopoulos NA, Bayer Pharma MSGWG, Steering Committees of Studies Evaluating I-b, et al. Genome-wide meta-analysis identifies novel multiple sclerosis susceptibility loci. Ann Neurol. 2011;70(6):897-912.
7. Jakimovski D, Kolb C, Ramanathan M, Zivadinov R, Weinstock-Guttman B. Interferon-β for Multiple Sclerosis. Cold Spring Harbor Perspectives in Medicine. 2018;8(11).
8. Rommer PS, Milo R, Han MH, et al. Immunological Aspects of Approved MS Therapeutics. Frontiers in Immunology. 2019;10(1564).
9. Ebrahimimonfared M, Ganji A, Zahedi S, Nourbakhsh P, Ghasami K, Mosayebi G. Characterization of Regulatory T-Cells in Multiple Sclerosis Patients Treated with Interferon Beta-1a. CNS Neurol Disord Drug Targets. 2018;17(2):113-118.
10. Schubert RD, Hu Y, Kumar G, et al. IFN-beta treatment requires B cells for efficacy in neuroautoimmunity. J Immunol. 2015;194(5):2110-2116.
11. Rizzo F, Giacomini E, Mechelli R, et al. Interferon-β therapy specifically reduces pathogenic memory B cells in multiple sclerosis

patients by inducing a FAS-mediated apoptosis. Immunology & Cell Biology. 2016;94(9):886-894.
12. Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of CD4⁺CD25⁺ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(18):6449-6454.
13. Haas J, Korporal M, Balint B, Fritzsching B, Schwarz A, Wildemann B. Glatiramer acetate improves regulatory T-cell function by expansion of naive CD4⁺CD25⁺FOXP3⁺CD31⁺ T-cells in patients with multiple sclerosis. Journal of Neuroimmunology. 2009;216(1):113-117.
14. Naismith RT, Wundes A, Ziemssen T, et al. Diroximel Fumarate Demonstrates an Improved Gastrointestinal Tolerability Profile Compared with Dimethyl Fumarate in Patients with Relapsing-Remitting Multiple Sclerosis: Results from the Randomized, Double-Blind, Phase III EVOLVE-MS-2 Study. CNS Drugs. 2020;34(2):185-196.
15. Cyster JG, Schwab SR. Sphingosine-1-Phosphate and Lymphocyte Egress from Lymphoid Organs. Annual Review of Immunology. 2012;30(1):69-94.
16. Oo ML, Thangada S, Wu M-T, et al. Immunosuppressive and Anti-angiogenic Sphingosine 1-Phosphate Receptor-1 Agonists Induce Ubiquitinylation and Proteasomal Degradation of the Receptor. Journal of Biological Chemistry. 2007;282(12):9082-9089.
17. Sykes DA, Riddy DM, Stamp C, et al. Investigating the molecular mechanisms through which FTY720-P causes persistent S1P1 receptor internalization. British Journal of Pharmacology. 2014;171(21):4797-4807.
18. Comi G, Kappos L, Selmaj KW, et al. Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis (SUNBEAM): a multicentre, randomised, minimum 12-month, phase 3 trial. Lancet Neurol. 2019;18(11):1009-1020.
19. Cohen JA, Comi G, Selmaj KW, et al. Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis (RADIANCE): a multicentre, randomised, 24-month, phase 3 trial. Lancet Neurol. 2019;18(11):1021-1033.
20. Kappos L, Fox RJ, Burcklen M, et al. Ponesimod Compared With Teriflunomide in Patients With Relapsing Multiple Sclerosis in the Active-Comparator Phase 3 OPTIMUM Study: A Randomized Clinical Trial. JAMA Neurology. 2021;78(5):558-567.
21. Janes K, Little JW, Li C, et al. The development and maintenance of paclitaxel-induced neuropathic pain require activation of the sphingosine 1-phosphate receptor subtype 1. J Biol Chem. 2014;289(30):21082-21097.
22. Shirani A, Stüve O. Natalizumab for Multiple Sclerosis: A Case in Point for the Impact of Translational Neuroimmunology. The Journal of Immunology. 2017;198(4):1381-1386.
23. Rao SP, Sancho J, Campos-Rivera J, et al. Human Peripheral Blood Mononuclear Cells Exhibit Heterogeneous CD52 Expression Levels and Show Differential Sensitivity to Alemtuzumab Mediated Cytolysis. PLOS ONE. 2012;7(6):e39416.
24. Chan A, Weilbach FX, Toyka KV, Gold R. Mitoxantrone induces cell death in peripheral blood leucocytes of multiple sclerosis patients. Clinical & Experimental Immunology. 2005;139(1):152-158.
25. Greenwood J, Steinman L, Zamvil SS. Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. Nature Reviews Immunology. 2006;6(5):358-370.
26. Chataway J, Schuerer N, Alsanousi A, et al. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. The Lancet. 2014;383(9936):2213-2221.
27. Metz LM, Li DKB, Traboulsee AL, et al. Trial of Minocycline in a Clinically Isolated Syndrome of Multiple Sclerosis. New England Journal of Medicine. 2017;376(22):2122-2133.
28. Chen X, Ma X, Jiang Y, Pi R, Liu Y, Ma L. The prospects of minocycline in multiple sclerosis. Journal of Neuroimmunology. 2011;235(1):1-8.
29. Fox RJ, Coffey CS, Conwit R, et al. Phase 2 Trial of Ibudilast in Progressive Multiple Sclerosis. N Engl J Med. 2018;379(9):846-855.
30. Willekens B, Presas-Rodríguez S, Mansilla MJ, et al. Tolerogenic dendritic cell-based treatment for multiple sclerosis (MS): a harmonised study protocol for two phase I clinical trials comparing intradermal and intranodal cell administration. BMJ Open. 2019;9(9):e030309.
31. Zubizarreta I, Flórez-Grau G, Vila G, et al. Immune tolerance in multiple sclerosis and neuromyelitis optica with peptide-loaded tolerogenic dendritic cells in a phase 1b trial. Proceedings of the National Academy of Sciences. 2019;116(17):8463-8470.
32. Vandenbark AA, Culbertson NE, Bartholomew RM, et al. Therapeutic vaccination with a trivalent T-cell receptor (TCR) peptide vaccine restores deficient FoxP3 expression and TCR recognition in subjects with multiple sclerosis. Immunology. 2008;123(1):66-78.
33. Iberg CA, Hawiger D. Natural and Induced Tolerogenic Dendritic Cells. J Immunol. 2020;204(4):733-744.
34. Iberg CA, Hawiger D. Targeting Dendritic Cells with Antigen-Delivering Antibodies for Amelioration of Autoimmunity in Animal Models of Multiple Sclerosis and Other Autoimmune Diseases. Antibodies (Basel). 2020;9(2).
35. Jones A, Hawiger D. Peripherally Induced Regulatory T Cells: Recruited Protectors of the Central Nervous System against Autoimmune Neuroinflammation. Front Immunol. 2017;8:532.
36. Miller SD, Karpus WJ. Experimental autoimmune encephalomyelitis in the mouse. Curr Protoc Immunol. 2007;Chapter 15:Unit 15 11.
37. Miller SD, Karpus WJ, Davidson TS. Experimental autoimmune encephalomyelitis in the mouse. Curr Protoc Immunol. 2010;Chapter 15:Unit 15 11.
38. Getts DR, Martin AJ, McCarthy DP, et al. Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis. Nat Biotechnol. 2012;30(12):1217-1224.
39. Miller SD, Turley DM, Podojil JR. Antigen-specific tolerance strategies for the prevention and treatment of autoimmune disease. Nat Rev Immunol. 2007;7(9):665-677.
40. Iberg CA, Jones A, Hawiger D. Dendritic Cells As Inducers of Peripheral Tolerance. Trends Immunol. 2017;38(11):793-804.
41. Jones A, Opejin A, Henderson JG, et al. Peripherally Induced Tolerance Depends on Peripheral Regulatory T Cells That Require Hopx To Inhibit Intrinsic IL-2 Expression. J Immunol. 2015;195(4):1489-1497.
42. Jones A, Bourque J, Kuehm L, et al. Immunomodulatory Functions of BTLA and HVEM Govern Induction of Extrathymic Regulatory T Cells and Tolerance by Dendritic Cells. Immunity. 2016;45(5):1066-1077.
43. Bourque J, Hawiger D. The BTLA–HVEM–CD5 Immunoregulatory Axis–An Instructive Mechanism Governing pTreg Cell Differentiation. Front Immunol. 2019;10:1163. .
44. Iberg CA, Hawiger D. Advancing immunomodulation by in vivo antigen delivery to DEC-205 and other cell surface molecules using recombinant chimeric antibodies. Int Immunopharmacol. 2019;73:575-580.
45. Opejin A, Surnov A, Misulovin Z, et al. A Two-Step Process of Effector Programming Governs CD4+ T Cell Fate Determination Induced by Antigenic Activation in the Steady State. Cell Reports. 2020;33(8):108424.

Disclosure

None reported. **MM**

RNA-Based Therapies for Neurodegenerative Diseases

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RNA-based therapies hold great promise for certain neurodegenerative diseases and may offer a tangible path to personalized medicine.





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Abstract

Most neurodegenerative disorders afflict the ageing population and are often incurable. Therefore, therapeutic development is a major focus in biomedical research. We highlight a new class of drugs, RNA molecules, to control gene expression and decrease neurotoxicity. Their efficacy is shown in pre-clinical studies, clinical trials and in cases of approved patient treatment. As the number of RNA-based strategies increases, so does the promise of targeting more disease-associated genes through a variety of different mechanisms.

Introduction

Neurodegenerative diseases encompass a heterogenous group of diseases that involve progressive degeneration of the nervous system. They include more common forms such as Alzheimer's disease (AD) and Parkinson's disease (PD), as well as less common forms including Huntington's disease (HD), frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and others. There are currently few therapeutic options for these neurological disorders and most are incurable. A common, but not universal feature, is aggregation of specific proteins including tau (in AD and FTD), amyloid beta (AD), α-synuclein (PD), huntingtin (HD), and TDP-43 (FTD and ALS). For many of these neurodegenerative diseases, inherited forms exist and multiple gene mutations have been identified. Generally, these mutations result in either gain-of-toxic function or loss-of-function.

At the cellular level, gain-of-toxic function mutations lead to production of aberrant mutant proteins (such as huntingtin with expanded polyglutamine repeats in HD, SOD1 point mutations in ALS, and C9ORF72 with repeat expansions in ALS and FTD), which have detrimental effects. On the other hand, loss-of-function mutations can lead to several different scenarios. Fundamentally, they can result in inadequate/insufficient production of the protein encoded by the mutated gene (such as progranulin or C9ORF72 in FTD). Loss-of-function mutations can also alter protein homeostasis, including protein processing (such as amyloid beta in AD), protein degradation (through autophagy and the ubiquitin proteasome system), and protein trafficking (such as nucleocytoplasmic transport and endolysosomal transport in ALS and FTD). Additionally, loss-of-function

mutations can result in changes in RNA metabolism, as is case when the functional levels of the RNA binding protein TDP-43 are reduced in ALS and FTD.

RNA-Based Therapies for Neurodegenerative Diseases

RNA-based therapies present as attractive therapeutic approaches for a number of these neurodegenerative diseases because they enable highly specific control of gene expression – either to reduce levels of target proteins in situations of gain-of-toxic function, or to increase levels of target proteins in situations of loss-of-function mutations. For RNA therapies, RNA molecules are designed to bind specifically to their target RNAs through Watson-Crick base pairing. In this way, RNA therapies can be used to precisely target the molecular causes of diseases and/or key pathogenic mechanisms. A major advantage of RNA-based therapy is that it can directly target any desired RNA, and thus it greatly expands the realm of possible targets beyond traditional druggable targets that are accessible to small molecule- and antibody-based approaches (Figure 1).

RNA therapies largely fall into two classes – antisense oligonucleotides (ASOs) and RNA interference (RNAi). ASOs are single-stranded RNAs that work by specifically binding to and modulating their target RNAs for translation or turnover. The use of ASOs to lower target protein levels was first reported in 1978 by Mary Stephenson and Paul Zamecnik,[1] but ASOs subsequently faced considerable challenges in the 1980s. Following major advances in ASO chemistry and delivery, ASOs made a strong resurgence in the 1990s. There is currently high enthusiasm for ASOs for neurodegenerative diseases, with the recent FDA approval of nusinersen (Spinraza) for SMA in 2016 and promising results in ongoing clinical trials with ASOs targeting huntingtin and SOD1.[2] In a more limited way, RNAi uses double stranded RNA molecules for gene silencing. RNAi was first reported in 1998 by Andrew Fire et al.,[3] who later shared the Nobel prize in 2006 with Craig Mello for this discovery. Two RNAi-based therapies – patisiran and inotersen – received FDA approval in 2018 for hereditary transthyretin-mediated amyloidosis.

Antisense drugs have been in used to study neurological disorders for more than two decades and improvements in the chemistry have extended the lifetime of these reagents. DNA and specially RNA are highly unstable due to degradation by nucleases present in all organisms. The efficacy of RNA oligonucleotides as drugs is made possible by chemical modifications to increase their stability, improve RNA binding activity and enhance distribution. To protect RNAs from nucleases, modifications are commonly designed to the phosphate backbone and ribose sugar. These and additional alterations have also been applied to increase RNA binding affinity and reduce toxicity.[4,5] The most common modification in therapeutic ASOs has been introduction of a phosphorothioate backbone. Sugar modifications are included at the ends of the oligonucleotides as an additional way of preventing nuclease degradation and enhancing RNA binding. The most common substitutions are to the naturally occurring 2'-O-methyl and the synthetic 2'-O-methoxyethyl (2'-MOE). These types of modifications have also been introduced for the drugs tested in clinical trials and those approved for patient use.

For neurological disorders, ASOs are commonly delivered directly into the cerebral spinal fluid (CSF). Clinically, ASOs are injected intrathecally to the spinal canal to reach the CSF. In pre-clinical animal models, such as rodents, ASOs are typically administered by direct intracerebroventricular injection. These forms of delivery result in widespread distribution of the molecules throughout the central nervous system (CNS).[6-10] Examination of ASO distribution in nonhuman primates via CSF shows distribution throughout the spinal cord and cortex[11] and deeper brain regions, such as the hippocampus, pons and amygdala.[9,11,12]

RNA-based therapies inherently have great potential as a form of personalized medicine. RNA therapies are versatile and can be designed to target specific genes, and potentially even to target specific patient mutations. In the case of ASOs, the most commonly applied approach is to design ASOs to downregulate protein levels (such as for SOD1, huntingtin, and tau). This type of mechanism is particularly useful for targeting mutant proteins with gain-of-toxic function and key cellular pathways/proteins involved in disease pathogenesis. ASOs can also be designed to modulate splicing of the target RNA (such as for the SMN2 RNA by nusinersen) to effectively increase the levels of functional SMN protein. Additionally, ASOs can be designed to block microRNAs and degradation of mutant mRNAs

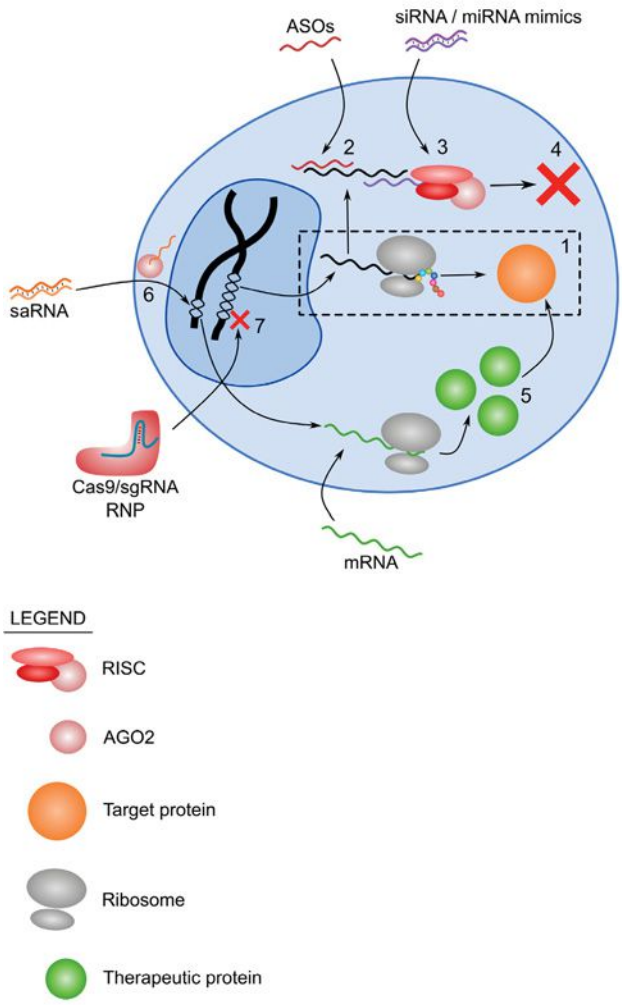


Figure 1. Overview of Different Mechanisms of Action of Different RNA Therapeutics.
(1) Without therapeutic RNA molecules, the translation of a pathogenic protein proceeds without inhibition (shown in the broken line box). (2) ASOs hybridize to the target mRNA, while the (3) siRNA/miRNA mimics utilize the RISC in the RNAi pathway to (4) inhibit translation of target mRNA. (5) Overexpression of a therapeutic protein that counteracts the function of the pathogenic protein can be done by delivering the mRNA of the therapeutic protein. (6) saRNA can be delivered to the cell where it binds to AGO2, is imported to the nucleus, and in turn activates an endogenous gene. (7) A more permanent approach to remove the pathogenic protein is by gene knockout using Cas9 and sgRNA RNPs.

Abbreviations: AGO2, argonaute 2; ASO, antisense oligonucleotide; RISC, RNA-induced silencing complex; RNP, ribonucleoprotein; saRNA, small activating RNA.
Source: Trends in Pharmacological Sciences

through the nonsense-mediated mRNA decay pathway. Below, we present these different ASO-based approaches in more detail in the context of specific neurodegenerative diseases.

ASOs Downregulate Protein Expression and Reduce Disease Toxicity

One of the most common uses for ASOs has been to lower the expression of proteins to reduce the levels of toxic aggregates. This is achieved by inducing targeted degradation of mRNA transcripts, such as in the case of SOD1, huntingtin, and tau. The oligonucleotides are designed to pair with the coding mRNA transcripts and trigger degradation by the enzyme ribonuclease (RNase) H1, which specifically degrades RNA:DNA hybrids. In this case, ASOs are designed with five to seven central DNA nucleotides. This approach has been successfully used to target superoxide dismutase 1 (SOD1), the first gene identified in connection with inherited ALS. Mutations in SOD1 account for 20% of familial ALS cases.[13] The mechanism by which SOD1 mutations cause disease is unknown, but most evidence suggests a gain-of-toxic function rather than loss of function. ASOs designed to downregulate SOD1 levels in preclinical research proved successful and moved on to testing in clinical trials. This was the first use of ASOs for neurodegenerative disease. The drug, Tofersen, is now in phase III clinical trials.[12,14] The collection of these studies showed efficacy in distribution of ASOs in the CNS using targeted therapy that proved safe and correlated with a measurable decrease in SOD1 levels and a reduction in disease in patients.

Based on the promising results for SOD1 in preclinical trials and in patient trials, the use of ASOs has been extended for another gene linked to familial and sporadic cases of ALS and frontotemporal dementia. The repeat expansion in the first intron of the gene C9ORF72 is linked to ALS and FTD (C9-ALS/FTD), resulting in a combination of gain and loss of function mechanisms.[15,16] This mutation is the most common genetic link in inherited ALS and FTD (approximately 40% of familial and 8-10% of sporadic ALS cases). Carriers of this repeat expansion accumulate RNA foci of the repeat transcript along with an increase in dipeptide repeats translated from the C9ORF72 mutation via repeat associated non-canonical (RAN) translation.[17] In addition, the repeat expansion causes haploinsufficiency as seen by reduced

C9ORF72 protein levels in patients. The main pathogenic process contributing to disease in C9-ALS/FTD remains under debate, and it is likely that all these defects synergize during disease. ASOs targeting the pathological C9ORF72 repeat expansion promote degradation of the noncoding region and reduction of RNA foci in patient-derived fibroblast and induced pluripotent stem neurons. Treatment of C9-ALS/FTD mouse models also showed a decrease in the toxic polypeptides derived from the repeat expansion and improved behavioral phenotype.[18] Importantly, an ASO with these characteristics does not affect C9ORF72 protein synthesis from the unaffected allele and is currently in patient clinical trials. The success of ASOs directed to SOD1 and C9ORF72 show the promise of ASO therapy for ALS and FTD, which may be applied to other targets associated with sporadic disease cases, which account for the majority of ALS and FTD patients.

Several laboratories within the Henry and Amelia Nasrallah Center for Neuroscience are currently carrying out research relating to RNA-based therapies in the context of different neurodegenerative diseases. Susan Farr's group has focused on using ASOs to target key proteins implicated in the pathogenesis of AD. Their studies have demonstrated that ASOs that decrease levels of amyloid precursor protein (APP), glycogen synthase kinase (GSK)-3β, and presenilin-1 improve learning and memory in mouse models of AD.[19-24] More recently, her laboratory is studying traumatic brain injury (TBI) and has found that post-injury administration of ASOs targeting GSK-3β prevents cognitive deficits.[25] These results strongly suggest that ASOs targeting these proteins could have similar beneficial cognitive effects in people with AD and TBI.

ASOs that Restore Gene Runction by Modulating mRNA Splicing

One of the most exciting developments in the ASO field has been the development of the RNA molecule nusinersen (Spinraza) to treat spinal muscular atrophy (SMA), which is the first FDA-approved drug for this disorder. SMA is a genetic disease involving death of motor neurons that had been the most common genetic cause of death in children before development of the drug. Life expectancy in the more severe cases was two years of age.[26,27] This defect is caused by decreased production of survival motor neuron (smn) protein of which there are two paralogous genes in humans, SMN1 and SMN2. The two genes are nearly identical; however, SMN2 is expressed at much lower levels than SMN1 because of a single site substitution in exon 7 in SMN2, which destroys an exonic splicing enhancer (ESE) sequence. Exon 7 skipping results in a truncated mRNA transcript and protein, which becomes degraded and generates loss of function. This results in 80% reduction in smn levels produced from SMN2. The downstream intronic region in SMN1 and SMN2 contains an intronic splicing silencer (ISS) sequence that binds the trans regulatory proteins hnRNP A1 and A2. Therefore, inhibition by ISS is counteracted by the ESE element in SMN1, resulting in sufficient inclusion/splicing of exon 7 and production of smn protein. SMA results from mutations in SMN1 that disrupt smn protein levels. Starting in the 1990s, the team of Dr. Adrian Krainer at Cold Spring Harbor Laboratories investigated the possibility of manipulating SMN2 splicing to enhance exon 7 inclusion and compensate for the loss of SMN1. The team tested the capability of ASOs to bind the SMN2 transcript and increase exon 7 inclusion. Their results showed that binding to the ISS was able to significantly enhance exon 7 inclusion by preventing the binding of hnRNP A1 and A2.[28,29] The research carried out by Krainer and others determined the molecular mechanisms of exon 7 splicing regulation using splicing reporters and other in vitro tools followed by the characterization of SMA mouse models to study the process in vivo. The ASO molecules designed based on the collection of the research worked in all pre-clinical trials in cells and in animal models. These also proved successful in clinical trials and warranted FDA approval. The use of nusinersen has already made a difference in the lives of many patients and their families. Similar methods have been used to control RNA splicing by ASOs for the treatment of Duchenne muscular dystrophy. In this case, the drugs promote exon skipping to restore the correct reading frame and generate the functional protein dystrophin, albeit smaller than normal.[30] The ASOs are approved for patients who benefit from having restored functional dystrophin at significantly levels. The results of these novel drugs show that ASOs may be used to modify RNA processing, not only to modulate transcript degradation, and that their use may be safe in patients.

RNA-Based Approach to Prevent Protein Aggregation and Restore Protein Function

One innovative proposal for the use of RNA for therapy is in the modulation of protein stability. This is

based on recent observations that RNA is very effective at maintaining RNA binding proteins in a soluble state, preventing them from misfolding and aggregation. Most neurodegenerative disease is characterized by the accumulation of protein aggregates, which may result in gain-of-toxic function. In the case of ALS and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U), the characteristic aggregates consist of the RNA binding protein TDP-43 (TAR DNA binding protein).[31,32] TDP-43 pathology is observed in other neurodegenerative diseases, including approximately half of Alzheimer's disease (AD) cases.[33-36] The prevalence of TDP-43 pathology is even more widespread in Limbic-predominant Age-related TDP-43 Encephalopathy (LATE), an AD-like disorder affecting the oldest population.[37] The pathogenesis of ALS and FTD is now widely viewed to be caused by dysfunction of RNA binding proteins and defects in RNA processing mechanisms. RNA binding proteins, like TDP-43, are extremely prone to aggregation. TDP-43 regulates RNA processing and thus controls the expression of hundreds of genes. Aggregation is linked to loss of protein function, which is highly deleterious in all cell types and in the model organisms tested. Therefore, approaches that alter TDP-43 levels, either by up or down-regulation, would negatively impact cell survival. Exciting new findings from different groups, including the Ayala lab, show that RNA acts as chaperone to these proteins under physiological conditions. Therefore, the Ayala lab and others aim to exploit RNA-based molecules to test their efficacy in preventing TDP-43 aggregation and restoring its function. If successful, this approach offers the advantage of preventing aggregation without altering normal levels of functional protein. Promising data suggest that RNA-based approaches may hold therapeutic promise for ALS and FTD, disorders associated with RNA-binding protein dysfunction, by specifically modulating protein function and homeostasis.

ASO-Based Approach to Increase Protein Levels

Another innovative use of ASOs that is being pursued in the Henry and Amelia Nasrallah Center for Neuroscience is for increasing protein levels. The Nguyen lab studies a progranulin-deficient form of FTD, in which one GRN (progranulin) allele is mutated and one intact GRN allele remains. The vast majority of disease-causing GRN mutations are nonsense mutations – which introduce a premature termination codon – and these mutant mRNAs are degraded by the nonsense-mediated mRNA decay (NMD) pathway. In 2016, the Krainer lab reported that ASOs can be designed to block NMD in cells.[38] Together with Ionis Pharmaceuticals, our lab developed an ASO-based strategy to block degradation of the mutant progranulin mRNA harboring the GRN^{R493X} mutation, which is the most common GRN mutation found in individuals with FTD. Our cell-based studies had established that the truncated progranulin protein produced from the GRN^{R493X} mRNA is functional, and thus we reasoned that increasing levels of the mutant protein would restore sufficient levels of functional progranulin protein. While we found that eight ASOs increased progranulin mRNA and protein levels in cells,[39] these ASOs did not increase progranulin levels in mice harboring the Grn^{R493X} mutation.

The Nguyen lab is currently testing a different ASO-based strategy that targets specific microRNA (miR) binding sites within the GRN mRNA. miRs regulate protein levels of about one third of all proteins in humans, and three microRNAs in particular (miR-29b, miR-107, and miR-659) have been reported to lower progranulin protein levels. We designed ASOs to target the binding sites of two of these miRs – with the rationale that ASOs that partially overlap with the miR binding sites would sterically hinder binding of the cognate miR and thereby increase progranulin levels. Our ongoing studies have identified a number of ASOs that effectively increase progranulin protein levels in human cells. We are excited to next test these ASOs in a humanized GRN mouse model. Overall, we are hopeful that ASOs can be used to increase progranulin levels and potentially lead to a treatment for progranulin-deficient FTD.

Conclusion

RNA-based therapies hold great promise for certain neurodegenerative diseases and may offer a tangible path to personalized medicine. These therapeutic efforts are largely informed by genetic studies that identify the genetic bases of disease and by basic science studies that identify specific molecular targets. As we continue to learn more about neurodegenerative diseases we can design and test additional RNA-based therapies.

References

1. Stephenson ML, Zamecnik PC. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc Natl Acad Sci U S A. 1978;75(1):285-288.
2. Leavitt BR, Tabrizi SJ. Antisense oligonucleotides for neurodegeneration. Science. 2020;367(6485):1428-1429.
3. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391(6669):806-811.
4. Bennett CF, Swayze EE. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu Rev Pharmacol Toxicol. 2010;50:259-293.
5. Bennett CF, Baker BF, Pham N, Swayze E, Geary RS. Pharmacology of Antisense Drugs. Annu Rev Pharmacol Toxicol. 2017;57:81-105.
6. Smith RA, Miller TM, Yamanaka K, et al. Antisense oligonucleotide therapy for neurodegenerative disease. J Clin Invest. 2006;116(8):2290-2296.
7. Kordasiewicz HB, Stanek LM, Wancewicz EV, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012;74(6):1031-1044.
8. Rigo F, Chun SJ, Norris DA, et al. Pharmacology of a central nervous system delivered 2'-O-methoxyethyl-modified survival of motor neuron splicing oligonucleotide in mice and nonhuman primates. J Pharmacol Exp Ther. 2014;350(1):46-55.
9. DeVos SL, Miller RL, Schoch KM, et al. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. Sci Transl Med. 2017;9(374):eaag0481.
10. Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1723-1732.
11. Southwell AL, Kordasiewicz HB, Langbehn D, et al. Huntingtin suppression restores cognitive function in a mouse model of Huntington's disease. Sci Transl Med. 2018;10(461).
12. McCampbell A, Cole T, Wegener AJ, et al. Antisense oligonucleotides extend survival and reverse decrement in muscle response in ALS models. J Clin Invest. 2018;128(8):3558-3567.
13. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993;362(6415):59-62.
14. Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N Engl J Med. 2020;383(2):109-119.
15. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011;72(2):245-256.
16. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72(2):257-268.
17. Nguyen L, Cleary JD, Ranum LPW. Repeat-Associated Non-ATG Translation: Molecular Mechanisms and Contribution to Neurological Disease. Annu Rev Neurosci. 2019;42:227-247.
18. Jiang J, Zhu Q, Gendron TF, et al. Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. Neuron. 2016;90(3):535-550.
19. Kumar VB, Farr SA, Flood JF, et al. Site-directed antisense oligonucleotide decreases the expression of amyloid precursor protein and reverses deficits in learning and memory in aged SAMP8 mice. Peptides. 2000;21(12):1769-1775.
20. Armbrecht HJ, Siddiqui AM, Green M, et al. Antisense against Amyloid-b Protein Precursor Reverses Memory Deficits and Alters Gene Expression in Neurotropic and Insulin-Signaling Pathways in SAMP8 Mice. J Alzheimers Dis. 2015;46(2):535-548.
21. Farr SA, Erickson MA, Niehoff ML, Banks WA, Morley JE. Central and peripheral administration of antisense oligonucleotide targeting amyloid-b protein precursor improves learning and memory and reduces neuroinflammatory cytokines in Tg2576 (AbPPswe) mice. J Alzheimers Dis. 2014;40(4):1005-1016.
22. Farr SA, Sandoval KE, Niehoff ML, Witt KA, Kumar VB, Morley JE. Peripheral administration of GSK-3b antisense oligonucleotide improves learning and memory in SAMP8 and Tg2576 mouse models of Alzheimer's disease. J Alzheimers Dis. 2016;54(4):1339-1348.
23. Farr SA, Ripley JL, Sultana R, et al. Antisense oligonucleotide against GSK-3b in brain of SAMP8 mice improves learning and memory and decreases oxidative stress: Involvement of transcription factor Nrf2 and implications for Alzheimer disease. Free Radic Biol Med. 2014;67:387-395.
24. Fiorini A, Sultana R, Förster S, et al. Antisense directed against PS-1 gene decreases brain oxidative markers in aged senescence accelerated mice (SAMP8) and reverses learning and memory impairment: a proteomics study. Free Radic Biol Med. 2013;65:1-14.
25. Farr SA, Niehoff ML, Kumar VB, Roby DA, Morley JE. Inhibition of glycogen synthase kinase 3beta as a treatment for the prevention of cognitive deficits after a traumatic brain injury. J Neurotrauma. 2019;36(11):1869-1875.
26. Finkel RS, McDermott MP, Kaufmann P, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology. 2014;83(9):810-817.
27. Rudnik-Schöneborn S, Berg C, Zerres K, et al. Genotype-phenotype studies in infantile spinal muscular atrophy (SMA) type I in Germany: implications for clinical trials and genetic counselling. Clin Genet. 2009;76(2):168-178.
28. Hua Y, Vickers TA, Okunola HL, Bennett CF, Krainer AR. Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. Am J Hum Genet. 2008;82(4):834-848.
29. Rigo F, Hua Y, Chun SJ, Prakash TP, Krainer AR, Bennett CF. Synthetic oligonucleotides recruit ILF2/3 to RNA transcripts to modulate splicing. Nat Chem Biol. 2012;8(6):555-561.
30. van Deutekom JC, Bremmer-Bout M, Janson AA, et al. Antisense-induced exon skipping restores dystrophin expression in DMD patient derived muscle cells. Hum Mol Genet. 2001;10(15):1547-1554.
31. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006;314(5796):130-133.
32. Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun. 2006;351(3):602-611.
33. Amador-Ortiz C, Lin WL, Ahmed Z, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol. 2007;61(5):435-445.
34. Robinson JL, Lee EB, Xie SX, et al. Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. Brain. 2018;141(7):2181-2193.
35. Wennberg AM, Tosakulwong N, Lesnick TG, et al. Association of Apolipoprotein E e4 With Transactive Response DNA-Binding Protein 43. JAMA neurology. 2018;75(11):1347-1354.
36. Yang HS, Yu L, White CC, et al. Evaluation of TDP-43 proteinopathy and hippocampal sclerosis in relation to APOE e4 haplotype status: a community-based cohort study. Lancet Neurol. 2018;17(9):773-781.
37. Nelson PT, Dickson DW, Trojanowski JQ, et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Brain. 2019;142(6):1503-1527.
38. Nomakuchi T, Rigo F, Aznarez I, Krainer A. Antisense oligonucleotide-directed inhibition of nonsense-mediated mRNA decay. Nat Biotechnol. 2016;34(2):164-166.
39. Nguyen AD, Nguyen TA, Zhang J, et al. Murine knockin model for progranulin-deficient frontotemporal dementia with nonsense-mediated mRNA decay. Proc Natl Acad Sci U S A. 2018;115(12):E2849-E2858.

Disclosure

None reported.

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Hydrogel-Based Spheroid Models of Glioblastoma for Drug Screening Applications

by Joey Bruns, MS & Silviya Petrova Zustiak, PhD



Hydrogel-grown GBM spheroids are promising tools for drug screening applications to address the growing backlog of drug candidates and the growing concerns with low drug efficacy or unexpected side effects.



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Abstract

Glioblastoma multiforme (GBM) is the most aggressive brain tumor, with median patient survival of 12-15 months even after treatment. To facilitate basic research as well as treatment development, bioengineered GBM models that adequately recapitulate aspects of the in vivo tumor microenvironment are greatly needed. Multicellular spheroids are a well-accepted model in tumor biology as well as drug screening because they recapitulate many of the solid tumor characteristics, such as hypoxic core and cell-cell communication. There are multiple approaches for growing GBM cells into tumor spheroids – non-adherent plastic dishes, hanging drop, bioreactors, and hydrogels, amongst others. Suspension spheroid models offer ease of growth, uniformity, and overall lower cost, but neglect the cell-matrix interactions, while hydrogel-based spheroids capture cell-matrix interactions and allow co-cultures with stromal cells. In this review, we summarize various approaches to fabricate GBM spheroid models as well as GBM spheroid characteristics and chemotherapeutic responsiveness as a function of hydrogel matrix encapsulation and properties, in order to advance therapies.

Glioblastoma, the Tumor Microenvironment, and Current Treatments

Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is a lethal disease with poor patient prognosis that represents 16% of all brain tumors.[1] GBM is the most aggressive type of brain tumor, with median patient survival of only 12-15 months. Over 23,000 new cases of central nervous system tumors are reported, and over 14,000 deaths occur annually due to GBM in the United States.[2] The initial GBM symptoms are non-specific, such as headaches or nausea and worsen rapidly.[3] The cause for most GBM cases is not known and there is no known method to prevent the disease. While the disease can happen at any age, it occurs most often in older adults and it is more common in males than females.[4]

GBM Microenvironment

The GBM microenvironment is extremely complex, consisting of various extracellular matrix (ECM) proteins, glucosaminoglycans (GAGs) and cell types. Healthy brain tissue contains low levels of fibrous proteins such as fibronectin, laminin and collagen and high levels of GAGs, both unbound and bound to fibrous proteins and proteoglycans.[5] The presence of GBM tumors greatly alters the

ECM both in concentration and composition, with the relative concentration of ECM compared to cellular components increasing from 20% to 48% in GBM.[5] In GBM, there is upregulation of primarily hyaluronic acid (HA), laminin, and collagen, along with other ECM components.[6,7] This increase in ECM components is due to upregulated secretion of fibrous proteins from healthy brain tissue and HA from glioma cells.[5,6] Other crucial non-cellular components of the tumor microenvironment are extracellular vesicles and soluble signals secreted from glioma cells, which trigger cell migration and ECM secretion from surrounding cells.[8] The GBM microenvironment also includes various cell types such as glioma cells, glial support cells (astrocytes and oligodendrocytes), and immune cells.[9,10] A simplified schematic of the GBM microenvironment is represented in Figure 1.

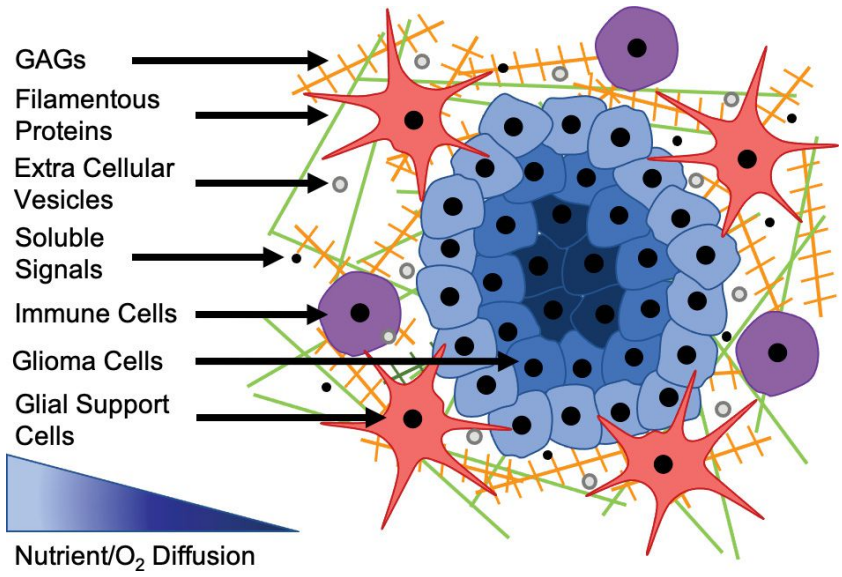


Figure 1. A simplified schematic of the GBM microenvironment.

The GBM microenvironment provides structural support and directs GBM cell behavior, disease progression and therapy responsiveness.[11] For example, presence of the proteoglycan tenascin-C correlates with angiogenesis and the progression of glioma.[12] Collagen IV, V, fibronectin, and laminin, which abound in the basolateral membrane of GBM-associated vessels, enhance cell survival, proliferation and migration.[13] Adhesion of integrins to their ECM ligands lessens GBM response to therapy.[14] In terms of mechanotransduction, increased tumor stiffness contributes to malignancy.[15] and alters integrin expression, which in turn regulates GBM cell survival, proliferation, migration and invasion.[16] In terms of support cells, endothelial cells in the perivascular niche secrete interleukin-8, which enhances the proliferation and migration of glioblastoma stem cells (GSCs).[17] Microglia, another support cell type, produce matrix metalloproteinases (MMPs) and modulate growth and migration of glioma cells.[18] Hence, mimicking key aspects of the glioblastoma microenvironment, such as biochemical and mechanical input as well as presence of support cells, is critical for the development of faithful in vitro GBM models that could lead to the discovery and development of successful therapies.

GBM Treatments

Typical GBM treatments include maximum safe surgical resection of the bulk tumor followed by chemotherapy with temozolomide (TMZ), coupled with radiation therapy.[19] TMZ is currently the only approved GBM chemotherapy, and provides only a modest two-month increase in median survival.[20] Overall, with current treatments, median survival rate is only 12-15 months with a two-year survival rate of only 25%.[21] Anti-angiogenesis therapy via bevacizumab (BEV) showed promising results at early clinical trials,[22] but phase III trials revealed increased toxic side effects without additional overall survival.[23] The challenge with successful chemotherapy is typically the complex tumor microenvironment and difficulty passing the blood brain barrier, which can lead to chemoresistance.[24] To address chemoresistance of individual chemotherapeutics, combination therapies are commonly used, but GBM recurrence is still a serious issue with current treatments.[25] Targeted therapies are currently being researched to help improve GBM treatment efficacy, including targeting epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and other receptor tyrosine kinase (RTK) pathways.[26,27]

GBM Subtypes

Perhaps one of the biggest contributors to GBM resistance to therapies is its heterogeneity. Recent large-scale genomic analyses of GBM by the Cancer

Genome Atlas Research Network identified four distinct molecular subtypes of GBM: neural, proneural, classical and mesenchymal.[28,29] Proneural GBM patients had the poorest response even to the most rigorous treatment, while patients with the other three subtypes had modest survival gains. Furthermore, each subtype had different genetic mutations and expression, suggesting the need for subtype-specific and individualized treatment. This and many follow-up studies have demonstrated GBM heterogeneity[30] and many others have addressed the critical importance of the microenvironment on GBM response to therapy.[31] However, it is yet to be interrogated how individual GBM subtypes respond to microenvironmental cues and how this interplay translates to therapeutic responsiveness. This is a potential new frontier in the search for GBM treatments.

Hydrogel-Based in vitro Spheroid Models for GBM

The Need for Hydrogel-Based Multicellular GBM Spheroid Models

Multicellular tumor spheroids could lead to a cancer therapy breakthrough by modeling avascular tumor nodules in the laboratory[32,33] providing a substrate for predictive high-throughput drug screening. The need for better screening has intensified as advances in genomics and organic chemistry created a backlog of candidate drugs.[34] Even with high-content screening, current methods frequently fail to eliminate poor candidates: roughly 90% of late-stage clinical trials fail, often due to unforeseen ineffectiveness or side effects.[35] Multicellular tumor spheroids could improve the predictive capacity of drug screening platforms, relieving cost and ethics concerns of human and animal trials.

Most commonly, spheroids are grown in a liquid - on non-adhesive substrates, hanging drops or in bioreactors.[36] While such suspension spheroids mimic many morphological, functional and mass transport features of naturally occurring tumors,[37] unlike natural tumors, these mimics exist in an attachment-free microenvironment which does not resemble the mechanical, physical, and biochemical properties of the native tumor ECM. To more faithfully mimic the native microenvironment, formation of spheroids in an external matrix has emerged as a promising alternative to suspension spheroids. Unlike suspension spheroids, matrix-grown ones can mimic tumor stroma, allowing tests of therapies that target the stroma, the immune system, or stromal cells.[38] Recent studies also demonstrate that growing spheroids in a matrix can produce more cancer stem cells,[39,40] which have been implicated as major contributors to therapy resistance and tumor recurrence.[41]

Tumor Spheroid Fabrication Methods

Tumor spheroids for drug screening can be formed via clonal expansion or through cell aggregation. For clonal expansion, single cells are immobilized in a hydrogel matrix such as Matrigel,[42] polyethylene glycol (PEG),[43] or fibrin[44] and allowed to grow into spheroids from a single cell. These spheroids require long growth times of one to two weeks, but are relatively simple to prepare, and the matrix provides critical physical, biochemical, and mechanical cues to the cells. However, they also result in spheroids of heterogenous sizes and shapes, where spheroid size is further limited by the formation of a hypoxic core. Alternatively, tumor spheroids can be formed in hydrogel matrices through aggregation either by loading cells at high density into hydrogel microspheres[45,46] or by seeding and allowing cells to aggregate in hydrogels with pre-templated spherical openings.[47] Such methods allow better control over spheroid size and heterogeneity, while still enabling cell-matrix interactions during spheroid maturation and growth.

For fast spheroid fabrication, cells could be seeded in specialty plates that prevent cell-surface interactions and forced to aggregate through forming strong cell-cell contacts. For example, the hanging drop method puts a high concentration of cells in a drop of cell culture media suspended in the air through surface tension, where cells aggregate and form spheroids.[48, 49] However, hanging drop plates are difficult to handle, are not amenable to robotic handling and do not allow spheroid characterizations directly in the plates. Micropatterned plates with ultra-low attachment surfaces (ULAP) have been developed for easier handling and spheroid characterizations, where cells are allowed to aggregate in the microwells.[50,51] Spheroids of uniform sizes can be produced robustly, and the spheroid size can be controlled via the microwell size. Upon formation, which happens within 24 hours, spheroid aggregates can be characterized directly in the plate, harvested and used as is, or harvested and seeded in an external matrix and allowed to grow further in the presence of cell-matrix interactions.[52]

Spheroid Models Using GBM Cell Lines

Cell lines have been extensively used in drug screening in general and for spheroid fabrication in

particular. For example, the National Cancer Institute uses a panel of 60 cells lines representing various cancers, namely NCI-60, to screen all U.S. Food and Drug Administration (FDA) approved chemotherapeutics.[53] Cell lines are less expensive than patient-derived primary cells and allow a high level of reproducibility and direct comparison of results between different labs. However, cells lines do not recapitulate the properties and heterogeneity of the primary tumor cells and are pre-conditioned to tissue culture plastics (TCP), thus possibly leading to altered cell-matrix interactions.

While simplistic, GBM spheroid models involving cell lines have taught us a lot about the effect of the mechanical, physical, and biochemical matrix properties on GBM spheroid characteristics and drug responsiveness. For example, Wang et al. demonstrated that U87 GBM spheroids proliferate more and show more infiltration in soft gels compared to stiff gels.[43] The researchers used PEG/HA hydrogels with MMP-cleavable crosslinkers and grew the spheroids for 14 days via clonal expansion. Varying the hydrogel stiffness also affected the expression of MMPs with MMP-1 upregulated in stiffer gels and MMP-9 upregulated in softer gels. MMP production is indicative of tumor progression, as invasion increases with increase in MMP production. Matrix stiffness has also been shown to affect the invasive capacity of tumor spheroids.[48] Specifically, U87 and U118 spheroids were formed and allowed to infiltrate into varying stiffnesses of gelatin/HA/PEG hydrogels (2 vs. 5 kPa). Cells preferentially infiltrated into the softer gels. Further, infiltrating cells were able to form secondary spheroids, demonstrating how cancer cells could infiltrate into brain tissue and create tumors in secondary locations. In another infiltration study, Stein et al. encapsulated U87 spheroids in collagen gels, using cells with (U87WT) and without (U87ΔEGFR) epidermal growth factor receptor (EGFR), present in malignant tumors, to model the spheroid invasive potential.[54] The authors found that the infiltrating periphery of the U87WT spheroids had higher invasion rate into the collagen gel, but the core radius of both U87WT and U87ΔEGFR grew at a steady rate of 27 μm/day. Interestingly, the core radius of U87WT decreased initially due to cells infiltrating into the collagen gels faster than the core was able to grow.

Multiple spheroid models have also been developed to understand how the surrounding matrix affects GBM spheroid drug responsiveness. For example, U87 spheroids were encapsulated in fibrin gels and allowed to infiltrate into the matrix.[55] When exposed to the chemotherapeutic atorvastatin, the infiltration capacity of the spheroids greatly decreased, and the number of apoptotic cells increased compared to the no drug control. It is important to note that while different hydrogel matrices can be used for spheroid encapsulation, the type of matrix itself could influence the cells' chemotherapeutic response. For example, U87 and U118 spheroids formed via clonal expansion within three to five days were treated with TMZ and Carmustin (BCNU).[42] The authors specifically compared spheroids formed in chitosan/PEG hydrogels vs. ones formed in Matrigel, and noted more invasion in Matrigel but increased chemoresistance in the chitosan/PEG hydrogels. Further, the matrix presence alone, compared to suspension spheroids, can affect spheroid characteristics and responsiveness to chemotherapeutics. For example, Erkoc et al. compared suspension U373 spheroids to spheroids encapsulated in gelatin methacryloyl (GelMA) hydrogels.[49] Spheroids grown in GelMA show increased diameter and cell viability compared to suspension spheroids over the course of 14 days with protrusions from the spheroid at day seven. MMP production, which contributes to spheroid invasiveness, is also dependent on matrix presence as MMP-3 expression increases in the gel matrix and MMP-9 increases in suspension spheroids. Anti-cancer drug treatment using Digitoxigenin (Dgx) results in decreased spheroid size and increased sensitivity to the anticancer drug compared to single cells in GelMA.

Spheroid Models Using Primary Cells and Tumor Explants

Primary cells and tumor explants address some concerns associated with cell lines, primarily introducing more tumor complexity. Primary cells are not pre-conditioned to the in vitro environment prior to drug screening, and the chances of mutations are greatly decreased. Tumor explants are even better at mimicking the tumor microenvironment as the cells contain some of the native ECM proteins as well as various support cells. In addition, using patient-derived cells and explants opens the door to personalized therapy, where drug and drug combinations can be pre-screened in vitro to determine efficacy prior to patient administration. While primary cells and tumor explants provide more physiologically relevant conditions compared to cell lines, they might not always be practical as they lack the reproducibility and verification potential of using cell lines.

In one study, Akay et al. created a microfluidic chip to be used on tumor explants for drug screening.[50]

Spheroids were formed in the microfluidic device on micropatterned PEGDA and treated with TMZ and BEV alone and in combination. The results showed that both drugs were effective in lowering cell viability, especially when used in combination. Of the three patient samples used, each sample had varying effects on tumor responsiveness due to the complex and heterogeneous tumor microenvironment. Another study specifically addressed the ability of tumor explants encapsulated in a physiologically relevant matrix, named VersaGel, to replicate patient drug responses.[51] Retrospective drug screening was performed on five patients and the results seen for explants in the VersaGel corroborated clinical responses: three patients were responsive to TMZ, one patient was partially responsive and one patient was non-responsive. With the non-responsive patient samples, three different chemotherapeutics were investigated and gave results with two drugs giving IC_{50}'s lower than maximum blood concentration and one drug giving an IC_{50} higher than maximum blood concentration, but patient response to the drugs was not determined.

Note that most spheroid models replicate avascular tumors and are usually cultured for up to 14 days due to diffusion and nutrient limitations. To further recapitulate the complexity of the tumor microenvironment, Ozturk et al. 3D printed a tumor organoid containing a tumor spheroid and blood vessel analogs.[56] Tumor spheroids were formed using patient-derived cells in a micropatterned plate, placed on top of a collagen hydrogel and sandwiched between two gelatin perfusion channels, with HUVEC cells perfused to mimic blood vessels, and subsequently covered with a collagen gel. The vascularization allowed for tumor spheroids to be cultured for two months, where spheroids infiltrated into the gelatin matrix. When treated with TMZ, the spheroids showed higher cell viability compared to single cell suspensions.

Conclusion and Future Perspectives

Hydrogel-grown GBM spheroids are promising tools for drug screening applications to address the growing backlog of drug candidates and the growing concerns with low drug efficacy or unexpected side effects. While there are a variety of preliminary studies describing the utility of such models and showing their ability to predict clinical outcomes, there are many venues available for future explorations. Those include studies to determine key hydrogel properties that affect spheroid responsiveness to chemotherapeutics or increasing model complexity by adding vascularization, perfusion, support cells and physiologically relevant ECM components. However, to be useful in drug screening, the complexity needs to be balanced by robustness and the amenability to high-throughput screening. In addition, while cell lines are useful for reproducibility, further research involving patient-derived cells would be useful in translational research and personalized medicine. Lastly, models need to take into account the different GBM subtypes and their specific microenvironment as they have shown differential drug responsiveness.

References

1. J.P. Thakkar, T.A. Dolecek, C. Horbinski, Q.T. Ostrom, D.D. Lightner, J.S. Barnholtz-Sloan, J.L. Villano, Epidemiologic and molecular prognostic review of glioblastoma, Cancer Epidemiology and Prevention Biomarkers 23(10) (2014) 1985-1996.
2. S.A. Langhans, Three-dimensional in vitro cell culture models in drug discovery and drug repositioning, Frontiers in pharmacology 9 (2018) 6.
3. A.M. Stark, J. van de Bergh, J. Hedderich, H.M. Mehdorn, A. Nabavi, Glioblastoma: clinical characteristics, prognostic factors and survival in 492 patients, Clinical neurology and neurosurgery 114(7) (2012) 840-845.
4. S.V. Ellor, T.A. Pagano-Young, N.G. Avgeropoulos, Glioblastoma: background, standard treatment paradigms, and supportive care considerations, SAGE Publications Sage CA: Los Angeles, CA, 2014.
5. A. Belousov, S. Titov, N. Shved, M. Garbuz, G. Malykin, V. Gulaia, A. Kagansky, V. Kumeiko, The extracellular matrix and biocompatible materials in glioblastoma treatment, Frontiers in Bioengineering and Biotechnology 7 (2019).
6. A.C. Bellail, S.B. Hunter, D.J. Brat, C. Tan, E.G. Van Meir, Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion, The international journal of biochemistry & cell biology 36(6) (2004) 1046-1069.
7. J.C. Knott, R. Mahesparan, I. Garcia-Cabrera, B. Bølge Tysnes, K. Edvardsen, G.O. Ness, S. Mørk, M. Lund-Johansen, R. Bjerkvig, Stimulation of extracellular matrix components in the normal brain by invading glioma cells, International journal of cancer 75(6) (1998) 864-872.
8. A. Yekula, A. Yekula, K. Muralidharan, K. Kang, B.S. Carter, L. Balaj, Extracellular Vesicles in Glioblastoma Tumor Microenvironment, Frontiers in Immunology 10 (2019).
9. W. Tomaszewski, L. Sanchez-Perez, T.F. Gajewski, J.H. Sampson, Brain tumor microenvironment and host state: implications for immunotherapy, Clinical Cancer Research 25(14) (2019) 4202-4210.
10. Z. Chen, D. Hambardzumyan, Immune microenvironment in glioblastoma subtypes, Frontiers in immunology 9 (2018) 1004.
11. N.A. Charles, E.C. Holland, R. Gilbertson, R. Glass, H. Kettenmann, The brain tumor microenvironment, Glia 60(3) (2012) 502-514.
12. D. Zagzag, D.R. Friedlander, D.C. Miller, J. Dosik, J. Cangiarella, M. Kostianovsky, H. Cohen, M. Grumet, M.A. Greco, Tenascin expression in astrocytomas correlates with angiogenesis, Cancer Research 55(4) (1995) 907-914.
13. T. Demuth, M.E. Berens, Molecular mechanisms of glioma cell migration and invasion, Journal of neuro-oncology 70(2) (2004) 217-228.
14. S. Hehlgans, M. Haase, N. Cordes, Signalling via integrins: implications for cell survival and anticancer strategies, Biochimica et Biophysica Acta (BBA)-Reviews on Cancer 1775(1) (2007) 163-180.
15. T.A. Ulrich, E.M. de Juan Pardo, S. Kumar, The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells, Cancer research 69(10) (2009) 4167-4174.
16. J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nature Reviews Cancer 10(1) (2010) 9-22.
17. D.W. Infanger, Y. Cho, B.S. Lopez, S. Mohanan, S.C. Liu, D. Gursel, J.A.

Boockvar, C. Fischbach, Glioblastoma stem cells are regulated by interleukin-8 signaling in a tumoral perivascular niche, Cancer research 73(23) (2013) 7079-7089.
18. D. Markovic, K. Vinnakota, S. Chirasani, M. Synowitz, H. Raguet, K. Stock, M. Sliwa, S. Lehmann, R. Kälin, N. Van Rooijen, Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion, Proceedings of the National Academy of Sciences 106(30) (2009) 12530-12535.
19. M.E. Hegi, A.C. Diserens, T. Gorlia, M.F. Hamou, N. de Tribolet, M. Weller, J.M. Kros, J.A. Hainfellner, W. Mason, L. Mariani, J.E. Bromberg, P. Hau, R.O. Mirimanoff, J.G. Cairncross, R.C. Janzer, R. Stupp, MGMT gene silencing and benefit from temozolomide in glioblastoma, The New England Journal of Medicine 352(10) (2005) 997-1003.
20. M.H. Cohen, J.R. Johnson, R. Pazdur, Food and Drug Administration Drug approval summary: temozolomide plus radiation therapy for the treatment of newly diagnosed glioblastoma multiforme, Clinical cancer research : an official journal of the American Association for Cancer Research 11(19 Pt 1) (2005) 6767-71.
21. O. Van Tellingen, B. Yetkin-Arik, M. De Gooijer, P. Wesseling, T. Wurdinger, H. De Vries, Overcoming the blood–brain tumor barrier for effective glioblastoma treatment, Drug Resistance Updates 19 (2015) 1-12.
22. H.S. Friedman, M.D. Prados, P.Y. Wen, T. Mikkelsen, D. Schiff, L.E. Abrey, W.K. Yung, N. Paleologos, M.K. Nicholas, R. Jensen, J. Vredenburgh, J. Huang, M. Zheng, T. Cloughesy, Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 27(28) (2009) 4733-40.
23. P. Fathpour, N. Obad, H. Espedal, D. Stieber, O. Keunen, P.Ø. Sakariassen, S.P. Niclou, R. Bjerkvig, Bevacizumab treatment for human glioblastoma. Can it induce cognitive impairment?, Neuro-oncology 16(5) (2014) 754-756.
24. M. Da Ros, V. De Gregorio, A.L. Iorio, L. Giunti, M. Guidi, M. De Martino, L. Genitori, I. Sardi, Glioblastoma chemoresistance: The double play by microenvironment and blood-brain barrier, International journal of molecular sciences 19(10) (2018) 2879.
25. S. Roy, D. Lahiri, T. Maji, J. Biswas, Recurrent glioblastoma: where we stand, South Asian journal of cancer 4(4) (2015) 163.
26. J.I. Bastien, K.A. McNeill, H.A. Fine, Molecular characterizations of glioblastoma, targeted therapy, and clinical results to date, Cancer 121(4) (2015) 502-516.
27. O.G. Taylor, J.S. Brzozowski, K.A. Skelding, Glioblastoma multiforme: An overview of emerging therapeutic targets, Frontiers in oncology 9 (2019) 963.
28. R.G. Verhaak, K.A. Hoadley, E. Purdom, V. Wang, Y. Qi, M.D. Wilkerson, C.R. Miller, L. Ding, T. Golub, J.P. Mesirov, Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1, Cancer cell 17(1) (2010) 98-110.
29. R. McLendon, A. Friedman, D. Bigner, E.G. Van Meir, D.J. Brat, G.M. Mastrogianakis, J.J. Olson, T. Mikkelsen, N. Lehman, K. Aldape, Comprehensive genomic characterization defines human glioblastoma genes and core pathways, Nature 455(7216) (2008) 1061-1068.
30. A. Sottoriva, I. Spiteri, S.G. Piccirillo, A. Touloumis, V.P. Collins, J.C. Marioni, C. Curtis, C. Watts, S. Tavaré, Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics, Proceedings of the National Academy of Sciences 110(10) (2013) 4009-4014.
31. A. Rape, B. Ananthanarayanan, S. Kumar, Engineering strategies to mimic the glioblastoma microenvironment, Advanced drug delivery reviews 79 (2014) 172-183.
32 X. Zhang, W. Wang, W. Yu, Y. Xie, X. Zhang, Y. Zhang, X. Ma, Development of an in vitro multicellular tumor spheroid model using microencapsulation and its application in anticancer drug screening and testing, Biotechnology progress 21(4) (2005) 1289-1296.
33. K.M. Yamada, E. Cukierman, Modeling tissue morphogenesis and cancer in 3D, Cell 130(4) (2007) 601-610.
34. A. Dove, Drug screening-beyond the bottleneck, Nature biotechnology 17(9) (1999) 859-864.
35. I. Kola, J. Landis, Can the pharmaceutical industry reduce attrition rates?, Nature reviews Drug discovery 3(8) (2004) 711-716.
36. J. Friedrich, C. Seidel, R. Ebner, L.A. Kunz-Schughart, Spheroid-based drug screen: considerations and practical approach, Nature protocols 4(3) (2009) 309-324.
37. I. Dufau, C. Frongia, F. Sicard, L. Dedieu, P. Cordelier, F. Ausseil, B. Ducommun, A. Valette, Multicellular tumor spheroid model to evaluate spatio-temporal dynamics effect of chemotherapeutics: application to the gemcitabine/CHK1 inhibitor combination in pancreatic cancer, BMC cancer 12(1) (2012) 15.
38. D.V. LaBarbera, B.G. Reid, B.H. Yoo, The multicellular tumor spheroid model for high-throughput cancer drug discovery, Expert opinion on drug discovery 7(9) (2012) 819-830.
39. F. Ordikhani, Y. Kim, S.P. Zustiak, The Role of Biomaterials on Cancer Stem Cell Enrichment and Behavior, JOM (2015) 1-7.
40. W. Rao, S. Zhao, J. Yu, X. Lu, D.L. Zynger, X. He, Enhanced enrichment of prostate cancer stem-like cells with miniaturized 3D culture in liquid core-hydrogel shell microcapsules, Biomaterials 35(27) (2014) 7762-7773.
41. C.T. Jordan, M.L. Guzman, M. Noble, Cancer stem cells, New England Journal of Medicine 355(12) (2006) 1253-1261.
42. F.C. Chang, S.L. Levengood, N. Cho, L. Chen, E. Wang, J.S. Yu, M. Zhang, Crosslinked Chitosan-PEG Hydrogel for Culture of Human Glioblastoma Cell Spheroids and Drug Screening, Advanced therapeutics 1(7) (2018) 1800058.
43. C. Wang, X. Tong, F. Yang, Bioengineered 3D brain tumor model to elucidate the effects of matrix stiffness on glioblastoma cell behavior using PEG-based hydrogels, Molecular pharmaceutics 11(7) (2014) 2115-2125.
44. C. Lee, E. Abelseth, L. De La Vega, S. Willerth, Bioprinting a novel glioblastoma tumor model using a fibrin-based bioink for drug screening, Materials Today Chemistry 12 (2019) 78-84.
45. A.S. Qayyum, E. Jain, G. Kolar, Y. Kim, S.A. Sell, S.P. Zustiak, Design of electrohydrodynamic sprayed polyethylene glycol hydrogel microspheres for cell encapsulation, Biofabrication 9(2) (2017) 025019.
46. S. Pradhan, C.S. Chaudhury, E.A. Lipke, Dual-phase, surface tension-based fabrication method for generation of tumor millibeads, Langmuir 30(13) (2014) 3817-3825.
47. M. Imaninezhad, L. Hill, G. Kolar, K. Vogt, S.P. Zustiak, Templated Macroporous Polyethylene Glycol Hydrogels for Spheroid and Aggregate Cell Culture, Bioconjugate chemistry 30(1) (2018) 34-46.
48. J.M. Heffernan, D.J. Overstreet, L.D. Le, B.L. Vernon, R.W. Sirianni, Bioengineered scaffolds for 3D analysis of glioblastoma proliferation and invasion, Annals of biomedical engineering 43(8) (2015) 1965-1977.
49. P. Erkoc, F. Seker, T. Bagci-Onder, S. Kizilel, Gelatin methacryloyl hydrogels in the absence of a crosslinker as 3D glioblastoma multiforme (GBM)-mimetic microenvironment, Macromolecular bioscience 18(3) (2018) 1700369.
50. M. Akay, J. Hite, N.G. Avci, Y. Fan, Y. Akay, G. Lu, J.-J. Zhu, Drug screening of human GBM spheroids in brain cancer chip, Scientific reports 8(1) (2018) 1-9.
51. K.C. Hribar, C.J. Wheeler, A. Bazarov, K. Varshneya, R. Yamada, P. Buckley, C.G. Patil, A simple three-dimensional hydrogel platform enables ex vivo cell culture of patient and PDX tumors for assaying their response to clinically relevant therapies, Molecular cancer therapeutics 18(3) (2019) 718-725.
52. M.F. Gencoglu, L.E. Barney, C.L. Hall, E.A. Brooks, A.D. Schwartz, D.C. Corbett, K.R. Stevens, S.R. Peyton, Comparative study of multicellular tumor spheroid formation methods and implications for drug screening, ACS biomaterials science & engineering 4(2) (2018) 410-420.
53. R.H. Shoemaker, The NCI60 human tumour cell line anticancer drug screen, Nature Reviews Cancer 6(10) (2006) 813-823.
54. A.M. Stein, T. Demuth, D. Mobley, M. Berens, L.M. Sander, A mathematical model of glioblastoma tumor spheroid invasion in a three-dimensional in vitro experiment, Biophysical journal 92(1) (2007) 356-365.
55. N. Bayat, S. Ebrahimi-Barough, A. Norouzi-Javidan, H. Saberi, R. Tajerian, M.M.M. Ardakan, S. Shirian, A. Ai, J. Ai, Apoptotic effect of atorvastatin in glioblastoma spheroids tumor cultured in fibrin gel, Biomedicine & Pharmacotherapy 84 (2016) 1959-1966.
56. M.S. Ozturk, V.K. Lee, H. Zou, R.H. Friedel, X. Intes, G. Dai, High-resolution tomographic analysis of in vitro 3D glioblastoma tumor model under long-term drug treatment, Science Advances 6(10) (2020) eaay7513.

Disclosure

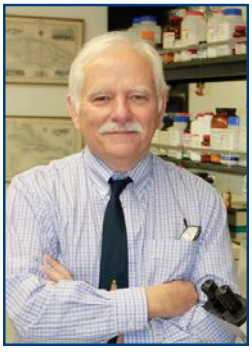
None reported. **MM**

The Deductive Reasoning Strategy Enables Biomedical Breakthroughs

by Gina L. C. Yosten, PhD, Grant R. Kolar, MD, PhD, Daniela Salvemini, PhD & Willis K. Samson, PhD

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Our Deductive Receptor Matching Strategy is being applied to identify the cognate receptors for several other known peptide hormones.



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Abstract

G protein-coupled receptors (GPCRs) transmit the signals of a variety of hormones and neurotransmitters and are targets of more than 30% of all FDA-approved drugs. We developed an approach for identifying the endogenous ligands for a family of orphan GPCRs that enables the development of novel therapeutics for the potential treatment of a wide variety of disorders including pain, diabetes, appetitive behaviors, infertility and obesity. With this approach, we have deorphanized five previously orphaned GPCRs.

G Protein Coupled Receptors as Potential Therapeutic Targets

It is estimated that 30-50% of all FDA-approved medications target G protein-coupled receptors (GPCRs).[1,2] There are three major classes of GPCRs in humans: Class A (rhodopsin-like), Class B (secretin-like), and Class C (metabotropic/glutamate-like). These are large proteins that reside in the cell membrane with a characteristic 7-transmembrane spanning domain and an extracellular terminus that may be glycosylated.[3] GPCRs also contain intracellular domains that interact with downstream signaling cascades that communicate the message imparted by a ligand (e.g. hormone) binding primarily to extracellular epitopes on the receptor. The selectivity of ligand-receptor binding makes them attractive candidates for drug development. Examples of existing therapeutics based upon knowledge of ligand structure/function relationships and receptor recognition motifs are numerous and include, for example, Class A receptor therapeutics targeting the angiotensin receptors, Class B receptor therapeutics targeting the GLP-1 receptor, and Class C receptor therapeutics targeting neurotransmitter receptors.

There may be as many as one thousand genes encoding GPCRs, but by far the largest number of these encode receptors in the Class A category. Remarkably, even with the development of the most modern molecular approaches, it is estimated that 130 or more of those identified genes encode GPCRs for which an endogenous ligand has yet to be identified. Think of these receptors as door locks, which protect entry into new

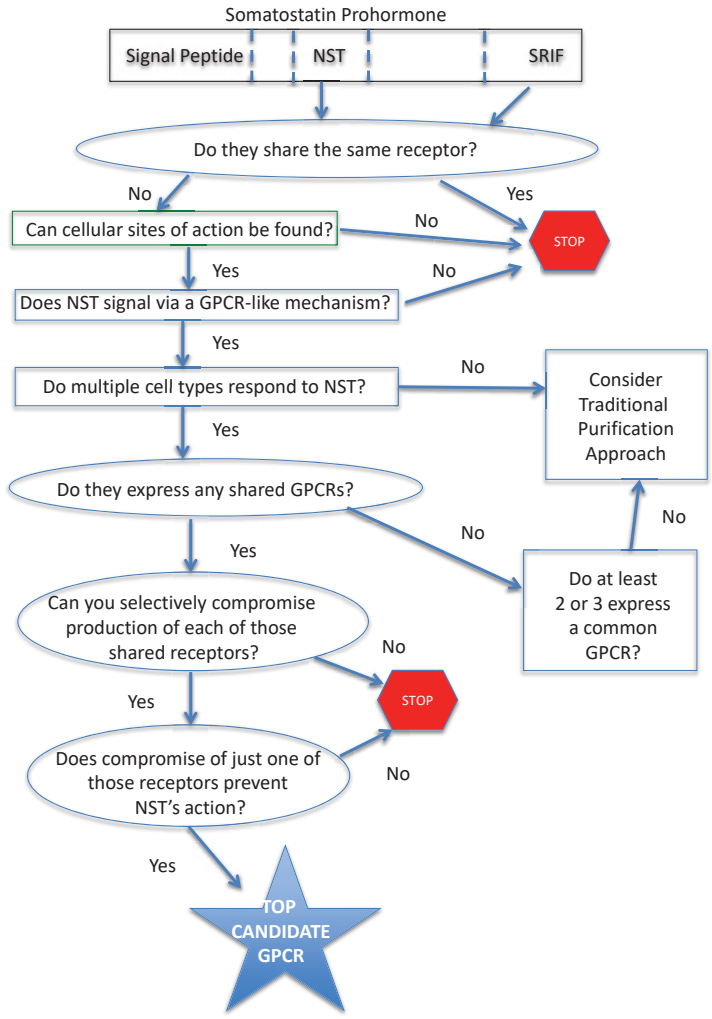


Figure 1. The Deductive Reasoning Strategy for the G protein-coupled receptor (GPCR) De-Orphanization. Demonstrated here is the identification of a top receptor candidate for neuronostatin (NST) a recently discovered, endogenous hormone,5 that is encoded in the same gene product as somatostatin (SRIF).

knowledge of cell function and potential avenues for the treatment of human diseases. This is exactly the reason why pharmaceutical companies and independent laboratories have expended such effort and capital in the search for the keys to those locks, the ligands that interact with those so-called "orphan receptors."

How do researchers identify endogenous ligands for these orphan receptors? The classic approach is to search tissue extracts for substances that interact with the receptor protein. This approach is handicapped by the need for large amounts of tissue, extensive protein purification steps and laborious ligand-receptor binding assays. The development of modern, robotic-based screening assays provided hope for increased success in the search for these endogenous ligands, but in fact this approach has been disappointing. All the same, those robotic screening assays have become critically important only once a candidate, endogenous ligand has been identified.[4] These may be proteins recently discovered or hormones that have been known to exist and have significant biologic activity for years, for which a cognate receptor has not been identified.

Our laboratory group recently discovered two previously unrecognized endogenous peptide hormones, neuronostatin, and phoenixin.[5,6] After validating the gene sequences and proteins encoded, we identified tissue sites where they are produced, physiologically relevant changes in their production, and finally tissues where they exert actions both in vitro and in vivo. Those foundational studies identified multiple sites of action, suggesting important contributions of the two hormones in such diverse functions as glucose homeostasis, growth, autonomic function, appetite/thirst, fluid and electrolyte homeostasis, and reproduction. The challenge then was to determine which of the myriad pharmacologic actions of these two hormones were physiologically relevant. To accomplish this task, we either had to create an animal model in which the gene expressing the hormone was ablated or find a population of animals or humans in which the gene product was missing or improperly produced. The first approach was impossible for

several reasons. For neuronostatin, creation of a knockout animal by embryonic gene deletion would not be instructive because encoded in the same gene as neuronostatin (NST) is the potent growth restricting hormone somatostatin (SRIF). Gene compromise would negate production of both hormones. We had to develop a unique, alternative approach.

We knew that neuronostatin exerted pharmacologic effects unique from those of somatostatin and that it did not bind to the known somatostatin receptors.[5] Thus, if we could identify the cognate receptor for neuronostatin, we might be able to compromise the production of that receptor and determine the importance of the peptide's pharmacologic actions. We established in cell culture-based experiments that neuronostatin activates cell-signaling cascades typical of other GPCRs and thus made what proved to be a "correct guess." We hypothesized that neuronostatin signals via one of the 130 orphan GPCRs and then developed a novel strategy to address that hypothesis, "The Deductive Reasoning Strategy."[7] (Figure 1)

The Strategy, which was patented by Dr. Yosten, basically assumes that all cell types responding to neuronostatin will share at least one (and hopefully not too many) GPCR. Multiple cell types, in this case cardiac myocytes, pancreatic alpha cells, hypothalamic neurons and KATO III cells (a gastric tumor cell line), were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) for the presence of messenger RNA encoding all the orphan GPCRs.[7] Commonly expressed receptors were then compromised in vitro by the use of small interfering RNA molecules that blocked expression each of those candidate GPCRs, one at a time, and then those compromised cells tested for their response to neuronostatin. Initially, we had four candidate shared receptors, and we used the KATO III cells for the siRNA (small interfering RNA molecules that block translation selectively of the mRNA encoding the targeted receptor protein) compromise study because those cells are readily transfected with the siRNA constructs and their response to neuronostatin (increased early gene, c-fos, expression) was readily detectable. Using this approach, we identified GPR107 as the cognate receptor for neuronostatin, at least in cells in culture.[7] Then, our task was to demonstrate the consequence of compromising GPR107 expression in vivo on the pharmacologic actions of the peptide and on basic physiologic mechanisms thought to require neuronostatin, such as autonomic function and glucose metabolism.

When approaching the in vivo experiments, it was important to choose a tissue site of action of neuronostatin that was well validated and approachable in vivo. We previously identified several central nervous system actions of the peptide and felt that intracerebroventricular delivery of the compromising siRNA would have the advantage over peripheral administration of sequestrating the siRNA construct within the targeted tissue,with less dilution by the general circulation. Indeed, we showed that neuronostatin acts pharmacologically in brain to raise blood pressure by actions on vasopressin release and increases sympathetic nerve trafficking. Could this action be blocked by prior compromise of GPR107 expression? Indeed it was, not only the pharmacologic effects of the peptide, but also normal baroreflex function in response to lowering and raising peripheral mean arterial pressure with a vasodilator (sodium nitroprusside) and a vasoconstrictor (phenylephrine), respectively. Non-GPR107-mediated central control of autonomic function, in this case the role of angiotensin II, was not compromised, demonstrating specificity.

Thus, we demonstrated that neuronostatin is the key that unlocks the door guarded by GPR107. But where does this lead us in terms of developing therapeutics? That promise lies in another action of neuronostatin, one related to glucose homeostasis. Neuronostatin is produced, along with somatostatin, in delta cells of the pancreas. We demonstrated the ability of neuronostatin to inhibit glucose-stimulated insulin secretion, at least in part by an initial action on the neighboring alpha cells. Importantly,

Table 1. Ligand-receptor pairs that have been de-orphanized to date using this approach.

LIGAND/HORMONE	COGNATE RECEPTOR	POSSIBLE APPLICATIONS
Pro-insulin Connecting Peptide (C-Peptide)[8]	GPR146	Diabetes, vascular diseases
Phoenixin[6,9,10]	GPR173	Reproductive assist, fluid and electrolyte homeostasis
Adropin[11]	GRP19	Metabolic disorders, obesity
Neuronostatin[5]	GPR107	Metabolic disorders, diabetes
Cocaine- and amphetamine regulated transcript peptide (CARTp)[16,17]	GPR160	Pain management, appetite regulation, obesity

neuronostatin is able to stimulate glucagon expression and secretion from an alpha cell line and isolated whole islets. We are currently extending those initial cell culture experiments into whole animal studies, both rodent and human protocols, with the hypothesis that neuronostatin or a stable analog might be of value as a prophylactic agent to prevent insulin overdose-induced hypoglycemia or as an adjunctive therapeutic in diabetics suffering from hypoglycemia unawareness. In fact, those studies are now underway, both here in St. Louis and in collaborations with clinical investigators in Copenhagen.

Expanding Discovery with New Molecular Tools

How applicable is the Deductive Reasoning Strategy for the identification of other ligand-receptor pairs? The answer is quite simple: very. Table 1 demonstrates the ligand-receptor pairs we have de-orphanized to date using this approach.

A second problem encountered when using traditional gene compromise strategies for testing the importance of either a ligand or receptor is one of embryonic lethality. If either the ligand or the receptor is essential for placentation or embryonic development, disruption of that protein by traditional means (global embryonic deletion) will not work. This has led to the development of conditional gene compromise strategies, knockouts that can be induced during the postnatal period or adulthood. The approach has two additional advantages: tissue/site-specific gene compromise can be affected, and the compromise is permanent. For instance, in the case of neuronostatin, if the goal is to compromise peptide signaling just on alpha cells of the pancreas, in order to determine the physiological relevance of the glucagon-releasing action of the peptide, a transgenic rodent line could be created in which the gene encoding GPR107 is targeted for deletion only in that cell type. This would then allow for the interaction of neuronostatin and GPR107 in other tissues, or even in other cell types in the pancreatic islet, without any interruption. Genetic "tricks" also have been developed for "on/off" switching of select gene deletion and re-expression in specific tissue.

Taking Discovery to Therapeutic Development

The goal in developing these state-of-the-art genetic manipulations is to not only determine whether a ligand is essential for normal physiologic function, but also the culprit in pathologic conditions resulting from loss of function of the ligand/receptor pair. However, these approaches require the initial identification of the receptor that transmits the ligand/hormone signal. This brings us back to the discovery process itself. Even after our Deductive Reasoning Strategy has identified a ligand for one of the orphan GPCRS, much characterization work is required. Physical association of the ligand with the receptor needs to be proven and, if possible, the epitopes (amino acid signatures) important for binding

of ligand to receptor need to be identified. This would facilitate the development of stable, efficacious analogs as agonists or antagonists. Stability of the endogenous ligand, and any analogs developed, would have to be established and the pharmacokinetic properties of these molecules established. Development of effective analogs can be approached in two ways. First, knowledge of the amino acid sequence of protein ligands and the epitope essential for receptor binding would allow modifications to the amino acid backbone to be made to enhance binding affinity and avidity, and to prolong biologic half-life. An example of this would be the exendins, analogs of the mammalian hormone GLP-1, which are prescribed widely now for appetite and glucose metabolism regulation (e.g. liraglutide and dulaglutide).

A second approach, once a ligand/receptor pair has been identified and proven to be important in the regulation of distinct tissue functions, is the development of small molecules, usually organic in composition, with optimal binding characteristics (e.g. losartan). Numerous extensive libraries of small molecules have been assembled over the years in attempts to develop better therapeutics. Many of these molecules failed to meet the essential criteria in the projects for which they were developed, only later to be found to target a completely different class of receptors. These molecules have the advantage of ready synthesis and easily modified carbon backbones. Thus, a wide variety of modifications can be made following principles of intelligent design that result in enhanced efficacy with less off-target effects (e.g. the V2 antagonists, including tolvaptan). Often these small molecules display the added advantage of being orally active.

What's next? As our discovery work has demonstrated, there are probably numerous, endogenously expressed peptides/proteins yet to be discovered that have important, physiologically relevant actions. Once identified, their cognate receptors will have to be identified. Similarly, peptides that are produced in a variety of human tissues and whose pharmacologic profiles have long been known still hold promise for the development of novel, efficacious therapeutics. Perhaps the best, recent of example of this is the peptide encoded by the cocaine- and amphetamine-regulated transcript, CARTp. First identified by classical peptide chemistry approaches in the 1980s and later re-discovered by more modern gene cloning approaches, this peptide is a potent inhibitor of food intake and reward-based behaviors, and more recently was found to act pharmacologically to induce pain.[12,13,14,15,16] Progress in developing agonists for treating eating disorders/obesity and antagonists for treating peripheral neuropathies had been blocked by fact that, even after extensive efforts using conventional receptor identification approaches, no CARTp receptor had been identified. Because of the peptide's interesting pharmacology, we teamed with Dr. Daniela Salvemini to identify the CARTp receptor using our Deductive Receptor Matching Strategy, identifying GPR160 as the CARTp receptor.[16] Using a variety of biochemical and cellular approaches, we defined the signaling cascade activated by CARTp-GPR160 interactions, demonstrated the requirement of GPR160 for CARTp-induced signaling in vitro and in vivo. Even more importantly, we demonstrated that compromise of GPR160 expression in laminae 1-3 of the dorsal horn of the lumbar spinal cord blocks the development of spinal injury-induced neuropathy and reverses already-established neuropathy.[16] More recently we demonstrated that compromise of GPR160 signaling in brainstem structures important for gut-brain feedback and termination of meals results in exaggerated eating and drinking under physiologically relevant conditions and that the anorexigenic action of CARTp in that tissue is prevented by GPR160 compromise.[17]

This work has opened new avenues for the development of novel therapeutics to treat peripheral neuropathies, if antagonistic analogs or small molecules can be developed that can be applied regionally over the lumbar

spinal column. Those goals are currently being pursued in collaboration with Dr. Salvemini in the Department of Pharmacology and Physiology at SLU. In terms of the anorexigenic action of CARTp, we envision structure-function relationships to reveal avenues for the development of CARTp-like, GPR160 agonists devoid of peripheral actions that can be used to control eating disorders and compulsive behaviors.

It doesn't stop there. Our Deductive Receptor Matching Strategy is being applied to identify the cognate receptors for several other known peptide hormones. An excellent example of our current studies is a search for the cognate receptor for nesfatin, the most potent anorexigenic peptide yet described, for which, like CARTp, even after extensive efforts a unique receptor has yet to be identified.[18,19,20] And there are more hormone targets to be identified, promising not only novel insight into the mechanisms of health and disease, but also providing a great training environment for the next generation of researchers who will carry our vision forward.

References

1. Tang, X.L., Wang, Y., Li, D.L., Luo, J., Liu, M.Y., 2012. Orphan G protein-coupled receptors (GPCRs): biological functions and potential drug targets. Acta Pharmacol Sin 33(3):363-371.
2. Fang, Y., Kenakin, T., Liu, C., 2015. Editorial: Orphan GPCRs As Emerging Drug Targets. Front Pharmacol 6:295.
3. Venkatakrishnan, A.J., Deupi, X., Lebon, G., Tate, C.G., Schertler, G.F., Babu, M.M., 2013. Molecular signals of G protein-coupled receptors. Nature 494: 185-194.
4. Ngo, T., Kufareva, I., Coleman, L.J., Graham, R.M., Abagyan, R., Smith, N.J. 2016. Identifying ligands at orphan GPCRs: current status using structure-based approaches. Br J Pharmacol 173(20): 2934-2951.
5. Samson, W.K., Zhang, J.V., Avsian-Kretchmer, O., Cui, K., Yosten, G.L.C., Klein, C., Lyu, R.-M., Wang, Y.X., Chen, X.Q., Yang, J., Price, C.J., Hoyda, T.D., Ferguson, A.V., Yuan, X.B., Chang, J.K., Hsueh, A.J., 2008. Neuronostatin encoded by the somatostatin gene regulates neuronal, cardiovascular , and metabolic functions. J Biol Chem 283(46): 31949-31959.
6. Yosten, G.L., Lyu, R.M., Hsueh, A.J., Avsian-Kretchmer, O., Chang, J.K., Tullock, C.W., et al., 2013. A novel reproductive peptide, phoenixin. J Neuroendocrinol 25(2):206-215.
7. Yosten, G.L., Redlinger, L.J., Samson, W.K., 2012. Evidence for an interaction of neuronostatin with the orphan G protein-coupled receptor, GPR107. Am J Physiol Regul Integr Comp Physiol 303(9):R941-949.
8. Yosten, G.L.C., Kolar, G.R., Redlinger, L.J., Samson, W.K., 2013. Evidence for an interaction between pro-insulin C-peptide and GPR146. Journal of Endocrinology 218: B1-B8.
9. Gasparini, S., L.M. Stein, S.P. Loewen, C.J. Haddock, J. Soo, A.V. Ferguson, G.R. Kolar, G.L.C. Yosten, W.K., 2017. Samson. Novel regulator of vasopressin secretion: phoenixin. American Journal of Physiology Regul Integr Comp Physiol 314: R623-R628.
10. Haddock, C.J., Almeida-Pereira, G., Stein, L.M., Yosten, G.L.C., Samson, W.K., 2020. A novel regulator of thirst behavior: phoenixin. American Journal of Physiology Regul Integr Comp Physiol 318(6): R1027-1035.
11. Stein, L.M, Yosten, G.L.C. , Samson, W.K., 2016. Adropin acts in brain to inhibit water drinking: possible interaction with the orphan G protein-coupled receptor, GPR19. American Journal of Physiology Regul Integr Comp Physiol 310: R476-480.
12. Spiess, J., Villarreal, J., Vale, W., 1981. Isolation and sequence analysis of a somatostatin-like polypeptide from ovine hypothalamus. Biochemistry 20(7):1982-1988.
13. Douglass, J., McKinzie, A.A., Couceyro, P., 1995. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci 15(3 Pt 2):2471-2481.
14. Aja, S., Sahandy, S., Ladenheim, E.E., Schwartz, G.J., Moran, T.H., 2001. Intracerebroventricular CART peptide reduces food intake and alters motor behavior at a hindbrain site. Am J Physiol Regul Integr Comp Physiol 281(6):R1862-1867.
15. Rogge, G., Jones, D. Hubert, G.W., Lin, Y., Kuhar, M.J., 2008. CART peptides: regulators of body weight, reward and other functions. Nat Rev Neurosci 9(10): 747-758.
16. Yosten, G.L., Harada, C.M., Haddock, C.J., Giancotti, L.A., Kolar, G.R., Patel, R., Guo, C., Chen, Z., Zhang, J., Doyle, T.M., Dickenson, A.H., Samson, W.K., Salvemini, D., 2020. GPR160 de-orphanization reveals critical roles in neuropathic pain in rodents. J Clin Invest 130(5):2587-2592.
17. Haddock C.J., Almeida-Pereira, G., Stein, L.M., Hayes, M.R., Kolar, G.K., Samson, W.K., Yosten, G.L.C., 2020. Signaling in rat brainstem via Gpr160 is required for the anorexigenic and antidipsogenic actions of cocaine- and amphetamine-regulated transcript peptide. American Journal of Physiology Regul Integr Comp Physiol in press. doi.org:10.1152/ajpregu.00096.2020
18. Yosten, G.L.C., Samson, W.K., 2009. Nesfatin exerts cardiovascular actions in brain: possible interaction with the central melanocortin system. American Journal of Physiology Regul Integr Comp Physiol 297: R330-R336.
19. Yosten, G.L.C., Samson, W.K., 2010. The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin antagonist. American Journal of Physiology Regul Integr Comp Physiol 298: R1642-R1647.
20. Pate A.T., Schnell, A.L., Ennis, T.A., Samson, W.K., Yosten, G.L.C., 2019. Expression and function of nesfatin-1 are altered by stage of the estrous cycle. American Journal of Physiology Regul Integr Comp Physiol 317: R328-336.

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Novel, Neuroscience-Informed Approaches to Trauma Care in Community Clinical Settings

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Post-traumatic growth (PTG) refers to the positive, transformative psychological adaptation a patient can experience after experiencing trauma, helping one gain an understanding of both the trauma and its negative psychosocial impacts.



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Abstract

Trauma exposure, a highly prevalent condition in the United States, results in the development of chronic physical and mental health disabilities, such as diabetes, cardiovascular disease and posttraumatic stress disorder. Bench science advances in neurobiology and neuroscience have been translated into exciting clinical interventions, which have been shown to improve mental functioning and stress response. This article highlights three such interventions in hospital, primary care and community settings.

Introduction

Studies show that between 51 and 89 percent of Americans have experienced trauma at some point in their life.[1] Individuals have been trauma-exposed if they have an emotional response to a terrible event, such as an accident, rape, natural disaster, or combat. Fear, intrusive recall, pervasive avoidance, depression, hopelessness, and anxiety are all common forms of psychological distress that accompany the physical pain and discomfort of trauma.[2] These psychological stressors can lead to the development of chronic mental health issues, including posttraumatic stress disorder (PTSD). This negative feedback loop can also lead to poor overall health outcomes, such as diabetes and cardiovascular disease, and interfere with crucial components of recovery and posttraumatic growth, such as physical therapy, social reintegration and a return to a productive life.[3]

Post-traumatic growth (PTG) refers to the positive, transformative psychological adaptation a patient can experience after experiencing trauma, helping one gain an understanding of both the trauma and its negative psychosocial impacts. Experiencing high levels of PTG can lead to a reduction in many of the psychosocial stressors associated with trauma, allowing patients to focus energy on thriving and having greater chance at a successful recovery.[4]

The Neurobiology of Trauma

Individuals with PTSD have substantial limitations when it comes to distinguishing between what is safe and what is not.[5] The reason for this is that a set of chemical changes are set in motion in the brain and body when trauma occurs. These are normal processes that occur as a result of threat, but when the threat is very severe, the processes go haywire. For instance, when an individual is a victim of an assault, epinephrine (adrenalin) or norepinephrine floods his/her

brain and gives that person a rush of energy via the sympathetic nervous system (SNS). This can cause a startle response, queasiness (stomach), and heightened blood pressure so the person is able to survive by fighting or fleeing the offender.[6] In addition, there is a release of cortisol (referred to as the hypothalamic-pituitary-adrenal [HPA] response), a naturally occurring steroid that reduces the inflammatory response, which also encourages survival, and beta-endorphins to reduce whatever pain the individual may feel. The sheer amount of these chemicals has a negative effect after the trauma occurs, however, because it affects memory and the capacity to learn and focus attention. It also encourages defensive responses across different situations. This leads to an inability to interpret different sensory inputs, high levels of anxiety, irritability, and hypervigilance. In other words, trauma-exposed individuals can become chronically hyper-aroused because their bodies are giving them the wrong messages about threat.

Physical pain resulting from traumatic injuries and distress are also mutually maintaining, with the heightened arousal causing muscles to become more tense and pain more intense. Increased levels of pain then act as a reminder of the traumatic events, intensifying the brains' response and making pain even worse.[2] This Cascade Model of PTSD[6] results in chronic mental health and behavioral consequences, such as increased stress-related discomfort, prolonged poor quality of life, and substance abuse.[1,2,7,8]

Current advances in translational science have used many of these important neuroscience findings to develop clinical interventions that correspond with existing limitations in short term memory, sensory input, anxiety, and hyperarousal on the part of individuals with PTSD. The following section reviews some of the research being done by members of the Henry and Amelia Nasrallah Center for Neuroscience (NCfN) at Saint Louis University (SLU) with trauma survivors in clinical settings.

Treating Severely Traumatized Patients in Hospital Outpatient Settings

Gun violence was responsible for over 15,000 deaths and 30,000 injuries in 2019 in the U.S., with gun-related deaths in Missouri ranking fifth in the country per capita and homicide being the leading cause of death for young people between the ages of 15 and 44 in St. Louis (https://giffords.org/). When individuals have been shot, they are generally transported to and treated in the hospital emergency department, transferred to an acute trauma care floor, and then discharged to post-acute care (inpatient rehabilitation, skilled nursing facility, or home) based on need, and additional follow-up medical care in an outpatient trauma clinic. Trauma-focused cognitive behavioral therapy (CBT) can be implemented as a first-line intervention for individuals experiencing the negative mental and behavioral health effects of gun violence trauma in these settings, which do not normally provide mental health services. Narrative exposure therapy (NET) is one form of CBT that has been gaining evidence for efficacy in reducing anxiety symptoms and hyperarousal in trauma survivors.[9] The SLU Oberle Institute has developed a grant-funded pilot program to further test this approach as part of their outpatient trauma services.

Neuroscientific research that focuses on physiological responses to memory informed the development of NET by incorporating an autobiographical-memory theoretical framework for understanding how intrusive symptoms and fear networks are activated in the brain.[10] Over the course of several NET sessions, participants are guided through a therapeutic process that encourages them to share a testimony of their traumatic experiences within a more general lifespan narrative that includes both positive and negative memories and thoughts on the future.

From a neurobiological perspective, NET provides a safe environment to recall the contextual information of traumatic "cold memories" associated with an event and the linked "hot memories" or fear structures, which consist of the sensory, cognitive and emotional perceptual information and physiological and motor responses associated with that same traumatic event. Flashbacks, a prime symptom of PTSD, are the result of all the cold and hot memories of the traumatic event being activated when the survivor encounters just one internal or external stimulus associated with the fear structure surrounding that event. PTSD causes the same SNS and HPA responses that become active when experiencing a traumatic event to become active and re-experienced during flashbacks, ultimately hindering the functioning of HPA. This increases the number and strength of associations between triggering cues, making it easier for traumatic memory to be activated. The negative effect of PTSD on the HPA response

also restricts memories from incorporating contextual spatio-temporal information, which makes recall of the event less coherent and more prone to activating the fear structure. The autobiographical nature of NET helps one reduce the sense of threat associated with hot memories by anchoring them to the spatio-temporal information of that individual's life. Continually sharing traumatic memories within the larger context of a life narrative with another individual helps one create a coherent lifeline that provides meaning to the recollection of traumatic events. It also allows new, non-traumatic information to become part of memory recall. This can enhance emotional processing of the traumatic event by helping to reduce the negative reinforcement of avoidance thoughts and behaviors. It can also improve emotional processing, reducing fear and anxiety associated with the SNS response and structuring traumatic memories in a way that changes the pathological associations surrounding them. This helps survivors understand that traumatic memories are distinct from the traumatic event itself, allowing recall to occur more safely and in a controlled manner.[9] Studies of NET and its effect on PTSD have focused almost exclusively on populations facing violence in or from developing countries. This pilot represents a novel approach to treating victims of violence in the U.S.

Treating Trauma Exposed Patients in Primary Care Settings

Studies show that higher lifetime trauma leads to impulsivity,[11] and a combination of the trauma and impulsivity are likely associated with a number of different behaviors such as fiscal imprudence,[12] substance use disorders (SUDs)[8,13,14] and other health impairing habits of behavior and lifestyle like reckless driving,[15] physical inactivity, poor diet,[16] risky sexual behavior,[17] medical non-adherence,[1] self-mutilation,[11] suicidality,[18] etc. Studies of delayed reward discounting (DRD), a category of impulsivity that is implicated in these behaviors, usually focus on SUDs and rarely consider trauma as a predictor of discounting. This is a particular concern among physicians in primary care settings where there is a need to negotiate with patients about decision-making related to health behaviors that contribute to chronic disease outcomes.

Meanwhile, neuroscience research has shown that trauma exposure may be an important predictor of discounting behavior. When high chronic stress occurs, such as traumatic stress, the amygdala's circuitry reacts by promoting defensive responding,[19]and there is increasing monoaninergic release that engages low affinity NA alpha1, dopaminergic (DA1), and serotonergic (5-HT2A) receptors, thereby interfereing with working memory.[20] Working memory acts as a temporary store for mental representations that are relevant to any decision or task that is currently taking place. It also sustains relevant information that can be retrieved from other areas of the brain during active processing. Several studies show a relationship between working memory and a measure of discounting.[21]

A behavioral economic framework called the Competing Neurobehavioral Decision System[22] is now being increasingly used to explain health-behavior decisions. These decisions are thought to be influenced by an impulsive decision-making system that leads individuals to choose immediate reinforcements, i.e., they "discount," and an executive decision system (i.e. working memory and other relevant areas of the brain) that leads individuals to inhibit impulses in favor of long-term rewards, i.e., they delay gratification.[22] Trauma-exposed patients' existing chronic stress and impaired cognitive functioning weakens these systems, causing them to discount (select smaller, short-term rewards over larger, long-term rewards).[23-25] This novel framework could have broad implications for how the medical community deals with trauma-exposed patients. Researchers affiliated with the SLUCare Physician Group, ARCHNet and SLU have already begun much of this important research.[23-29]

Also, important translational work prompted by researchers at Virginia Tech's Addiction Recovery Research Center has resulted in a number of interventions that can be used in primary care settings.[22] These interventions include modified behavioral economic approaches to motivational interviewing and mindful attention, choice bundling/bracketing (patient asked to make a series of decisions rather than one discrete decision), episodic future thinking (projecting self into plausible future scenarios), working memory training, contingency management and contracting, which seek to alter how people structure choices.[30]

One such intervention, episodic future thinking, has shown to be the most effective in reducing discounting in laboratory research.[31] Because sessions of episodic future thinking are very brief, they have great potential for primary care because they involve prompting individuals to develop goals and generate

positive cues about the future over pre-determined periods of time, which can be used by primary care providers and care teams to revisit during check-ups. In addition, episodic future thinking interventions also aid what can become negative thinking about the future, which is common among trauma-exposed patients.[32] Like NET, which was introduced in the previous section, episodic future thinking interventions also are used to improve patients' ability to establish narratives – and these narratives help with coping and behaviors that require decisions between short and long term rewards. This has led to the development of a research protocol to test an episodic future thinking intervention at SLUCare. This intervention would essentially focus on whether episodic future thinking reduces not just discounting, but also chronic disease outcomes (in this case chronic disease outcomes related to diabetes).[33]

University-Community Partnership To Address Trauma-Related Cognitive-Communicative Decline

Traumatic Brain Injuries (TBIs) are prevalent in older African Americans of low socio-economic status, most commonly caused by falls.[34] The presence of a TBI increases risk for dementia.[35-37] Given the overwhelming evidence that minority elders experience greater risk but inadequate and frequently delayed detection of dementia, this group experiences increased numbers of preventable hospitalizations, and higher healthcare cost burden.[38,39] Therefore, culturally competent strategies are necessary for reducing health disparities that systematically and disproportionately affect underserved elderly African Americans with signs and symptoms of cognitive-communicative decline. The following paragraphs describe one such strategy.

In 2017, the SLU School of Medicine's Geriatric Workforce Enhancement Program partnered with CareSTL Health and Northside Youth and Senior Service Center, Inc., two community health centers serving minority and economically disadvantaged residents of North St. Louis, to establish the Senior Social Group For Brain Health As We Age. The NCfN provided substantial support for the group, which was operational until the onset of the COVID-19 pandemic. As indicated by the group name, sessions focused upon aging brain health issues, including trauma-related topics such as prevention and management of TBIs. The group of 10 to 12 community-dwelling African American elders was led by a cognitive neuroscientist from SLU with clinical credentials in speech-language pathology (WP), in collaboration with the medical, nursing, and behavioral health staff of CareSTL Health and Northside.

Weekly gatherings were conducted in the North St. Louis neighborhood of The Ville, on the hallowed grounds of the former Homer G. Phillips Hospital (HGPH). Opened in 1937, HGPH welcomed African American patients and was the premiere medical training center for African American healthcare professionals before its forcible closing in 1979.[40] Later. CareSTL Health transformed a corner of the HGPH building complex into a day health center; group participants gathered at this historic site for weekly sessions. As a familiar and cherished symbol of this African American community's response to racial health disparities, it was conducive to candid conversations about health that inspired participants' revelations of personal experiences of trauma.

Culturally responsive activities were led with input and approval from participants and community health center staff to sensitively address the subject of TBIs resulting from falls, vehicular accidents, gun-shot wounds and domestic violence, among other causes experienced by group participants.[40-42] Their learning of trauma-related concepts was promoted by introducing functional phrases and terms such as "chronic traumatic encephalopathy" (and the acronym "CTE"), "cognitive reserve" and "concussion" in guided group discussions based on collective readings of lay-friendly materials by the National Institute on Aging, the Alzheimer's Association and the Centers for Disease Control and Prevention, among other authoritative sources. These take-home materials in the forms of handouts, brochures and booklets were distributed to participants as accessible and appealing external memory aids that could be shared with family and professional caregivers.

Because falls are a leading cause of trauma-related hospitalizations among the elderly and are especially common among low-income African American elders, we dedicated multiple sessions to falls prevention (i.e., http://www.cdc/gov/homeandrecreationalsafety/falls). Participants' learning about dementia risk associated with TBI from falls and other causes was assessed and consolidated through group games which tested pre-training knowledge, such as Brain Health Jeopardy, Brain Health Trivia Tournament, and word-

search contests. Participants reported that their newly acquired actionable knowledge about trauma enhanced their interactions with health professionals, family caregivers and peers, and contributed to their sense of control over their health and quality of life as they age.

Conclusion

The commitment to improving the lives of individuals by translating groundbreaking bench science to novel approaches to health care is paramount as the NCFN continues to gain prominence in academia and academic medicine as a leading Center of Excellence. The three examples of trauma care in community clinical settings described in this article represent how members of the NCfN are fulfilling this imperative and working to improve care across the continuum using a neuroscience-informed approach.

References

1. Kronish IM, Edmondson D, Li Y, Cohen BE. Post-traumatic stress disorder and medication adherence: results from the Mind Your Heart study. J Psychiatr Res. 2012;46(12):1595-1599.
2. Leserman J, Whetten K, Lowe K, Stangl D, Swartz MS, Thielman NM. How trauma, recent stressful events, and PTSD affect functional health status and health utilization in HIV-infected patients in the south. Psychosom Med. 2005;67(3):500-507.
3. Mitchell KS, Aiello AE, Galea S, Uddin M, Wildman D, Koenen KC. PTSD and obesity in the Detroit neighborhood health study. Gen Hosp Psychiatry. 2013;35(6):671-673.
4. Mark KM, Stevelink SAM, Choi J, Fear NT. Post-traumatic growth in the military: a systematic review. Occup Environ Med. 2018;75(12):904-915.
5. James LM, Strom TQ, Leskela J. Risk-taking behaviors and impulsivity among veterans with and without PTSD and mild TBI. Mil Med. 2014;179(4):357-363.
6. Davidson JR. Long-term treatment and prevention of posttraumatic stress disorder. J Clin Psychiatry. 2004;65 Suppl 1:44-48.
7. Hall KS, Hoerster KD, Yancy WS, Jr. Post-traumatic stress disorder, physical activity, and eating behaviors. Epidemiol Rev. 2015;37:103-115.
8. Jacobsen LK, Southwick SM, Kosten TR. Substance use disorders in patients with posttraumatic stress disorder: a review of the literature. Am J Psychiatry. 2001;158(8):1184-1190.
9. Neuner F, Schauer M, Klaschik C, Karunakara U, Elbert T. A comparison of narrative exposure therapy, supportive counseling, and psychoeducation for treating posttraumatic stress disorder in an african refugee settlement. Journal of consulting and clinical psychology. 2004;72(4):579.
10. Robjant K, Fazel M. The emerging evidence for Narrative Exposure Therapy: a review. Clin Psychol Rev. 2010;30(8):1030-1039.
11. Zlotnick C, Shea MT, Recupero P, Bidadi K, Pearlstein T, Brown P. Trauma, dissociation, impulsivity, and self-mutilation among substance abuse patients. Am J Orthopsychiatry. 1997;67(4):650-654.
12. Henegar JM, Archuleta K, Grable J, Britt S, Anderson N, Dale A. Credit card behavior as a function of impulsivity and mother's socialization factors. Journal of Financial Counseling and Planning. 2013;24(2):37.
13. Bickel W, Odum A, Madden G. Impulsivity and cigarette smoking: Delay discounting in current, never, and ex-smokers. Psychopharmacology. 1999;146(4):447-454.
14. Madden G, Petry N, Badger G, Bickel W. Impulsive and self-control choices in opiod-dependent patients and non-drug-using control participants: Drug and money rewards. Experimental and Clinical Psychopharmacology. 1997;5(3):256-262.
15. Bıçaksız P, Özkan T. Impulsivity and driver behaviors, offences and accident involvement: A systematic review. Transportation research part F: traffic psychology and behaviour. 2016;38:194-223.
16. Thamotharan S, Lange K, Zale EL, Huffhines L, Fields S. The role of impulsivity in pediatric obesity and weight status: a meta-analytic review. Clin Psychol Rev. 2013;33(2):253-262.
17. Moore AA, Overstreet C, Kendler KS, Dick DM, Adkins A, Amstadter AB. Potentially Traumatic Events, Personality, and Risky Sexual Behavior in Undergraduate College Students. Psychol Trauma. 2016.
18. Krysinska K, Lester D. Post-traumatic stress disorder and suicide risk: a systematic review. Arch Suicide Res. 2010;14(1):1-23.
19. Martijena ID, Rodriguez Manzanares PA, Lacerra C, Molina VA. Gabaergic modulation of the stress response in frontal cortex and amygdala. Synapse. 2002;45(2):86-94.
20. Robbins TW, Arnsten AF. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annu Rev Neurosci. 2009;32:267-287.
21. Finn PR, Gunn RL, Gerst KR. The Effects of a Working Memory Load on Delay Discounting in Those with Externalizing Psychopathology. Clinical psychological science : a journal of the Association for Psychological Science. 2015;3(2):202-214.
22. Bickel W. A competing neurobehavioral decision system model of SES-related health and behavioral disparities. Preventitive Medicine (in press). 2014.
23. Van den Berk-Clark C, Patterson Silver Wolf D. Mental Health Help Seeking Among Traumatized Individuals: A Systematic Review of Studies Assessing the Role of Substance Use and Abuse. Trauma, violence & abuse. 2017;18(1):106-116.
24. Van den Berk Clark C. The role of impulsivity on health behavior related to cardiovascular disease among young adults. Psychological Trauma: Theory, Research, Practice, and Policy. 2020.
25. Van Den Berk-Clark C, Myerson J, Green L, Grucza RA. Past trauma and future choices: differences in discounting in low-income, urban African Americans. Psychological medicine. 2018;48(16):2702-2709.
26. Van Den Berk-Clark C, Balan S, Shroff MV, Widner G, Price RK. The impact of hazardous alcohol use on behavioral healthcare utilization among National Guard service members. Substance use & misuse. 2016;51(5):625-636.
27. Van den Berk-Clark C, Secrest S, Walls J, et al. Association between posttraumatic stress disorder and lack of exercise, poor diet, obesity, and co-occuring smoking: A systematic review and meta-analysis. Health Psychology. 2018;37(5):407.
28. Scherrer JF, Salas J, Norman SB, et al. Association Between Clinically Meaningful Posttraumatic Stress Disorder Improvement and Risk of Type 2 Diabetes. JAMA Psychiatry. 2019;76(11):1159-1166.
29. Scherrer JF, Salas J, Schneider FD, et al. PTSD improvement and incident cardiovascular disease in more than 1000 veterans. J Psychosom Res. 2020;134:110128.
30. Ashe ML, Newman MG, Wilson SJ. Delay discounting and the use of mindful attention versus distraction in the treatment of drug addiction: a conceptual review. J Exp Anal Behav. 2015;103(1):234-248.
31. Stein JS, Wilson AG, Koffarnus MN, Daniel TO, Epstein LH, Bickel WK. Unstuck in time: episodic future thinking reduces delay discounting and cigarette smoking. Psychopharmacology (Berl). 2016.
32. Zimbardo P, Sword R, Sword R. The time cure: Overcoming PTSD with the new psychology of time perspective therapy. John Wiley & Sons; 2012.
33. Epstein LH, Paluch RA, Stein JS, et al. Role of delay discounting in predicting change in HBA1c for individuals with prediabetes. Journal of behavioral medicine. 2019;42(5):851-859.
34. Kisser J, Waldstein SR, Evans MK, Zonderman AB. Lifetime prevalence of traumatic brain injury in a demographically diverse community sample. Brain Inj. 2017;31(5):620-623.
35. Crane PK, Gibbons LE, Dams-O'Connor K, et al. Association of Traumatic Brain Injury With Late-Life Neurodegenerative Conditions and Neuropathologic Findings. JAMA Neurol. 2016;73(9):1062-1069.
36. Livingston G, Huntley J, Sommerlad A, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396(10248):413-446.
37. Tripodis Y, Alosco ML, Zirogiannis N, et al. The Effect of Traumatic Brain Injury History with Loss of Consciousness on Rate of Cognitive Decline Among Older Adults with Normal Cognition and Alzheimer's Disease Dementia. J Alzheimers Dis. 2017;59(1):251-263.
38. Chin AL, Negash S, Hamilton R. Diversity and disparity in dementia: the impact of ethnoracial differences in Alzheimer disease. Alzheimer Dis Assoc Disord. 2011;25(3):187-195.
39. Glymour MM, Manly JJ. Lifecourse social conditions and racial and ethnic patterns of cognitive aging. Neuropsychol Rev. 2008;18(3):223-254.
40. Dilworth-Anderson P, Pierre G, Hilliard TS. Social justice, health disparities, and culture in the care of the elderly. J Law Med Ethics. 2012;40(1):26-32.
41. Fortune DA, Lang R, Cook S, Byrd GS. African Americans and Alzheimer's Disease: Role of Health Educators in Addressing this Silent Epidemic. American Journal of Health Studies. 2013;28(3).
42. Harris JL, Fleming VB. Toward model-driven interventions for African Americans with cognitive-communicative disorders. Paper presented at: Seminars in speech and language2009.

Disclosure

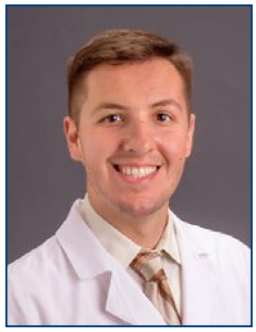
None reported.

MM

Plastic Bronchitis in Adult and Pediatric Patients: A Review of its Presentation, Diagnosis, and Treatment

by Niel Patel, BS, Mayur Patel, BS, Ravali Inja, BS, Armin Krvavac, MD & Andrew J. Lechner, PhD

Given the broad range of PB etiologies, the most direct common pathway leading to the formation of casts is yet to be determined, and indeed may not exist.



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Abstract

Purpose of Study

Plastic Bronchitis (PB) is a rare pulmonary condition characterized by the presence of casts in the trachea or bronchial tree. While there are many individual cases reported in pediatric and adult populations, no thorough reviews of pediatric and adult cases of PB exist in the literature. The purpose of this article is to conduct a comprehensive review of PB regarding presentation, diagnosis, pathophysiology, and treatments.

Etiology

In the pediatric population, PB can be attributed to pediatric cardiothoracic surgeries such as Fontan procedures, infections, inflammatory processes, acute chest syndrome, or iatrogenic processes. In the adult population, PB can be idiopathic or due to infections, anatomic variations in lymphatic vessels, surgeries, medications, or other comorbidities.

Pathophysiology

The pathophysiology of PB is still widely unknown; however, associations with inflammatory diseases and cardiac surgery have been proposed. There are two types of cast formations found in plastic bronchitis: Type I casts are associated with inflammatory diseases and Type II casts are associated with surgical procedures.

Treatment

Historically, PB has been treated by a variety of pharmacological methods including the use of corticosteroids and mucolytics. Recently, the treatment paradigm has shifted towards procedures such as lymphatic embolization, duct ligation, and stent grafting.

Conclusions

The information available regarding PB is still sparse, hence future research is necessary for further understanding of the disease. Due to its numerous presentations and disease associations, awareness of plastic bronchitis, and its treatment options is essential for primary care providers and respiratory specialists.

Introduction

Plastic bronchitis (PB), also known as cast bronchitis, is a rare respiratory condition consisting of branching intrabronchial cast formation that leads to the blockage of distal and intermediate airways. Patients presenting with PB may show signs of productive cough,

Table 1: Plastic Bronchitis Etiologies

Adults (≥ 18)	Pediatrics (<18)
Anatomic • Thoracic Duct Stenosis[21] • Lymph flow variation[22] • Thoracic Duct Duplication[23]	Acute Chest Syndrome[10] • Endobronchial Injury
Idiopathic[17-18]	Iatrogenic[45] • Prolonged chest tube drainage • Chylothorax • Formation of cardiopulmonary collateral vessels
Infectious • H1N1 Influenza[24] • AIDS/Kaposi Sarcoma[25]	Infectious • Adenovirus[11] • Adenovirus Serotype 7[12] • Mycoplasma pneumonia[13] • Influenza B[14]
Miscellaneous • ACE Inhibitors[28] • Silicosis[26] • Beta Thalassemia Minor[27]	Inflammatory Processes • Atopy or Asthma[16] • Idiopathic Chronic Eosinophilic Pneumonia[15]
Surgery • Coronary Artery bypass graft[19] • Bilateral Lung Transplantation[20] • Fontan procedure; adult onset[8]	Surgery[6] • Fontan procedure • Glenn Shunt • Tetralogy of Fallot repair • Arterial switch operation

Table 1: Shown above is a comparison of the variety of causes of plastic bronchitis between adult and pediatric populations.

dyspnea, fever, or pleuritic chest pain. Due to the rarity of the disorder, the true prevalence of PB is unknown.

The majority of pediatric cases of PB are associated with surgical correction of congenital heart disease, although many other etiologies have been identified. In pediatric populations, most causes of plastic bronchitis are slightly more common in males, excepting allergic causes in which females predominate.[1]

Though more prevalent overall in children, many cases of plastic bronchitis have been described in adults. From idiopathic cases to abnormal lymphatic circulation, case reports within the literature describe a similarly wide variety of etiologies of plastic bronchitis in adults.

Despite its rarity, plastic bronchitis presents with common and persistent symptoms that can result in severe respiratory complication such as respiratory failure and death.[2,3]

In addition to pharmacological treatment options, several novel surgical procedures have been recently developed that completely alleviate patients' PB-associated symptoms. Considering its occurrence in patients of all ages and potential for severe but treatable respiratory failure, plastic bronchitis is a condition of which clinicians should be aware.

Within the current literature, no comprehensive review of plastic bronchitis in adult and pediatric populations exists. The purposes of this discussion are to illuminate differences in etiology between the two patient populations, present the current understanding of the pathophysiology causing cast formation, and discuss emerging treatment options for management, ultimately providing clinicians with a framework for identifying and treating plastic bronchitis.

Due to the multiple names describing plastic bronchitis, the literature search for this review

Table 2: Plastic Bronchitis Case Studies in Pediatric Patients

Study	Number of Patients	Mean Age (years)	Diagnostic Procedures	Etiology	Treatment
Singhi et al.[6]	1	5	Expectorated mucus casts and Histologic examination of casts	Fontan procedure	Pulmonary vasodilators
Grutter et al.[7]	4	2.6 - 4	Bronchoscopy and Histologic examination of casts	Fontan Procedure	Sildenafil, Bosentan, Inhaled steroids, Aerosolized tPA, Nebulized N-acetylcysteine
Feray et al.[10]	6	2 - 18	Chest X-ray, Chest CT, and Bronchoscopy	Sickle cell disease	Aerosolized mucolytic agents and 2-mercapto-ethane sulfonate and N-acetylcysteine
Lu et al.[11]	1	3	Chest X-ray, Chest CT. Bronchoscopy, and Bronchoalveolar lavage	Adenovirus infection	Direct extraction of the cast during bronchoscopy
Kirito et al.[14]	3	8	Chest X-ray, Chest CT, and Bronchoscopy	Influenza B infection	Direct extraction of casts, IV peramivir, glucocorticoid
Hussain et al.[15]	1	15	Chest X-ray, Chest CT, Bronchoscopy, Fine needle biopsy of casts, and Bronchoalveolar lavage	Eosinophilic lung disease	Fluticasone, Albuterol, Mucomyst, 7% hypertonic saline, chest physiotherapy, Azithromycin, and direct extraction of casts
Chhawchharia et al.[16]	1	9 months	Chest X-ray. Chest CT, and Bronchoscopy	Dander (buffalo and cow) hypersensitivity	Salbutamol, Nebulized ipratropium bromide, Glucocorticoids,3% saline with chest physiotherapy, and Oral montelukast

Table 2: Shown above is a summary of the diagnostic procedures, etiology, and treatment of select pediatric cases of plastic bronchitis.

examined the key words "plastic bronchitis,", "Hoffman bronchitis," and "cast bronchitis" on PubMed and included only the English literature. Articles were differentiated between pediatric and adult cases by age, with patients over 18 years considered as adults.

Etiology

While the etiology of plastic bronchitis (PB) is poorly described, there are multiple reports that provide insight on the presentation of PB in pediatric and adult populations. Emerging cases of PB have revealed many causes and likely pathologies of this rare disease, with only some shared etiologies of PB between the two populations. A summary of plastic bronchitis etiologies in depicted in Table 1.

The most detailed cases of PB in the pediatric population are from surgical corrections of congenital heart diseases and other cardiac procedures. For instance, the Fontan procedure may lead to PB in about 4% of patients.[4]

In these cases, cast formation may be related to the development of an abnormal lymphatic physiology (called Fontan physiology) and inflammation.[5,6]

Plastic bronchitis may also be observed in pediatric patients after undergoing a Glenn shunt (hemi-Fontan) procedure, Tetralogy of Fallot repair, arterial switch operation, and other cardiac surgeries.[6,7] The time of PB presentation after surgery may vary widely, as patients can present with casts within two months or several decades after surgery.[6,8,9]

Aside from cardiac surgeries, cast formations have been described in patients with sickle cell disease. In a report by Feray,[10] bronchial casts were found in six

children and one young adult with homozygous sickle cell disease presenting with acute chest syndrome. In that report, one patient developed severe acute respiratory distress syndrome due to airway obstruction by voluminous casts occluding the left main bronchus.[10]

PB cases have also been reported in pediatric patients with bacterial and viral infections. For example, a three-year-old male patient presented with cast occlusion of his left lower bronchus, fever and cough. Bronchoalveolar lavage (BAL) fluid analyzed with immunofluorescence confirmed an adenovirus B1 infection.[11]

Bronchial cast formation also seems to be a plausible scenario in patients with adenovirus serotype 7 infections. In one report, two children with adenovirus serotype 7 infection presented with a high fever, cough, dyspnea, and bronchial casts in the left lower lobe.[12] Rarely, casts have been described in patients with atypical bacterial infections, particularly with Mycoplasma pneumoniae.[13] Similarly, additional cases of PB were described in children infected with the influenza B virus, although some of these patients had various underlying diseases such as asthma and nephrotic syndrome. However, all of these patients developed casts within one week after onset of the influenza infection (1 to 6 days after onset).[14]

Therefore, influenza B should also be considered as a potential cause of cast formation in children with PB.

Lastly, PB in children may be secondary to inflammatory processes such as asthma and idiopathic chronic eosinophilic pneumonia. One report describes a 15-year-old male with pulmonary eosinophilia complicated by bronchiectasis and left upper lobe casts. BAL fluid analysis from the left upper and lower lobes revealed eosinophilia and Charcot-Leyden crystals.[15] A similar pathophysiology was also noted in a nine-month-old male with type II pneumocyte dysfunction, which was suggested to occur from a hypersensitivity reaction to animal dander.[16] A summary of select pediatric cases is further described in Table 2.

Unlike PB in children, cast formation in adults is usually idiopathic. When etiologies can be identified, they have an infectious origin, or are associated with cardiopulmonary procedures, anatomic variations in lymphatic flow, medications, or environmental exposures. Casts in adults have been found to vary in shape, size, location, and symptom presentation.[17,18]

Although many cases can be ascribed as idiopathic, PB in adults can rarely present as a complication of thoracic surgeries. One report describes a 54-year-old male with non-ST-elevation myocardial infarction required a three-vessel coronary artery bypass that developed acute respiratory distress four hours after surgery. Bronchoscopy revealed a cast at the level of the carina, possibly occurring because of an acute inflammatory response in the post-operative period.[19] In another case, a 32-year-old male underwent bilateral lung transplantation and developed bronchial casts in the left lower lobe on post-operative day one and day three.[20]

Abnormal lymphatic flow because of anatomic variations or pathologies may explain the appearance of PB in some adults. In a 42-year-old male presenting with bronchial casts, a diagnostic lymphangiogram revealed thoracic duct stenosis which caused abnormal lymph flow in lymphatic collateral vessels in the supraclavicular region.[21] In another case, a 35-year-old pregnant female developed casts because of an abnormal thoracic duct anatomy, and a lymphangiogram revealed the thoracic duct making a "hairpin" turn at the left supraclavicular region and emptying aberrantly into the left pulmonary hilum. In the same patient, another duct was present which separated from the main thoracic duct and emptied into the right hilum.[22] Lastly, a 32-year-old female developed three bronchial casts, and her lymphangiogram revealed duplication of the thoracic duct with a smaller duct on the left side, a larger duct of the right, and other small tributary vessels below the carina, collectively compromising lymphatic flow.[23]

Similar to the etiology of PB in children, adults can present with casts formation as a result of pulmonary infection. A 55-year-old male infected with H1N1 influenza A presented with cardiac arrest and respiratory failure. After undergoing bronchoscopy, bloody casts were found in the right main bronchus. In this patient, Okada et al. proposed that anticoagulation and infection exacerbated the patient's symptoms and led to the formation of bronchial casts.[24] AIDS and Kaposi Sarcoma, an angioproliferative disorder, have led to the formation of casts as well. For example, in a 25-year-old male with HIV, a large cast was found at the carina that extended to occlude the right main bronchus. It is suspected that these casts formed as a consequence of lymphatic leakage and stasis secondary to Kaposi Sarcoma.[25]

Lastly, PB in adults may have miscellaneous causes including environmental exposures, drug reaction, and

Table 3: Plastic Bronchitis Case Studies in Adult Patients

Study	Number of Patients	Mean Age (years)	Diagnostic Procedures	Etiology	Treatment
Jett et al.[18]	1	40	Chest X-ray, Bronchoscopy, Transbronchial biopsy, and Histologic examination of casts	Idiopathic	Corticosteroids, Nebulized Acetylcysteine, Albuterol
Eberlein et al.[20]	1	32	Bronchoscopy	Bilateral lung transplantation	Direct extraction of casts
Albitar et al.[21]	1	42	Bronchoscopy, Bronchoalveolar lavage, and Diagnostic lymphangiogram	Thoracic duct stenosis	Thoracic duct embolization
Stoddart et al.[23]	1	32	Bronchoscopy, Bronchoalveolar lavage, and Diagnostic lymphangiogram	Abnormal lymphatic drainage-duplication of the thoracic duct	Thoracic duct ligation and removal of subcarinal lymphatics
Okada et al.[24]	1	55	Chest X-ray. Chest CT, Bronchoscopy, and Histological examination of casts	H1N1 infection	Direct extraction of casts
Habib et al.[25]	1	25	Chest CT, Bronchoscopy and Histological examination of casts, Diagnostic Lymphangiogram, and Lymphoscintigraphy	AIDS associated Kaposi Sarcoma	Failed trial of nebulized dornase alpha, chest physiotherapy, Ipratropium, Albuterol
Watanabe et al.[26]	1	48	Chest CT, MRI, and Pulmonary angiography	Silicosis	Thoracoscopic resection of lymph node
Plumacher et al.[28]	1	26	Chest CT, Histologic and microbiologic analysis of the casts	ACE inhibitor-Ramipril	Switching from ramipril to candesartan

Table 3: Shown above is a summary of select adult cases of plastic bronchitis and associated diagnostic procedures, etiologies, and treatments.

genetic predispositions. One case describes a 48-year-old male with lymphadenopathy and a right middle lobe cast, in whom resection of lymph nodes revealed lymphadenopathy due to silicosis that may have impaired lymphatic flow.[26] PB was also reported in a 33-year-old female with beta thalassemia minor.[27]

Bronchial casts were also noted in a 26-year-old female patient with congestive heart failure who had been treated with ramipril, an angiotensin converting enzyme-inhibitor (ACE-I), which was hypothesized to lead to the development of casts. Switching to candesartan, an angiotensin receptor blocker, and performing chest massages to loosen bronchial casts resulted in cast expectoration and termination of new cast formation.[28] A summary of select adult case studies is displayed in Table 3.

The etiologies of PB differ between adult and pediatric populations. In children, many cast formations can be associated with surgery for congenital heart defects. Casts can also be formed through inflammatory processes and respiratory infections. Rarely, PB can be caused by acute coronary syndrome (ACS) or iatrogenic processes. Similarly, adults can develop casts through infections or cardiac and pulmonary surgeries. Anatomic variations in the lymphatic system may also be related to cast formations. Nevertheless, most cases in adults remain idiopathic at present.

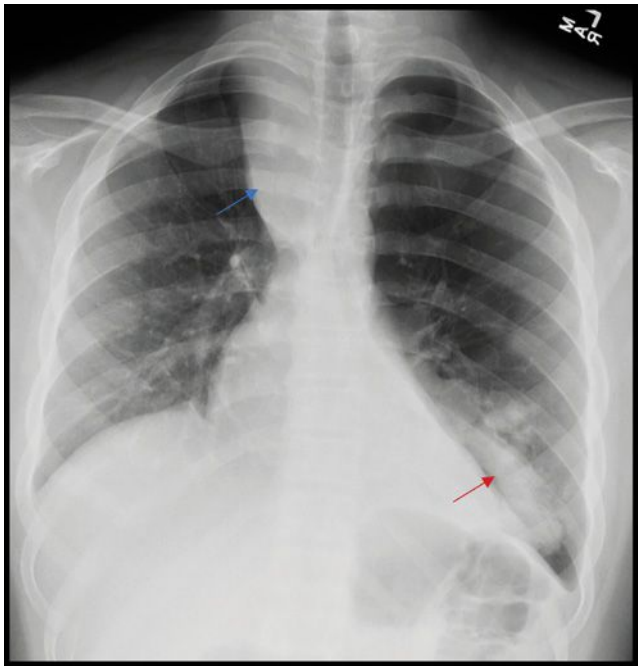


Figure 1. Chest Radiograph.
Shown are signs of airway obstruction from the bronchial cast. Complete right upper lobe collapse (blue arrow) with right hemi-diaphragm elevation due to volume loss. Nodular opacity in lingual (red arrow) representing bronchial cast.

Clinical Presentation

There are no definitive diagnostic criteria or tests for plastic bronchitis. The diagnosis of PB is mainly clinical and is based on clinical presentation, bronchoscopic, and imaging findings. The clinical presentation of plastic bronchitis is comparable to other chronic respiratory illnesses such as asthma, cystic fibrosis, or bronchiectasis. General symptoms include productive cough, wheezing, and dyspnea. Physical examination may reveal decreased breath sounds at lung bases with simultaneous wheezing.[32] PB is distinguished from other chronic respiratory illness by presence of the branching casts that are expectorated, commonly containing inflammatory and proteinaceous material. Episodes of expectoration could go on for years before the diagnosis is made due to the similarity of symptoms to disease processes such as cystic fibrosis. Chest radiographs can demonstrate bronchial obstruction with possible lobar atelectasis; presenting as patchy opacities in chest radiographs (Figure 1).[49] Since signs and symptoms may not be specific, it emphasizes the importance of acquiring a detailed history and physical examination along with the need for chest imaging and possible bronchoscopic evaluation.

Pathophysiology

The phenomena underlying plastic bronchitis are still widely unresolved. Plastic bronchitis is characterized as a condition seen most commonly in children (but also can be found in adults) in which thick casts are expectorated (Figure 2A). The mechanisms behind cast formation remain unknown, but associations with asthma, congenital cardiac history status post cardiac surgery, and cystic fibrosis (CF) have been made.[29] Many mechanisms have been proposed to play a part in cast formation.

Casts have been characterized into two types. Type I casts are inflammatory with components including neutrophils, eosinophils, and Charcot Leyden crystals seen in asthma and CF (Figure 2B and 2C). These casts are believed to have been formed due to decreased mucociliary clearance mechanisms.[30] Type II casts are associated with congenital heart disease status post Fontan procedure and are found histologically to be acellular with proteinaceous lymph material as the major component. With the inferior vena cava (IVC) being connected to the pulmonary artery (PA) for passive redirection of blood flow in a Fontan procedure, casts can accumulate over time from insufficient lymph drainage secondary to higher pulmonary venous pressures. The accumulation of casts and consequent airway obstruction acutely increases the pulmonary venous pressure and further propagates cast formation.[30] The majority of these associated conditions are diagnosed in childhood, but plastic bronchitis should be considered in adults when individuals have a history of cardiac surgery or chronic inflammatory lung disease.[32] An all-encompassing answer has not been found for what underlies plastic bronchitis, but these proposed mechanisms provide a framework for a deeper understanding of the disease.

Treatment

Despite their many differing etiologies, adult and pediatric plastic bronchitis populations have greatly overlapped in their treatment methods. Further, the lack of in-depth knowledge of the underlying pathophysiology has not prevented the gradual evolution of effective treatment options for both symptomatic relief and correction of underlying causes of plastic bronchitis. Treatment for PB was described as early as 1960 by Johnson et al. who stated that the manual removal of casts was the only source of relief for an adult patient with idiopathic plastic bronchitis.[31] Both pharmacologic and surgically therapies for PB have greatly evolved since those early efforts.

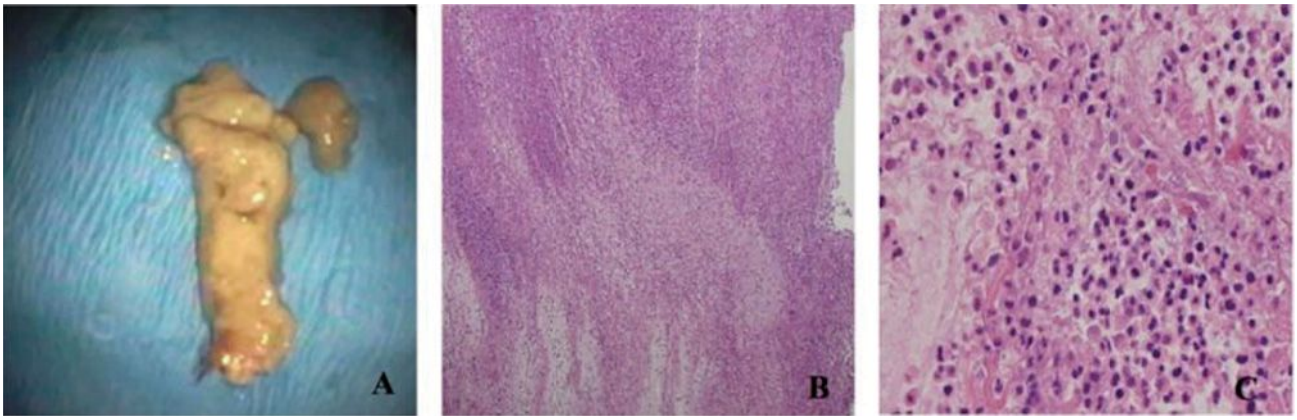


Figure 2. Gross and Histological Appearance of Bronchial Cast.
(A) Bronchial cast expectorated by pediatric patient. (B) Hematoxylin and eosin stain displaying inflammatory cells.
(C) Neutrophils and macrophages seen within the cast.

Pharmacological treatment options for plastic bronchitis are similar to those used in the management of other obstructive and restrictive lung diseases. Moreover, the mode of effective treatment has been shown to vary depending on the cast type present and its location in the patient. Although no comparative studies are available to guide treatment, some would argue that pharmacological treatment is more helpful for patients with Type I casts of an inflammatory origin.[32] In contrast, plastic bronchitis cases with Type II, noninflammatory casts require treating the underlying cause, with surgical options showing more improved outcomes and prevention of recurrent cases.

One pharmacological treatment option includes corticosteroids to target underlying inflammation. Both inhaled and systemic corticosteroid have been deemed effective in reducing inflammatory cast formation and alleviating PB symptoms in pediatric and adult populations.[32,33] Additionally, corticosteroids were found to reduce hemoptysis in patients who presented with hemoptysis as a symptom of PB.[34] However, corticosteroids have not been shown to provide significant reduction in plastic bronchitis-associated complications or mortality.

For patients in whom corticosteroids are ineffective or contraindicated, other anti-inflammatory medications remain available. Low-dose, long term therapy with macrolide antibiotics have demonstrated anti-inflammatory effects which reduce the formation of Type I casts in plastic bronchitis.[33,35] For patients with an underlying infectious cause of plastic bronchitis or whose casts demonstrate microbial growth in culture, additional antibiotic therapy directed towards the causal organisms is also indicated as part of the treatment course.[32,33] More recently, sirolimus, an immunosuppressant drug used in the prevention of organ transplant rejection, has been presented as an emerging treatment for adult cases of PB.[36] Sirolimus is hypothesized to reduce the formation of chyle, a digestion-related bodily fluid consisting of fat droplets and lymph, which in cases of overproduction, can lead to obstruction and leakage causing plastic bronchitis.

N-acetylcysteine, a mucolytic agent used in the management of chronic obstructive pulmonary disease, has also been used in both pediatric and adult patients with plastic bronchitis.[32,37] Further, N-acetylcysteine may a be safe option in the treatment of pregnant patients with plastic bronchitis. It can be administered via nebulization to assist with dissolving casts prior to bronchoscopy.[4]

Several other medications have been described as possible plastic bronchitis treatments by preventing cast formation or accelerating cast dissolution. The use of intravenous unfractionated heparin in an inpatient setting in adults with PB may slow the rate of Type I cast formation.[32] In bronchial casts with fibrin present, plasminogen activators including urokinase and tPA were demonstrated to reduce cast size and lung obstruction.[32,35] DNAse, an enzyme used to degrade DNA products in patients with cystic fibrosis, has also been used to attempt cast dissolution. Nevertheless, in terms of effectiveness, N-acetylcysteine appears to be more efficient in performing this objective when compared to DNAse or plasminogen activators.[38] Lastly, octreotide, a synthetic somatostatin analog, has been theorized to reduce cast formation via the

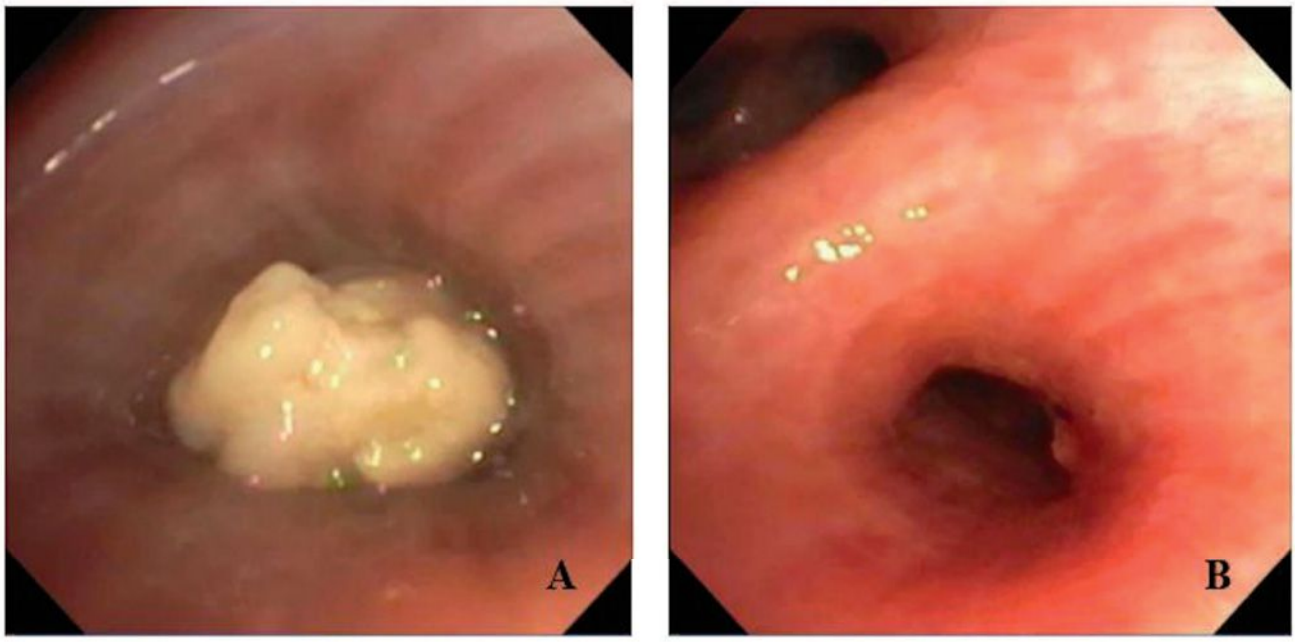


Figure 3. Airway Bronchoscopy.
The images above were captured via flexible bronchoscopy. (A) Shows complete obstruction of the airway (lingula) by a mucous cast. (B) Demonstrates patent airway (lingula) after complete extraction of the cast.

vasoconstriction of splanchnic vessels and subsequent reduction in intestinal secretions. These changes lead to a decreased amount of intestinal absorption, ultimately reducing lymph flow and leading to a corresponding decreased production of lymphatic casts. However, this medication was not effective in the adult case of plastic bronchitis in which it has been attempted.[36]

Despite the variety of pharmacological treatment options available, no single medication has been proven to effectively and consistently treat patients with plastic bronchitis. In addition, certain drug classes have been shown to be ineffective in treating PB. Bronchodilators such as beta-agonists, guaifenesin, and hypertonic saline, while used in the treatment of other pulmonary conditions such as CF, have not exhibited any significant improvement in plastic bronchitis patients in either pediatric or adult populations.[39,40]

In addition to such pharmacologic therapies, surgical interventions have emerged as effective treatment options which address both the underlying cause of plastic bronchitis and provide symptomatic relief. Procedural methods used in the management of PB have one of two underlying objectives: (1) the resection of existing bronchial casts; or (2) the prevention of new cast formation via lymphatic ductal intervention.

Bronchial cast removal via flexible bronchoscopy has elicited successful outcomes in patients with plastic bronchitis (Figure 3). In a case described by Sheikh et al., bronchoscopy was performed daily for removal of casts until the adult patient gradually recovered and cast formation ceased.[19] Further reports on flexible bronchoscopy in PB indicated that the implementation of cryoextraction, a method by which bronchial casts are solidified by cooling at the moment of removal, was also effective at procedurally treating plastic bronchitis.[41] Respiratory kinesiotherapy was also found to help facilitate bronchoscopy for cast removal. In an adult case of idiopathic plastic bronchitis, high-frequency oscillation was used to vibrate the chest wall, allowing for the loosening of bronchial casts prior to bronchoscopy.[42]

While bronchoscopic cast removal has acutely benefited patients with plastic bronchitis, emerging surgical treatment options that permanently modify lymphatic drainage present a new and effective method for resolving the underlying causes of some forms of the disease. As previously mentioned, patients with abnormal lymphatic flow and drainage show increased risk for developing plastic bronchitis. In direct response to these findings, several surgical options are available for treatment. The first was described by Itkin et al. and used imaging to identify aberrant

Table 4: Summary of Treatment Modalities and Corresponding Evidence

Study	Level of Evidence	Treatment Modality	Target Population
Pharmacological Treatments			
Coen et al[32] Kim et al[33] Wang et al[34]	Case report Case report Case series	Corticosteroids	Adults and Pediatrics
Coen et al[32] Kim et al[33] Madsen et al[35]	Case report Case report Review	Macrolide antibiotics	Adults and Pediatrics
Shah et al[36]	Case report	Sirolimus	Adults
Coen et al[32] Kumar et al	Case report Case series	N-acetylcysteine	Adults and Pediatrics
Coen et al[32] Madsen et al[36] Brogan et al[38]	Case report Case series Case series	Unfractionated heparin and Tissue plasminogen activators (tPA)	Adults and Pediatrics
Surgical Treatments			
Sheikh et al[19]	Case report	Flexible bronchoscopy + cast extraction	Adults
Sriratanaviriyakul et al[41]	Retrospective study	Cryoextraction	Adults
Raoufi et al[42]	Case report	Respiratory Kinesiotherapy	Adults
Itkin et al[43] Dori et al[44] Peyton et al[45] Geanacopoulos et al[46]	Case series Retrospective case series Case series Retrospective study	Lymphatic embolization surgery	Adults and Pediatrics
Hess et al[40]	Case report	Thoracic duct ligation	Adults
Majdalany et al[47]	Case report	Thoracic duct stent grafting	Adults
Albitar et al[21] Yung et al[48] Hess et al[40]	Case report Case report Case report	Dietary modifications	Adults and Pediatrics

Table 4: Shown above is summary of pharmacological and surgical treatment options for plastic bronchitis.

thoracic duct branches that drained directly into the lung. By occluding extraneous lymphatic drainage via embolization, the authors reported complete resolution of symptoms and the prevention of future complications of plastic bronchitis in five of six patients.[43] Furthermore, patients undergoing lymphatic embolization experienced no additional obstruction or bleeding and required fewer post-surgical medications or respiratory support. This novel and minimally invasive surgical method of percutaneous embolization has also been applied to pediatric patients with plastic bronchitis stemming from Fontan circulation or congenital heart disease resulting in abnormal lymphatic circulation. In such patients, the procedure equaled its success in adults in alleviating symptoms and preventing additional exacerbations.[44-46]

Cases of plastic bronchitis have been described in which patients were unable to undergo lymphatic embolization for treatment due to higher risk or to failed previous attempts. In such cases, alternative

surgical procedures have been attempted. In one patient for which cannulation of lymphatic vessels was unsuccessful, thoracic duct ligation was performed, yielding similar results to percutaneous embolization.[40] Another report describes the use of thoracic duct stent-grafting to re-direct lymphatic flow through the main thoracic duct and thereby exclude flow to aberrant lymphatic branches.[47]

A summary of pharmacological and surgical treatment options for PB is outlined in Table 4. Even as pharmacological regimens and surgical treatment methodologies continue to evolve, alternative therapies including lifestyle modification remain options to be considered for PB patients. Albitar et al. described a 42-year-old male patient whose PB symptoms worsened upon consumption of meals with high fat content and were improved by reduction of fat intake.[21] Indeed, Yung et al. recommended a complete fat-free diet for patients with severe plastic bronchitis leading to ventilatory failure.[48] It was hypothesized that decreased fat consumption leads to a reduction in chyle production, ultimately reducing lymph volume and cast formation.

Discussion

Plastic bronchitis presents as a severe respiratory condition with a broad spectrum of etiologies. In children, the surgical correction of congenital heart disease[6] and immune-mediated inflammatory[16] and infectious causes prednominate.[11] In comparison, adults with plastic bronchitis exhibit additional etiologies such as anatomical lymphatic abnormalities[22] or idiopathic causes.[17] Additional etiologies of plastic bronchitis are continually being reported, and the full scope of the origin of this condition remains unknown. Regardless of underlying causes, patients experience productive cough, dyspnea, and wheezing which is easily misdiagnosed as another pulmonary condition such as chronic bronchitis or atypical asthma.

Due to the potential for severe cardiopulmonary compromise in patients with plastic bronchitis, it is essential for primary care physicians and respiratory specialists to understand the clinical presentation and available treatment options. The treatment for plastic bronchitis has evolved with the introduction of novel lymphatic surgical techniques.[43] Prior to the innovation of lymphatic embolization, stent grafting, and duct ligation techniques for plastic bronchitis, clinicians focused on the extensive variety of pharmacological measures for treating the condition. From corticosteroids to reduce inflammation to mucolytics such as N-acetylcysteine for loosening and dislodging casts, some patients experienced at least temporary improvement of symptoms, but no single medication has provided consistent efficacy within the current literature. However, the implementation of new bronchoscopic techniques along with the aforementioned lymphatic procedures have led to a dramatic reduction or even elimination of patient symptoms and recurrence rates for plastic bronchitis.[43] Moving forward, the best therapeutic approach for physicians may be to consider surgery as a primary intervention, with medications and lifestyle modifications serving as ancillary therapies targeted towards continued symptomatic improvement.

Despite these advancements, there is a dire need for further investigation. Given the broad range of PB etiologies, the most direct common pathway leading to the formation of casts is yet to be determined, and indeed may not exist. Moreover, the advent of many treatments for plastic bronchitis is recent and none of them have been evaluated in randomized control trials. Given the rarity of the disease and a current lack of controlled trials, the long-term outcomes of surgery, recurrence rates within different treatment groups, and overall demographic risk factors for the development of plastic bronchitis remain unknown.

While rare, plastic bronchitis has emerged as a treatable condition. With further clinician education, research, and awareness, many PB patients can look forward to improved outcomes and more durable and effective care.

References

1. Li Y, Williams RJ, Dombrowski ND, et al. Current evaluation and management of plastic bronchitis in the pediatric population. Int J Pediatr Otorhinolaryngol. 2020;130:109799.
2. Park JY, Elshami AA, Kang DS, Jung TH. Plastic bronchitis. Eur Respir J. 1996;9(3):612-614.
3. Sharma VJ, Iyengar AJ, Zannino D, et al. Protein-losing enteropathy and plastic bronchitis after the Fontan procedure. J Thorac Cardiovasc Surg. 2020.
4. Patil MH, Siddiqi A, Jeffrey Mador M. Successful bronchoscopy in a pregnant patient with plastic bronchitis. Respir Med Case Rep. 2016;18:8-9.
5. Larue M, Gossett JG, Stewart RD, Backer CL, Mavroudis C, Jacobs ML. Plastic bronchitis in patients with fontan physiology: review of the literature and preliminary experience with fontan conversion and cardiac transplantation. World J Pediatr Congenit Heart Surg. 2012;3(3):364-372.
6. Singhi AK, Vinoth B, Kuruvilla S, Sivakumar K. Plastic bronchitis. Ann Pediatr Cardiol. 2015;8(3):246-248.
7. Grutter G, Di Carlo D, Gandolfo F, et al. Plastic bronchitis after extracardiac Fontan operation. Ann Thorac Surg. 2012;94(3):860-864.
8. Liptzin DR, Di Maria MV, Younoszai A, et al. Pulmonary Screening in Subjects after the Fontan Procedure. J Pediatr. 2018;199:140-143.

9. Grizales CL, Gonzalez LM, Castrillon MA, et al. Plastic bronchitis: A case report. Respir Med Case Rep. 2019;28:100876.
10. Feray S, Mora P, Decavele M, Pham T, Hafiani EM, Fartoukh M. Plastic bronchitis: An unusual complication of acute chest syndrome in adult. Respir Med Case Rep. 2017;21:93-95.
11. Lu Z, Zheng Y. Plastic bronchitis associated with adenovirus infection. Lancet Infect Dis. 2018;18(4):474.
12. Yuan L, Huang JJ, Zhu QG, Li MZ, Zhuo ZQ. Plastic bronchitis associated with adenovirus serotype 7 in children. BMC Pediatr. 2020;20(1):268.
13. Zhang X, Vinturache A, Ding G. Plastic bronchitis in a 3-year-old boy. CMAJ. 2019;191(48):E1336.
14. Kirito Y, Matsubayashi T, Ohsugi K. Plastic bronchitis: Three cases caused by influenza B virus Yamagata lineage. Pediatr Int. 2019;61(4):421-423.
15. Hussain H, Kherallah N. Eosinophilic lung disease with plastic bronchitis and bronchiectasis in an adolescent male. Clin Case Rep. 2018;6(12):2407-2410.
16. Chhawchharia R, Gupta N, Gupta D, Agarwal P. Plastic Bronchitis: A Manifestation of Dander Hypersensitivity. Indian Pediatr. 2020;57(8):760-761.
17. Eberlein MH, Drummond MB, Haponik EF. Plastic bronchitis: a management challenge. Am J Med Sci. 2008;335(2):163-169.
18. Jett JR, Tazelaar HD, Keim LW, Ingrassia TS, 3rd. Plastic bronchitis: an old disease revisited. Mayo Clin Proc. 1991;66(3):305-311.
19. Sheikh AY, Ahmadi-Kashani M, Mohindra V, Friedenberg A, Pramanik SB, Ogden WD. A Rare Case of Plastic Bronchitis in an Adult Patient After Cardiopulmonary Bypass. Ann Thorac Surg. 2016;101(3):1176-1178.
20. Eberlein M, Parekh K, Hansdottir S, Keech J, Klesney-Tait J. Plastic bronchitis complicating primary graft dysfunction after lung transplantation. Ann Thorac Surg. 2014;98(5):1849.
21. Albitar HAH, Vassallo R. Lymphatic Plastic Bronchitis Secondary to Thoracic Duct Stenosis. Mayo Clin Proc. 2019;94(7):1141-1142.
22. Siddiqi NH, Kraman SS, Pressman B. Adult-Onset Plastic Bronchitis with Novel Lymphatic Anatomy: Cured with Endo-Lymphatic Embolization. Respiration. 2019;98(2):171-173.
23. Stoddart A, Dincer HE, Iber C, Tomic R, Bhargava M. Chyloptysis causing plastic bronchitis. Respir Med Case Rep. 2014;13:4-6.
24. Okada Y, Okada A, Narumiya H, Iiduka R, Katsura K. Bloody Bronchial Cast Formation Due to Alveolar Hemorrhage Associated with H1N1 Influenza Infection. Intern Med. 2017;56(20):2747-2751.
25.Habib SA, Vasko RC, Badawy J, Anstead GM. Plastic Bronchitis in an AIDS Patient with Pulmonary Kaposi Sarcoma. Case Rep Pulmonol. 2018;2018:9736516.
26. Watanabe K, Ishida T, Sugawara A, Tachihara M, Munakata M. An adult case of plastic bronchitis. Intern Med. 2008;47(17):1549.
27. Yadav M, Tirpude S, Joshi JM. Plastic bronchitis in beta thalassemia minor. Lung India. 2013;30(3):206-208.
28. Plumacher KS, Paul T, Sigler M. Expectoration of bronchial casts in association with Ramipril treatment. Cardiol Young. 2019;29(12):1565-1566.
29. Kunder R, Kunder C, Sun HY, et al. Pediatric plastic bronchitis: case report and retrospective comparative analysis of epidemiology and pathology. Case Rep Pulmonol. 2013;2013:649365.
30. Verghese S, Jackson M, Vaughns J, Preciado D. Plastic bronchitis in a child with Fontan's physiology presenting for urgent rigid bronchoscopy. Anesth Analg. 2008;107(4):1446-1447.
31. Johnson RS, Sita-Lumsden EG. Plastic bronchitis. Thorax. 1960;15:325-332.
32. Coen M, Daniel L, Serratrice J. An adult case of plastic bronchitis: a rare and multifactorial disease. J Thorac Dis. 2018;10(1):E16-E19.
33. Kim EJ, Park JE, Kim DH, Lee J. Plastic bronchitis in an adult with asthma. Tuberc Respir Dis (Seoul). 2012;73(2):122-126.
34. Wang G, Wang YJ, Luo FM, et al. Effective use of corticosteroids in treatment of plastic bronchitis with hemoptysis in Chinese adults. Acta Pharmacol Sin. 2006;27(9):1206-1212.
35. Madsen P, Shah SA, Rubin BK. Plastic bronchitis: new insights and a classification scheme. Paediatr Respir Rev. 2005;6(4):292-300.
36. Shah A, Donovan J, Marino P, et al. A lesson in plasticity: a 74-year-old man with plastic bronchitis. Thorax. 2017;72(11):1055-1057.
37. Kumar A, Jat KR, Srinivas M, Lodha R. Nebulized N-Acetylcysteine for Management of Plastic Bronchitis. Indian Pediatr. 2018;55(8):701-703.
38. Brogan TV, Finn LS, Pyskaty DJ, Jr., et al. Plastic bronchitis in children: a case series and review of the medical literature. Pediatr Pulmonol. 2002;34(6):482-487.
39. Muller AF, Mithal A. A case of plastic bronchitis. J R Soc Med. 1988;81(6):360.
40. Hess NR, Piercecchi C, Desai N, Fisher MR, Lee EH, Force SD. Successful Thoracic Duct Ligation for Plastic Bronchitis in an Adult. Ann Thorac Surg. 2017;103(6):e539-e540.
41. Sriratanaviriyakul N, Lam F, Morrissey BM, Stollenwerk N, Schivo M, Yoneda KY. Safety and Clinical Utility of Flexible Bronchoscopic Cryoextraction in Patients With Non-neoplasm Tracheobronchial Obstruction: A Retrospective Chart Review. J Bronchology Interv Pulmonol. 2015;22(4):288-293.
42. Raoufi M, Achachi L, Mrabet FZ, et al. A Rare Case of Idiopathic Plastic Bronchitis. Case Rep Pulmonol. 2017;2017:4789751.
43. Itkin MG, McCormack FX, Dori Y. Diagnosis and Treatment of Lymphatic Plastic Bronchitis in Adults Using Advanced Lymphatic Imaging and Percutaneous Embolization. Ann Am Thorac Soc. 2016;13(10):1689-1696.
44. Dori Y, Keller MS, Rome JJ, et al. Percutaneous Lymphatic Embolization of Abnormal Pulmonary Lymphatic Flow as Treatment of Plastic Bronchitis in Patients With Congenital Heart Disease. Circulation. 2016;133(12):1160-1170.
45. Peyton C. Protein-Losing Enteropathy and Plastic Bronchitis After the Fontan Operation. Crit Care Nurse. 2018;38(6):e5-e12.
46. Geanacopoulos AT, Savla JJ, Pogoriler J, et al. Bronchoscopic and histologic findings during lymphatic intervention for plastic bronchitis. Pediatr Pulmonol. 2018;53(11):1574-1581.
47. Majdalany BS, Khayat M, Sanogo ML, Saad WE, Khaja MS. Direct trans-cervical endolymphatic thoracic duct stent-graft for plastic bronchitis. Lymphology. 2018;51(3):97-101.
48. Yung H, Sharma K, Flowers W, et al. Food for Thought: Dietary Intervention in a Rare Cause of Severe Ventilatory Failure. Eur J Case Rep Intern Med. 2020;7(5):001523.
49. Robinson M, Smiley M, Kotha K, Udoji T. Plastic Bronchitis Treated With Topical Tissue-Type Plasminogen Activator and Cryotherapy. Clin Pediatr (Phila). 2016;55(12):1171-1175.

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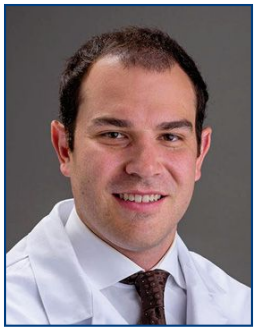
The impetus for writing this review with preclinical medical students as its primary authors was provided by an insightful "Diagnosis" article by Dr. Lisa Sanders in the February 12, 2020, issue of the *New York Times Sunday Magazine*. That article, "He had trouble breathing and inhalers didn't work. What was going on?" stimulated considerable student discussion during their team-based learning assignment to 'reverse engineer' this article written for a non-medical audience into a clinically meaningful synopsis of a rare and probably under-reported medical condition.

Disclosure

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What Are the Risk Factors for an Upper Extremity Deep Venous Thrombosis After Orthopaedic Irrigation and Debridement and Peripherally Inserted Central Catheter Placement?

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Abstract

Background

Peripherally inserted central catheter (PICC) placement is necessary for delivery of intravenous (IV) antibiotics to treat bone and soft tissue infections. Upper extremity deep venous thrombosis (DVT) after PICC placement is a complication with unknown incidence in the orthopaedic literature. The major objectives of this study are

(1) Identifying the rate of upper extremity PICC-associated DVTs after orthopaedic procedures;

(2) Which orthopaedic subspecialties are most likely to encounter an upper extremity PICC-associated DVT?

(3) What surgeries or medical comorbidities are risk factors for upper extremity PICC-associated DVTs?

(4) Does type of DVT chemoprophylaxis decrease the risk of an upper extremity PICC-associated DVT?

Methods

A retrospective review of electronic medical records (EMR) was performed to include all patients undergoing irrigation and debridement (I&D) for treatment of orthopaedic surgery-related infections over a 10-year period. All patients with PICC placement were included for analyses. Age, sex, and medical comorbidities were extracted from the EMR. Mann-Whitney non-parametric tests, Fisher's exact tests, Chi-square tests, and Cochran-Mantel-Haenszel (CMH) tests were used to determine associations with DVT events for those with PICCs based on medical comorbidities, PICC lumen size, team placing the PICC, impact of implant removal, and protective effect of DVT chemoprophylaxis. Significance was set at $p<0.05$.

Results

Twenty-one of 660 patients (3.18% rate) were found to have an upper extremity PICC-associated DVT. A history of DVT (OR=8.99 [95% CI: 3.39, 49.42]) was significantly associated with an upper extremity PICC-associated DVT. The greatest risk for an upper extremity PICC-associated DVT

was intramedullary implant removal (OR=12.43 [95% CI: 3.13, 49.52]). The type of DVT chemoprophylaxis did not significantly affect the likelihood of an upper extremity PICC-associated DVT.

Conclusion

Intramedullary implant removal and a history of DVT are risk factors for an upper extremity PICC-associated DVT. The results of this study should be of particular interest to surgeons who do not typically give DVT prophylaxis and plan to perform surgery on patients with CHF, a history of a DVT, or plan to manipulate the intramedullary canal.

Introduction

Long-term antibiotic treatment for deep bacterial infections involving bone and/or soft tissues often involves placement of peripherally inserted central catheters (PICC). The antibiotic selection is often managed by our infectious disease colleagues, while orthopedics often prescribes deep vein thrombosis (DVT) chemoprophylaxis if it is indicated. Previously identified risk factors for upper extremity PICC-associated deep venous thrombosis is elevated in patients with congestive heart failure (CHF),[1] malignancy,[2] and those who are in critical condition.[3] However, little peer-reviewed evidence exists in the orthopaedic literature. Although the insertion of an indwelling catheter causes endothelial damage—the other two parts of Virchow's triad—the patient's hypercoagulable state and increased venous stasis are increased during orthopaedic surgery. The influence of hypercoagulability can be appreciated in the 5% rate of PICC-associated DVTs in ambulatory patients without cancer, which is 15 times lower than the 75% rate reported for cancer patients.[4,5] Accurate delineation of at-risk patients and appropriate use of chemoprophylaxis are critical for providing evidence-based care as DVT events are associated with significant morbidity and mortality and increase health care expenditures ranging from $10,000 to $16,000 per event.[6-8]

This study was designed to assess all patients undergoing I&D for treatment of orthopaedic surgery-related infections at our institution over a 10-year period in order to determine the likelihood of occurrence of an upper extremity PICC-associated DVT based on medical comorbidities, PICC lumen size, the team placing the PICC, post-operative weight bearing status, impact of intramedullary implant removal, and type of DVT chemoprophylaxis. We hypothesized the following patients would be at significantly greater risk for PICC-associated DVT: 1) patients with a history of DVT, cancer, or CHF; 2) patients without DVT chemoprophylaxis; 3) patients undergoing surgery with a surgeon who infrequently prescribes DVT chemoprophylaxis; and 4) patients undergoing more complex surgery including intramedullary explant with debridement.

Participants and Methods

With institutional review board approval, electronic medical records (EMR) were reviewed for all patients who underwent I&D performed by the orthopaedic surgery department at our Midwestern, US-located institution during the years 2008 to 2018. Billing information for CPT codes associated with "irrigation and debridement" or "incision and drainage" were used to identify the cohort of patients included for analysis. The following information was extracted from the EMR to determine risk factors for an upper extremity PICC-associated DVT: age, sex, medical comorbidities, lumen size, PICC placement by interventional radiology or nurse IV team, orthopaedic subspecialty performing I&D, arthroplasty explant during I&D (intramedullary implant), post-operative weight bearing status, days of admission after DVT diagnosis, and post-operative DVT chemoprophylaxis including type of chemical prophylaxis. Eighty-one mg aspirin was not differentiated from 325 mg aspirin when including aspirin as the type of DVT chemoprophylaxis. Clopidogrel was included as DVT chemoprophylaxis if the patient was taking this preoperatively and it was continued post-operatively. If a patient had a polyethylene swap without intramedullary arthroplasty explant, this was not included in the arthroplasty explant cohort. If the patient was on DVT chemoprophylaxis for any reason prior to surgery, and the chemoprophylaxis regimen was continued post-operatively, it was included as their

Table 1. Patient demographics and PICC characteristics, means ±standard deviation.

	Category	PICC-associated DVT	No DVT	OR	95% OR CI	P-value
Age		53.67±23.4	50.48±21.55			0.50
Sex	Female	11	270	1.34	(0.56, 3.19)	0.51
	Male	10	328	1		
Lumen size	single	2	29	1		0.39
	double	18	611	0.43	(0.95, 1.93)	
	triple	0	7	-		
PICC placement	Nurse	14	564	0.41	(0.1537, 1.0912)	0.11

DVT chemoprophylaxis regimen. Only patients who had a DVT at the site of PICC placement, which was confirmed with non-compressibility of the vein on venous duplex ultrasound, were considered to have an upper extremity PICC-associated DVT. Other inclusion criteria included any patient who underwent an I&D procedure and had an upper extremity PICC placement during the same hospital visit. All patients who had the PICC removed during the same hospital visit were included in the analysis. If it was unclear if the medical comorbidity was present at the time of PICC placement, the medical comorbidity was not recorded to minimize bias.

Statistical Analyses

Data were validated and summarized using frequency tables and percentages for categorical variables and means and standard deviations or medians and ranges, for continuous variables. A two-tailed unequal variance student t-test or Mann-Whitney non-parametric test was used to determine if there was a significant difference between patient demographics and PICC characteristics with significance set at $p < 0.05$. Fisher's exact test was used to test the following conditions: if there was an association between DVT chemoprophylaxis and implant removal resulting in an upper extremity PICC associated DVT, to determine if all types of anticoagulation are equally efficacious at preventing DVT events, and to determine if certain orthopaedic subspecialties are at increased risk of having an upper extremity PICC-associated DVT. Additionally, Chi-square tests were used to determine which medical comorbidities are risk factors for a PICC associated DVT event, with dichotomous comparisons performed using Fisher's Exact method. A contingency table was used to compare implant removal status and DVT status at fixed level of anticoagulation status. The Cochran-Mantel-Haenszel (CMH) test was used to test the conditional association between implant removal status and DVT status in the presence of the anticoagulation status.

Results

A total of 1,279 unique patients undergoing I&D during the years 2008 to 2018 were identified and had non-missing PICC status. Of these patients undergoing I&D, 626 patients (48.94%) were identified as having at least one PICC placed during the same hospital stay as the I&D. Fifty-nine (9.42%) of these patients had multiple PICC placements with 12 (1.92%) having three or more PICC placements, yielding a total of 709 PICC placements. Of the 709 PICC placements identified in our cohort, a total of 21 upper extremity PICC-associated DVTs were identified. None of the 21 patients with a PICC-associated DVT had multiple PICC line placements. The rate of upper extremity PICC related DVTs were 3.35% per patient and 2.96% per PICC line placement. Neither age ($p = 0.3037$) nor sex ($p = 0.514$) was significantly different between patients who developed an upper extremity PICC-associated DVT versus those who did not. Eight of the 21 patients diagnosed with an upper extremity PICC-associated DVT were non-weight bearing or partial weight bearing, while the remaining 13 were designated as "weight bearing as tolerated". Non-weight bearing status was not significantly different in the patients with a PICC-associated DVT compared to those without (38.1%) an upper extremity PICC-associated DVT ($p=0.9731$). The average length of PICC duration was 34.3 ± 10.5 days (range 2-48 days), while the average number of days until diagnosed upper extremity PICC-associated DVT was 11.7 ± 5.6 days. A diagnosis of an upper extremity

Table 2. Likelihood of a PICC-associated DVT based on presence of DVT chemoprophylaxis and intramedullary implant removal.

	For all *PICC placements*				
	No DVT (N=673)	**DVT present (N=19)**	**Odds ratio**	**95% OR CI**	**p-value**
No implant removal and had anticoagulation	663	16	1		0.004
Implant removal and no anticoagulation	10	3	12.43	(3.13, 49.52)	
	For distinct *patients*				
	No DVT (N=594)	**DVT present (N=19)**	**Odds ratio**	**95% OR CI**	**p-value**
No implant removal and had anticoagulation	587	16	1		0.003
Implant removal and no anticoagulation	7	3	15.72	(3.72, 66.42)	

PICC-associated DVT was associated with immediate readmission in all patients with a resulting 3.2 ± 3.1 days of hospital stay until discharge. PICC lumen size and team placing the PICC were not significantly different between DVT cohorts (Table 1). Three patients with DVT did not have information in the EMR regarding PICC lumen size or the team who placed the PICC.

We identified 92 patients (14.65%) in the PICC cohort who had an intramedullary implant removal with a total of 115 total intramedullary implant removals. Of the 92 patients with implant removal, 10 patients (10.87%) did not receive chemical DVT prophylaxis. When including each explant event, 13 of the 115 implant removals did not receive chemical DVT prophylaxis. This led to a 12.43 OR [95% CI: 3.13, 49.52] for patient specific and a 15.72 OR [95% CI: 3.72, 66.42] for total incidence of upper extremity PICC-associated DVTs when an intramedullary implant removal occurred during I&D (Table 2). When we specifically identified patients who had chemical DVT prophylaxis with implant removal compared to no implant removal, there was still an elevated, although lower risk for an upper extremity PICC-associated DVT (OR = 3.62 [95% CI: 1.12, 11.76]) (Table 3). With respect to medical comorbidities, history of DVT (OR = 8.99 [95% CI: 3.39, 23.86]) was significantly associated with an upper extremity PICC-associated DVT. Hypertension, CHF, cancer, smoking, diabetes, and other comorbidities were not found to be significant.

When comparing orthopaedic subspecialties, rate of upper extremity PICC-associated DVT events in shoulder surgery (25%) (p = 0.0161) and spine surgery (8.16%) (p = 0.0410) were significantly higher than rate of PICC-associated DVT in orthopaedic trauma (2.01%). Shoulder surgery also had a significantly higher rate of upper extremity PICC-associated DVT events compared to hip and knee arthroplasty (3.10%) (p = 0.0347). Of note, both shoulder surgery patients who experienced upper extremity PICC-associated DVTs underwent total shoulder arthroplasty explant.

Finally, when comparing enoxaparin, factor Xa inhibitors, 325 mg aspirin, warfarin, and clopidogrel, we did not find significant differences in efficacy among these common types of DVT chemoprophylaxis. Additionally, DVT chemoprophylaxis did not result in a significantly lower rate of upper PICC-associated DVT events when compared to not using DVT chemoprophylaxis for orthopaedic patients undergoing I&D, p=0.1344.

Discussion

Data from the present study documented upper extremity PICC-associated DVTs in 3.2% of orthopaedic surgery patients who underwent I&D at a tertiary care center. Our upper extremity PICC-associated DVT rate closely aligns with the 5% rate

Table 3. Odds ratio of developing a DVT based on anticoagulation status and intramedullary implant removal.

All PICC placements		No DVT	DVT present	Odds ratio	95% OR CI	P-value
No anticoagulation given	No implant removal	191	4	1		0.0058
	implant removal	10	3	14.33	(2.82, 72.85)	
Anticoagulation given	No implant removal	388	7	1		0.0508
	implant removal	84	5	3.30	(1.02, 10.64)	
CMH test result: Common Odds ratio estimate = 4.81; common odds ratio 95% confidence interval = (1.86, 12.43); CMH test p-value = 0.0009.						
Distinct patients		**No DVT**	**DVT present**	**Odds ratio**	**95% OR CI**	**P-value**
No anticoagulation given	No implant removal	179	4	1		0.0032
	implant removal	7	3	19.18	(3.69, 102.56)	
Anticoagulation given	No implant removal	340	7	1		0.0386
	implant removal	67	5	3.62	(1.12, 11.76)	
CMH test result: Common Odds ratio estimate = 5.41; common odds ratio 95% confidence interval = (2.07, 14.13); CMH test p-value = 0.0003.						

reported by Lobo et al.[9] Importantly, the present study delineated risk factors for upper extremity PICC-associated DVTs in orthopaedic surgery patients. Of the variables assessed, intramedullary implant removal, shoulder, and spine surgery, and history of DVT were significant risk factors for an upper extremity PICC-associated DVT, while age, sex, post-operative weight bearing status, lumen size, number of PICC placement attempts, cancer, smoking, diabetes mellitus, and DVT chemoprophylaxis type were not. Taken together, these data suggest that orthopaedic surgeons should consider prescribing DVT chemoprophylaxis for patients who have a history of DVT, patients who are undergoing I&D for spine surgery or a shoulder arthroplasty explant.

Similar to Liem et al. and Evans et al., patients in the present study with a history of DVT had significantly greater (8x) odds for upper extremity PICC-associated DVTs.[2,10] Interestingly, data from the present study did not show cancer to be predictive comorbidity for DVT. Perhaps patients with cancer who are healthy enough to undergo orthopaedic procedures are not at significantly increased risk for sustaining a DVT, however, this study may be underpowered for this analysis based on inclusion of only 34 patients with cancer requiring PICC placement.

We also hypothesized that more complex surgeries (intramedullary arthroplasty explants) would be associated with greater risk for upper extremity PICC-associated DVTs. While Evans et al.[10] examined the effects of surgery duration (< 1 or >1 hr) on risk for DVT, the present study assessed relative surgical complexity based on the assumption that it is a more accurate and standardized assessment of operative burden on the patient. This assumption appeared to hold true as Evans et al. reported only a slightly greater risk (1.7x) for DVT with longer duration surgeries, whereas arthroplasty explant surgeries were associated with significantly greater (12.4x) odds of experiencing an upper extremity PICC-associated DVT in the present study.

A strength of the present study is its analysis of a full spectrum of orthopaedic patients undergoing I&D, regardless of subspecialty. This allowed us to compare patients who are frequently treated with DVT chemoprophylaxis (67% of our trauma cohort and 90% of our arthroplasty service) and compare them to patients who infrequently are prescribed DVT chemoprophylaxis (44% of our shoulder and 44% of our spine service). Interestingly, the trauma and arthroplasty services, who frequently prescribe DVT chemoprophylaxis had a lower rate of PICC-associated DVT events than the shoulder and spine services who infrequently prescribe DVT chemoprophylaxis. Even when prescribed DVT chemoprophylaxis, arthroplasty explant was associated with a significantly greater (3.6x) odds for an upper extremity PICC-associated DVT. This data may be useful to help identify patients who could benefit from DVT prophylaxis after PICC placement.

An additional strength of the study is that every patient in the study was seen either by a member of our orthopaedic team, infectious disease team, or by the home health service after the I&D and prior to removal of the PICC. We consider this to be a

100% follow up rate. This was likely possible as the PICC line required removal by either home health, infectious disease, or the orthopaedic team. Each of these teams communicated with our orthopaedic team if there were any concerns regarding the PICC not working appropriately or there was concern for a DVT and this was documented in the EMR.

In contrast to Alnimer et al.[13] who reported PICC-associated DVTs were minimized with DVT chemoprophylaxis, our study did not show administration of DVT chemoprophylaxis or type of DVT chemoprophylaxis significantly diminished the occurrence for an upper extremity PICC-associated DVT when analyzing all patients. We hypothesize this was due to many I&Ds in healthy patients undergoing minor bone or soft tissue I&Ds.

Although we identified potential risks for upper extremity PICC-associated DVTs, there is an inherent risk of wound complications with administration of DVT chemoprophylaxis (14). These complications can range from increased bleeding, readmission to the hospital (4.7% rate), return to the operative suite (3.4% rate), or prolonged hospital stay (5.1% rate) (15). These risks should be weighed against the 3.2% rate of upper extremity PICC-associated DVTs, which resulted in hospital admission with an average hospital length of stay of 3.2 ± 3.1 days. These patients were admitted to the internal medicine service who managed the DVT anticoagulation treatment regimen. This typically consisted of either immediate oral anticoagulation with a single overnight hospital stay or immediate use of a heparin drip and subsequent transition to oral apixaban, subcutaneous weight-based enoxaparin or an enoxaparin bridge to warfarin. The second option was typically assigned to patients who were deemed to be less healthy that those treated only with an oral regimen.

We recognize there are limitations inherent with all retrospective studies including the accuracy of data input in the EMR. If it was unclear if a medical comorbidity was present at the time of PICC placement, the medical comorbidity was not included in our analysis. It is also possible upper extremity PICC-associated DVTs were not identified because the patient was asymptomatic. Only symptomatic patients who had pain at the PICC site or whose PICC line was not functioning appropriately were identified as having a DVT after ultrasound diagnosis. Further, our method of identifying patients with PICC line placements after I&Ds may have missed some patients with PICC placements. One potential alternative would have been to cross-reference all PICC placements in our hospital system by searching for CPT codes associated with PICC placements and then identify all orthopaedic providers who ordered PICC placement. Due to available hospital resources, this was not feasible to perform. Additionally, there were only eight patients in our hospital system who had a shoulder I&D with subsequent PICC placement. Compared to 49 spine, 292 joints, and 219 trauma patients, the small subgroup of shoulder patients could lead to high variability in the percentage of shoulder patients with a PICC-associated thrombus causing high variation in our confidence interval and results. Hospital systems with a larger number of patients undergoing shoulder I&D and PICC placement, especially if they underwent an arthroplasty explant, would help confirm our findings. Further, although we collected all patients over a 10-year period who had an I&D by the orthopaedic service, we had inadequate power to determine if the type of DVT chemoprophylaxis affected the rate of PICC associated DVT chemoprophylaxis. This was an expected finding as it requires large studies with >100,000 patients to identify appropriate efficacy of different types of DVT chemoprophylaxis to prevent lower extremity DVTs in the joint reconstruction literature.[14]

Conclusion

Upper extremity PICC-associated DVTs are relatively infrequent occurrences after orthopaedic irrigation and debridement, occurring at a 3.2% rate. A history of previous DVT increases the likelihood for upper extremity PICC-associated DVTs. Additionally, patients undergoing shoulder or spine surgery appear to be at increased likelihood for experiencing an upper extremity PICC-associated DVT. However, intramedullary explant procedures had the largest impact in elevating the risk for an upper extremity PICC-associated DVT. This data should be helpful for orthopaedic surgeons who do not routinely prescribe DVT chemoprophylaxis, but who operate on patients with a history of DVT, patients who require a complex surgery, or an intramedullary explant.

In these patients we recommend they be prescribed DVT chemoprophylaxis to mitigate the risk of a PICC-associated DVT. Of course, a risk-benefit ratio should be considered prior to administration of DVT chemoprophylaxis in all patients as increased bleeding and post-operative complications are a concern with DVT chemoprophylaxis agents.

References

1. Dean SM, Abraham W. Venous thromboembolic disease in congestive heart failure. Congest Heart Fail. 2010;16:164–169.
2. Liem TK, Yanit KE, Moseley SE, et al. Peripherally inserted central catheter usage patterns and associated symptomatic upper extremity venous thrombosis. J Vasc Surg. 2012;55:761–767.
3. Chopra V, Anand S, Hickner A, et al. Risk of venous thromboembolism associated with peripherally inserted central catheters: A systematic review and meta-analysis. Lancet. 2013;382:311–325.
4. Fallouh N, McGuirk HM, Flanders SA, Chopra V. Peripherally inserted central catheter-associated deep vein thrombosis: A narrative review. Am J Med. 2015;128:722–738.
5. Saber W, Moua T, Williams EC, et al. Risk factors for catheter-related thrombosis (CRT) in cancer patients: a patient-level data (IPD) meta-analysis of clinical trials and prospective studies. J Thromb Haemost. 2011;9:312–319.
6. Lefebvre P, Laliberté F, Nutescu EA, et al. All-cause and potentially disease-related health care costs associated with venous thromboembolism in commercial, Medicare, and Medicaid beneficiaries. J Manag Care Pharm. 2012;18:363–374.
7. MacDougall DA, Feliu AL, Boccuzzi SJ, Lin J. Economic burden of deep-vein thrombosis, pulmonary embolism, and post-thrombotic syndrome. Am J Health Syst Pharm. 2006;63:S5–S15.
8. Spyropoulos A, Lin J. Direct medical costs of venous thromboembolism and subsequent hospital readmission rates: an administrative claims analysis from 30 managed care organizations. J Manag Care Pharm. 2007;13:475–486.
9. Lobo BL, Vaidean G, Broyles J, et al. Risk of venous thromboembolism in hospitalized patients with peripherally inserted central catheters. J Hosp Med. 2009;4:417–422.
10. Evans RS, Sharp JH, Linford LH, et al. Risk of symptomatic DVT associated with peripherally inserted central catheters. Chest. 2010;138:803–810.
11. Piazza G, Goldhaber SZ, Lessard DM, et al. Venous thromboembolism in heart failure: preventable deaths during and after hospitalization. Am J Med. 2011;124:252–259.
12. Chopra V, Ratz D, Kuhn L, et al. Peripherally inserted central catheter-related deep vein thrombosis: contemporary patterns and predictors. J Thromb Haemost. 2014;12:847–854.
13. Alnimer Y, Haddad RA, Abdalla A, Bachuwa G, Rios-Bedoya C. PICC line-related thrombosis; Does pharmacological deep vein thrombosis help? J Hosp Med. 2017;12(2).
14. Shorr AF, Kwong LM, Sarnes M, Happe L, Farrelly E, Mody-Patel N. Venous thromboembolism after orthopedic surgery: implications of the choice for prophylaxis. Thromb Res. 2007; 121(1):17-24.

Disclosure

Author Contributions Statement

All authors have read and approved the final submitted manuscript. The following is the author contribution: MJL, EVL, MJS, JLC: substantial contributions to research design, acquisition, analysis and interpretation of data; MJL, BSS, SMH, AG, JM, ZS, BTM, JLC, EVL, MJS: drafting the paper and revising it critically; MJL, BSS, SMH, AG, JM, ZS, BTM, JLC, EVL, MJS: approval of the final and submitted versions.

Ethical Review Statement

This study was approved and executed under approval from the University of Missouri's Institutional Review Board (#2014081). No external funding was used in the execution of this study.

Conflict of Interest Statement

The author group reports the following conflicts:

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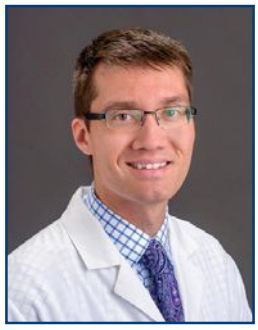
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Current Hybrid Interventions in Vascular Surgery: Merging Past and Present

by Jennifer Huffman, MD, W. Kirt Nichols, MD & Jonathan Bath, MD

Hybrid interventions in vascular surgery have evolved significantly in the last 50 years and have become a mainstay treatment option in re-vascularization of complex vascular disease.



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Abstract

As the United States population continues to age, the prevalence of vascular disease continues to increase and thus the role of vascular surgeons in disease intervention has become more important than ever. Over the last 30 years, endovascular techniques have become the mainstay for management of vascular disease. However, open operations continue to play a role in management of complex vascular disease. This article provides an update and discussed the role of hybrid interventions in vascular surgery with a focus on its use in femoral endarterectomy and peripheral stenting, retrograde open mesenteric stenting.

Introduction

Hybrid interventions in vascular surgery are defined as the utilization of both open surgical and endovascular techniques simultaneously in a single setting operation. The first hybrid procedure dates back to 1973 when dilation of pelvic arteries was performed along with a femoral-femoral bypass procedure for limb salvage.[1] Since that time, the use of hybrid procedures has grown popularity as it allows additional management options for complex revascularization procedures in patients with significant comorbidities and complex disease. Hybrid procedures were initially utilized for the management of peripheral artery disease and in 2008 accounted for between five and 21% of all vascular reconstructions.[2] As the use of hybrid interventions has grown, indications for use have grown beyond peripheral arterial vascularization and now include visceral vessel revascularization as well as carotid revascularization.

Endovascular techniques were initially developed in the 1970s with the advent of percutaneous recanalization after chronic arterial occlusion.[3] Since their introduction, utilization of endovascular techniques has grown exponentially, and they have become integral in the routine clinical management of vascular disease with progressive replacement of open surgery by endovascular procedures.[4] However, the need for open interventions remain. Hybrid procedures have combined elements of both open surgery and endovascular techniques as an integral strategy in re-vascularization operations. Benefits of hybrid intervention include decreasing delays to complete revascularization, the ability to immediately repair inadequate endovascular results and vice

versa, immediate angiographic confirmation of revascularization, and shortened hospital stays, making them an attractive option for modern vascular surgical intervention.[5] This review aims to review three of these hybrid interventions in vascular surgery.

Femoral Endarterectomy and Peripheral Stenting

Introduction

Aortoiliac and common femoral occlusive disease is a variation of peripheral artery disease that affects the lower aorta and can result in debilitating claudication and tissue loss.[6] These lesions can be exceedingly difficult to manage as they involve multiple levels of the arterial tree. The gold standard for treatment of these lesions involves open surgical reconstruction with aortobifemoral or iliofemoral bypass.[7] These operations are extensive, requiring laparotomy or flank incision and often necessitate aortic cross clamping resulting in high operative morbidity and mortality that is often poorly tolerated in patients with high operative risk.[8] The advent of endovascular procedures including iliac balloon percutaneous transluminal angioplasty and stenting have allowed an alternative to open surgery for focal iliac occlusive disease in high risk patients.[7] Meanwhile, femoral endarterectomy for occlusive disease of the common femoral artery has remained standard of practice for over 50 years.[9] A hybrid approach combining endovascular iliac stenting and common femoral endarterectomy presents a viable alternative option to open aortoiliofemoral reconstruction.

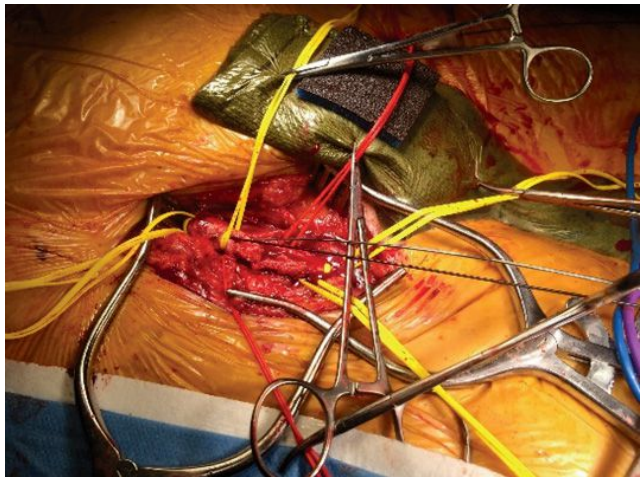


Figure 1. Demonstrates open access of the groin with the endovascular sheath in place

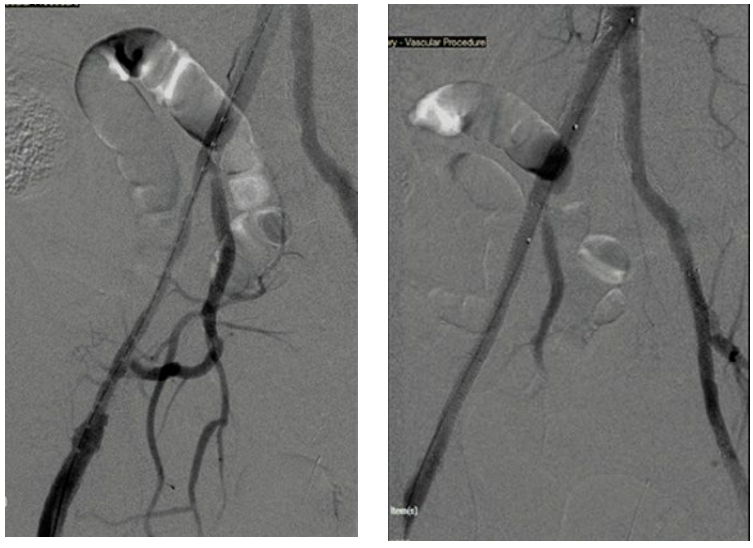


Figure 2. Panel A (left) demonstrates the pre iliac stenting angiography and Panel B (right) demonstrates post stenting iliac angiography.

Case Report 1

A representative case study involves a 68-year-old male with four-week history of foot ulceration who had absent right femoral pulse on physical examination. CTA was performed demonstrating severe atherosclerotic disease of the right common iliac artery as well as the right common femoral artery. Traditional open revascularization in this patient would require aorto-bifemoral bypass with femoral endarterectomy. However, a hybrid intervention with femoral endarterectomy and peripheral stenting is also an option. The patient was taken to the operating room for hybrid intervention with plan for femoral endarterectomy and peripheral stenting. The procedure was performed as follows. Using ultrasound, the femoral artery was accessed, and a 7 French sheath placed. The iliac lesion was able to be recanalized and wire access was maintained into the distal aorta. The endarterectomy of the common femoral and profunda femoris was then performed, and a patch angioplasty completed using Bovine pericardium. The entire iliac system was then treated with balloon-expandable stents placed into

the common iliac artery and self-expanding stents from the external iliac artery down into the femoral patch. Completion angiography demonstrated three vessel run off to the lower extremity at the completion of the operation. See Figure 1 and Figure 2.



Figure 3. Demonstrates the open view of the superior mesenteric artery with the transverse colon at the superior aspect and the small bowel at the inferior aspect. The small bowel mesentery is opened transversely to expose the SMA.

Discussion

Hybrid approaches combining elements of endovascular iliac stenting and open common femoral endarterectomy are a viable surgical option for the management of complex aortoiliofemoral peripheral vascular disease. As these alternative hybrid approaches have gained popularity, studies have aimed to demonstrate their safety, efficacy, and durability when compared to traditional open repair options. When compared to the gold standard open operative repair, hybrid repair options are particularly attractive option for high-risk operative patients. Traditional open repair operations are morbid operations associated with 8.3% risk of morbidity and 3.3% risk of mortality for all comers.[10] Hybrid repair options are less invasive and present an option for repair in older patients with more comorbidities without a prohibitive level of operative mortality. A hybrid approach eliminates the invasiveness of an open operation and decreases the physiological stress associated with aortic cross-clamping and laparotomy.[11] Additionally, this repair option is associated with shortened ICU and hospital length of stays also contributing to a reduced 30-day perioperative mortality in these patients.[11] Overall survival after three years is higher in the open repair patients when compared to hybrid repair patients which likely represents a selection bias where the sickest patients were preferentially treated with hybrid repair and therefore had lower long term survivability as a result of their comorbid disease.[11] Therefore, hybrid repair is a viable option in high-risk patients unable to tolerate an open operation.



Figure 4. Panel A, (left), represents pre stenting CTA of the SMA and Panel B, (right), represents post stenting CTA of the SMA.

Review of the literature demonstrates that hybrid repair options are a durable repair option. Studies have shown that hybrid repair options have an overall technical success rate of 99% compared to 100% success rate in open repair resulting in post-operative improvement in claudication symptoms and ABI.[11] Both open and hybrid repair options are associated with a greater than 90% patency. Multiple studies have demonstrated no statistically significant difference between primary graft patency in both groups at both one- and three-year intervals.[11,12] Review of the literature demonstrates a one-year patency of 94% in open repair and 92% in hybrid repair patients.[12] Three-year patency is cited at 95% for open repair patients, and 97% for hybrid repair patients.[11] Hybrid repair options for complex aortoiliofemoral peripheral

vascular disease are technically successfully, safe, and equivalent in post-operative patency rates when compared to traditional open approaches.

Retrograde Open Mesenteric Stenting

Introduction

Acute mesenteric ischemia is a rare and challenging problem that often leads to delayed diagnosis and comes with an associated mortality rate the range of 50%.[13] Traditionally, acute mesenteric ischemia was managed with a combined approach utilizing open mesenteric bypass and bowel resection with second look operation in 24 to 48 hours.[14] Meanwhile, the rise of endovascular therapy has also presented alternative options for management especially in elderly patients with multiple comorbidities who would otherwise not tolerate an open operation. However, some lesions remain incompatible with a completely endovascular approach.[15] The advent of retrograde open mesenteric stenting has emerged as an alternative option that allows faster revascularization, is effective for embolic or thrombotic disease, and reduces intraoperative time.

Case Report 2

A representative case study involves an 83-year-old male with history of weight loss and abdominal pain with eating who presented with acute worsening of his abdominal pain. CTA was performed demonstrating severe atherosclerotic disease of the superior mesenteric artery with completely occluded blood flow. He was taken to the operating room with plan for hybrid revascularization with retrograde open mesenteric stenting. Anterior approach to the SMA was performed via a midline laparotomy. The transverse colon was mobilized superiorly, and the small bowel was mobilized interiorly. The small bowel mesentery was then opened transversely exposing the SMA. Attempted thrombectomy was unsuccessful. The SMA was then accessed and a 7 French sheath placed. The SMA lesion was able to be recanalized and position in the aortic lumen was confirmed. Stent was then placed across the SMA with extension several millimeters into the aorta. Post intervention CTA demonstrated restoration of blood flow through the SMA. See Figures 3 and 4.

Discussion

Hybrid approaches combining open laparotomy with retrograde open mesenteric stenting are a viable surgical alternative to open embolectomy or percutaneous stenting in the management of acute mesenteric ischemia. Several factors contribute to poor suitability for percutaneous stenting including patients with lesions that are difficult to visualize because of flush occlusions or that are difficult to cross with standard guidewire techniques.[16,17] Acute occlusions are also prone to distal embolization because of fresh thrombosis, and therefore poorly suited for purely endovascular repair approaches.[18] Direct exposure of the vessel allows rapid access for puncture, improved ability to cross the lesion, and less risk of distal embolization.[5] Stenting also avoids the need to reconstruct the vessel by surgical bypass and avoids the need for vein harvesting.[17] Additionally, this approach allows immediate assessment of bowel viability which is impossible with purely endovascular approach.

Multiple studies have sought to demonstrate the safety and efficacy of these hybrid procedures in the management of mesenteric ischemia. Retrograde open mesenteric stenting is technically successful in 98% of patients.[16] Despite technical success, early mortality rates remain high around 45%, which likely reflects a delay in diagnosis and treatment. This mortality rate is similar to existing literature for traditional repair approaches.[1,9] Primary patency rates at two years are 76%.[16] Freedom from symptom recurrence and freedom from re intervention are 72% and 74% over a two-year period.[16] Therefore, retrograde open mesenteric stenting should be considered over percutaneous stenting in patients with flush occlusion or peritoneal signs as these patients require laparotomy for assessment of bowel viability.[20] This approach is equivalent in safety and efficacy when compared to traditional approaches.

Transcarotid Artery Revascularization

Introduction

Carotid artery stenosis accounts for 7% to 10% of all ischemic strokes.[21] The gold standard

operation for management of carotid artery stenosis is a traditional open carotid endarterectomy due to its excellent durability and low intraoperative stroke risk.[22] Transfemoral carotid stenting has emerged as an option for endovascular repair however this procedure is riddled with issues including a high stroke rate, difficulty with navigating the aortic arch and crossing the lesion, post deployment emboli and risk of restenosis.[23] Hybrid option of transcarotid artery revascularization has emerged as it allows a less invasive approach while eliminating many of the issues associated with transfemoral carotid stenting.

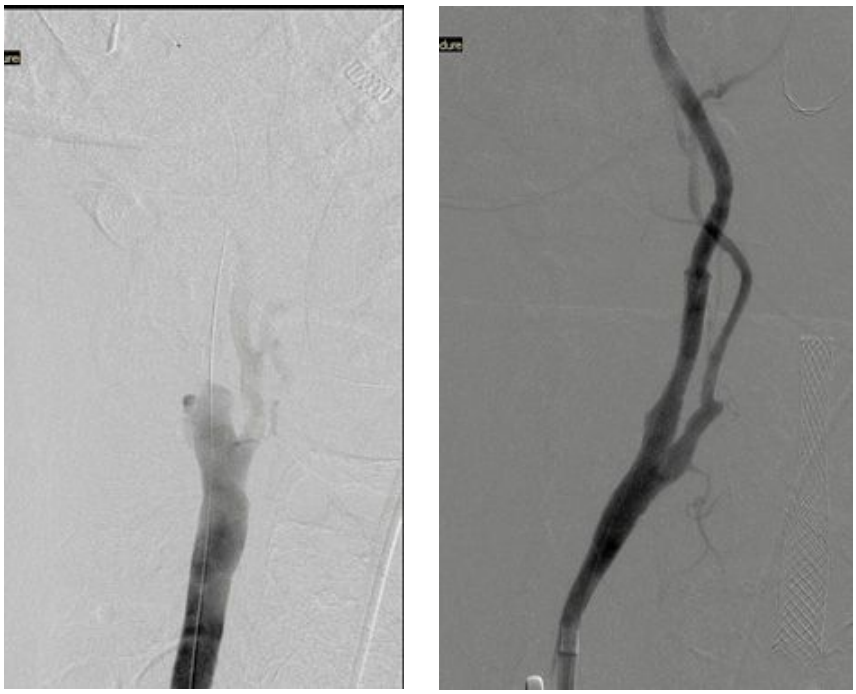


Figure 5. Panel A represents pre stenting angiography of the carotid and Panel B represents post stenting angiography of the carotid.

Case Report 3

A representative case study for the procedure involves a 60-year-old female with multiple medical comorbidities including a history of laryngeal cancer with neck radiation, lung cancer, COPD on three liters of home oxygen, and carotid stenosis. Given her multiple comorbidities and history of neck irradiation, she was a poor candidate for open revascularization and therefore presented to the OR for transcarotid artery revascularization. A neck incision is made along the sternocleidomastoid muscle and the carotid artery is exposed and then cannulated with placement of a sheath. This catheter is externally connected a flow reversal device that connects to a femoral vein cannulation. This allows reversal of flow in the carotid artery thus preventing embolization to the brain in the stenting process. Once flow reversal is established, a wire is passed across the stenotic lesion. Pre-dilation of the lesion is performed followed by stent placement across the stenosis. Completion angiography confirms good position of the stent and the artery is then decannulated. See Figure 5.

Discussion

With the advent of hybrid interventions for the management of carotid atherosclerotic disease, literature has sought to compare this novel procedure to traditional open carotid endarterectomy to demonstrate its safety and efficacy. Carotid endarterectomy remains the gold standard for treatment of patients with carotid artery disease, mostly secondary to high rates of morbidity and mortality associated with purely endovascular interventions for carotid disease.[24] The advent of carotid flow reversal systems has allowed hybrid approaches to carotid repair while providing a neuroprotection system not available with traditional endovascular approaches.[25] When comparing CEA to TCAR approaches, TCAR patients had higher rates of pre-operative co-morbidities including diabetes mellitus, hyperlipidemia, coronary artery disease, and renal insufficiency when compared to carotid endarterectomy patients, owing to a selection for less invasive intervention strategies in higher risk patient populations.[6] Stroke rates were similar between the two groups and 30 days (1.0% TCAR vs 0.3%) and one-year (stroke, 2.8% vs 2.2%).[26] Mortality rates were also similar between the two groups at 30 days (0.3% TCAR vs 0.7%) and one-year (stroke, 2.8% vs 2.2%).[26] TCAR was

associated with a decreased rate of cranial nerve injury 0.3% vs 3.8%).[26] Therefore, TCAR achieves similar outcomes compared with CEA while mitigating cranial nerve injury.

Studies have also sought to compare outcomes in patients undergoing traditional transfemoral carotid artery stenting versus hybrid TCAR procedures. Patients undergoing TCAR procedures were significantly older, had more cardiac comorbidities and were more likely to be asymptomatic. Trans-femoral carotid artery stenting was associated with twice the odds of in hospital adverse neurological events and TIA/stroke/death when compared to TCAR independent of symptom status.[27]

Conclusion

Hybrid interventions in vascular surgery have evolved significantly in the last 50 years and have become a mainstay treatment option in re-vascularization of complex vascular disease. Hybrid procedures remain important in contemporary vascular practice and will certainly continue to evolve in the future of vascular surgery.

References

1. Porter JM, Eidemiller LR, Dotter CT et al: Combined arterial dilatation and femorofemoral bypass for limb salvage. Surg Gynecol Obstet 1973;137:409–412.
2. Ebaugh JL, Gagnon D, Owens CD et al: Comparison of costs of staged versus simultaneous lower extremity arterial hybrid procedures. Am J Surg 2008;196:634–640.
3. Gruentzig, A, Hopff, H: Percutaneous recanalization after chronic arterial occlusion with a new dilator-catheter (modification of the Dotter technique). Dtsch Med Wochenschr 1974; 99: 2502–2511.
4. Lepore MR Jr, Yoselevitz M, Sternbergh WC 3rd, Money SR. Minimally invasive vascular techniques. Ochsner J. 2000;2(3):145-152.
5. Balaz P, Rokosny S, Bafrnec J, Björck M. The Role of Hybrid Procedures in the Management of Peripheral Vascular Disease. Scandinavian Journal of Surgery. 2012;101(4):232-237.
6. Clair DG, Beach JM. Strategies for managing aortoiliac occlusions: access, treatment and outcomes. Expert Rev Cardiovasc Ther. 2015;13(5):551-563.
7. Norgren L, Hiatt WR, Dormandy JA, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II) J Vasc Surg. 2007;45(Suppl S):S5–67.
8. Dimick JB, Cowan JA, Henke PK, et al. Hospital volume-related differences in aorto-bifemoral bypass operative mortality in the United States. J Vasc Surg. 2003;37(5):970–5
9. J.L. Kang, V.I. Patel, M.F. Conrad, G.M. Lamuraglia, T.K. Chung, R.P. Cambria. Common femoral artery occlusive disease: contemporary results following surgical endarterectomy. J Vasc Surg, 48 (2008), pp. 872-877
10. S.O. de Vries, M.G. Hunink. Results of aortic bifurcation grafts for aortoiliac occlusive disease: a meta-analysis. J Vasc Surg, 26 (1997), pp. 558-569
11. Piazza M, Ricotta JJ 2nd, Bower TC, Kalra M, Duncan AA, Cha S, Gloviczki P. Iliac artery stenting combined with open femoral endarterectomy is as effective as open surgical reconstruction for severe iliac and common femoral occlusive disease. J Vasc Surg. 2011 Aug;54(2):402-11.
12. Zamor KC, Hoel AW, Helenowski IB, Beck AW, Schneider JR, Ho KJ. Comparison of Direct and Less Invasive Techniques for the Treatment of Severe Aorto-Iliac Occlusive Disease. Ann Vasc Surg. 2018 Jan;46:226-233.
13. M. Bala, J. Kashuk, E.E. Moore, Y. Kluger, W. Biffl, C.A. Gomes, et al. Acute mesenteric ischemia: Guidelines of the world society of emergency surgery. World J Emerg Surg, 12 (2017), p. 38.
14. G.S. Oderich, R. Malgor, J.J. Ricotta II. Open and endovascular revascularization for chronic mesenteric ischemia: A tabular review of the literature. Ann Vasc Surg, 23 (2009), pp. 700-712.
15. Arthurs ZM, Titus J, Bannazadeh M, Eagleton MJ, Srivastava S, Sarac TP, Clair DG. A comparison of endovascular revascularization with traditional therapy for the treatment of acute mesenteric ischemia. J Vasc Surg. 2011 Mar;53(3):698-704; discussion 704-5.
16. Oderich GS, Macedo R, Stone DH, Woo EY, Panneton JM, Resch T, Dias NV, Sonesson B, Schermerhorn ML, Lee JT, Kalra M, DeMartino RR, Sandri GA, Ramos Tenorio EJ; Low Frequency Vascular Disease Research Consortium Investigators. Multicenter study of retrograde open mesenteric artery stenting through laparotomy for treatment of acute and chronic mesenteric ischemia. J Vasc Surg. 2018 Aug;68(2):470-480.e1.
17. Scali ST, Ayo D, Giles KA, Gray S, Kubilis P, Back M, Fatima J, Arnaoutakis D, Berceli SA, Beck AW, Upchurch GJ, Feezor RJ, Huber TS. Outcomes of antegrade and retrograde open mesenteric bypass for acute mesenteric ischemia. J Vasc Surg. 2019 Jan;69(1):129-140.
18. E.J. Ryer, M. Kalra, G.S. Oderich, A.A. Duncan, P. Gloviczki, S. Cha, et al.Revascularization for acute mesenteric ischemia. J Vasc Surg, 55 (2012), pp. 1682-1689.
19. J.T. Blauw, T. Bulut, G.S. Oderich, B.R. Geelkerken, Dutch Mesenteric Ischemia Study GroupMesenteric vascular treatment 2016: from open surgical repair to endovascular revascularization. Best Pract Res Clin Gastroenterol, 31 (2017), pp. 75-84.
20. D.G. Clair, J.M. Beach. Mesenteric ischemia. N Engl J Med, 374 (2016), pp. 959-968.
21. Otite FO, Khandelwal P, Malik AM, Chaturvedi S. National Patterns of Carotid Revascularization Before and After the Carotid Revascularization Endarterectomy vs Stenting Trial (CREST). JAMA Neurol. 2018;75(1):51–57.
22. Barnett HJM, Taylor DW, Haynes RB, et al; North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med. 1991;325(7):445-453.
23. AbuRahma AF, Mousa AY. Current Status of Carotid Stenting Versus Endarterectomy. Adv Surg. 2016 Sep;50(1):235-56.
24. A. Alpaslan, M. Wintermark, L. Pinter, S. Macdonald, R. Ruedy, R. KolvenbachTranscarotid artery revascularization with flow reversal. J Endovasc Ther, 24 (2017), pp. 265-270.
25. C.J. Kwolek, M.R. Jaff, J.I. Leal, L.N. Hopkins, R.M. Shah, T.M. Hanover, et al.Results of the ROADSTER multicenter trial of transcarotid stenting with dynamic flow reversal. J Vasc Surg, 62 (2015), pp. 1227-1234.
26. Kashyap VS, King AH, Foteh MI, Janko M, Jim J, Motaganahalli RL, Apple JM, Bose S, Kumins NH. A multi-institutional analysis of transcarotid artery revascularization compared to carotid endarterectomy. J Vasc Surg. 2019 Jul;70(1):123-129.
27. Malas MB, Dakour-Aridi H, Wang GJ, Kashyap VS, Motaganahalli RL, Eldrup-Jorgensen J, Cronenwett JL, Schermerhorn ML. Transcarotid artery revascularization versus transfemoral carotid artery stenting in the Society for Vascular Surgery Vascular Quality Initiative. J Vasc Surg. 2019 Jan;69(1):92-103.e2.

Disclosure

None reported.

MM

Home-Based Arm Cardiac Rehabilitation in Disabled Veterans: A Pilot Study

by Anand Chockalingam, MD, Albert Chan, MD, Jill A. Kanaley, PhD & Kul Aggarwal, MD

Home-based tele-monitored individually- tailored cardiac rehabilitation is promising for disabled patients.



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Abstract

One of the cornerstones of treatment after acute coronary syndromes is cardiac rehabilitation (CR). However, traditional CR remains underused in the United States due to comorbidities and geographical limitations. To evaluate feasibility and safety of our individually tailored CR program, we evaluated twelve weeks of tele-monitored home-based arm ergometer and weight training exercises in seven Veterans. Prior to beginning our CR program, all Veterans underwent an arm ergometer stress test and training in the proper techniques for arm exercises and weight training. Seattle Angina Questionnaire (SAQ) and the MacNew Heart Disease Health-related Quality of Life (MacNew) questionnaire were administered at the beginning and conclusion of the program. Six patients completed the study. One withdrew due to generalized weakness. There were no adverse events during the study period. There was a perceived improvement in heart disease related global (4.47 to 4.61), physical, emotional, and social well-being by the MacNew questionnaire. The SAQ showed improvement in physical limitation, angina frequency, treatment satisfaction, and overall quality of life (36.1 to 51.7) after completion of our tailored CR program. There was a decrease in average blood pressure and patients were able to exercise seven minutes longer and workload increased eight additional watts. This pilot study demonstrates the safety and feasibility of a home-based arm cardiac rehabilitation program. These tailored programs may improve quality of life in coronary artery disease patients with disabilities.

Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in the United States.[1] The care of CAD patients has undergone significant improvement over the past several decades with newer imaging modalities, percutaneous coronary interventions (PCI), coronary artery bypass grafting (CABG) surgery and pacing therapies.[2] However, to truly control atherosclerosis, reduce mortality and improve quality of life, lifestyle changes with diet and exercise remain crucial.[3] We now have direct evidence that atherosclerosis can be reversed[4, 5] and this requires long-term adherence to a healthy diet, regular exercise and aggressive medical therapy.[6, 7]

Cardiac rehabilitation (CR) programs focus on these lifestyle issues and can reduce cardiac events by about 20%.[8, 9] Unfortunately, CR programs remain underused in the United States, with an estimated participation rate of only 10% to 20% of the over two million eligible patients annually.[3, 10] Contributing to the vast underuse of these services are a low patient referral rate, older age, poor patient motivation and geographic limitations to CR center access.[3] Arthritis, peripheral artery disease, neurologic disorders (e.g., stroke, paraplegia and gait disorders), COPD, amputations, as well as psychological factors such as depression are further barriers to CR participation.[11-13]

To potentially rectify these concerns, home-based CR programs with a nurse supervising the various aspects of patient care and progress through CR have been developed over last 3 decades.[14-17] A recent meta-analysis of 12 studies concluded that in stable CAD patients, home-based CR programs were safe and as effective as center based programs in improving health and quality of life.[18] Arm exercises are encouraged when there are walking difficulties, but this has not been systematically explored as the central CR exercise modality.[19] 'Transfer effect' is an important concept whereby training in one muscle group produces cardiac and general circulatory benefits.[20-22] Arm training improves exercise capacity in COPD.[23] In randomized studies in patients with peripheral vascular disease, arm ergometry compared with treadmill exercise training showed equivalent improvement in walking distance.[24, 25] The aim of our home-based arm CR program is to determine compliance and effectiveness of an individually tailored home-based arm-exercise CR program among disabled Veterans unable to participate in traditional CR. With ongoing COVID distancing concerns, many CR programs are embracing virtual home based CR methods worldwide.

Methods

The HACR study was a prospective single center pilot study at the Harry S Truman Memorial Veterans Hospital (HSTMVH) in Columbia, MO. All veterans at HSTMVH diagnosed with CAD and one of the following presentations were eligible for enrollment: stable angina, acute myocardial infarction (ST elevation MI and NSTEMI), post-percutaneous coronary intervention (PCI), or post-coronary artery bypass grafting (CABG). Veterans meeting these criteria were then screened to see if they could participate in conventional treadmill-based cardiac rehabilitation. Those who could not perform leg exercises because of arthritis, stroke or other limiting factors were considered for HACR. Patients were excluded if they had decompensated heart failure, unstable angina, complex ventricular arrhythmias, uncontrolled hypertension (systolic blood pressure >200 mmHg, diastolic blood pressure >100mmHg), severe symptomatic valvular disease, dementia, or expected life-expectancy of less than two years. All veterans provided written informed consent to enroll in the study. The study was approved by the local Institutional Review Board and the VA Research Committee.

Initial Visit

During the initial visit, baseline parameters including vital signs, weight, height, body fat analysis, and waist circumference were obtained. Medical history and current medications were reviewed with each Veteran in a manner similar to the initial entry visit for traditional phase II CR. Laboratory tests were performed to measure initial complete metabolic panel, complete blood count, B-natriuretic peptide (BNP), fasting lipid profile, fasting blood sugar, high-sensitivity C-reactive protein (hs-CRP), and hemoglobin A1c (HbA1c). Functional status and heart disease related quality of life was measured using the Seattle Angina Questionnaire (SAQ) and MacNew Heart Disease Health-related Quality of Life (MacNew) questionnaire respectively.[26, 27]

A supervised arm ergometer stress test was performed using a calibrated Monark Rehab Trainer (Monark Exercise AB, Vansbro, Sweden) to determine baseline functional capacity, exclude ischemia and unstable arrhythmias, and set targets for the home-based arm exercise program using the Borg scale of Perceived Exertion.[28] Veterans performed arm ergometry in the seated position with the axle adjusted to shoulder height. Initial work load was without any added resistance for one minute. Resistance was increased by 15-20 Watts every two minutes. Blood pressure and a

12-lead ECG were obtained during a 30-second break between each increase in resistance. Veterans were asked to rate perceived exertion (RPE) on the Borg Scale of 6 to 20 at each level of arm ergometry. Each patient's increase in resistance was customized to target total exercise duration of 10-20 minutes. The workload reached by the patient in the last complete minute was considered the peak workload. Predetermined end points for terminating the stress test were failure to maintain 50 rotations per minute, angina or dyspnea or generalized fatigue requiring the Veteran to cease exercise, ST segment depression of at least 2mm, serious ventricular arrhythmias, or a decrease of 20 mmHg or more in systolic blood pressure.

Upon completion of the arm ergometer stress test, the participants were provided and instructed in the proper use of a home arm ergometer and a heart rate monitor (Polar Electro, Kempele, Finland) by cardiac rehabilitation nurses. Muscle strength was tested using dumbbells for four upper body resistance exercises (chest press, shoulder press, biceps curl, triceps extension). The Veterans were tested for 1-Repitition Maximum (RM) Assessment. The 1-RM is calculated by having the Veteran perform the exercise with the maximum amount of weight tolerated with proper form and range of motion. Once the 1-RM was obtained for each exercise, dumbbells were provided for the participants at a weight that was safe to perform two sets of 15 repetitions with proper form and range of motion. Education regarding the participant's heart disease, exercise plan, diet, and smoking cessation were provided as well at the initial visit.

Home Rehabilitation

The participants were prescribed an arm ergometry regimen that consisted of a warm up period of two minutes at 40-50% of age-predicted maximum heart rate (or Borg perceived exertion score of 7 to 9) followed by an increase to 50-70% of maximum heart rate (or Borg score of 13 to 15) for 20 minutes. The participants adjusted the resistance of their home arm ergometer to reach this target. The aerobic training session would then conclude with a two-minute cool down at 40-50% of maximum heart rate (or Borg score of 7 to 9). Following a short period of rest, participants were instructed to perform two sets of 15 repetitions for each of the four upper body resistance exercises using the dumbbells provided. The Veterans were encouraged to perform the prescribed exercises three times per week and were given a diary to record exercise adherence and heart rates. They were also instructed to stop exercise for any new cardiac symptoms and seek medical attention.

Follow-Up

Weekly phone calls were performed by research personnel to monitor progress, encourage compliance and offer lifestyle education as well as to address any patient concerns. Veterans were seen at the HSTMVH CR center once in every four weeks during the 12-week HACR study for supervised arm ergometry and resistance training and to ensure proper technique and safety. If the Veterans were tolerating and safely able to increase the weights, they were given new sets of dumbbells. CR nurses addressed patient concerns, reviewed medications provided education about CAD risk factors similar to traditional CR. At the end of the 12-week HACR study, the Veteran had an exit visit with a review of clinical information and vital signs. Laboratory tests, SAQ and MacNew questionnaires and a supervised arm ergometer stress test with the same protocol as the initial arm ergometer stress test were repeated to estimate HACR effect.

Results

Seven patients enrolled in the study. All participants were male with ages ranging from 58 to 65 years old. Of the seven, six completed the study in its entirety. One patient voluntarily dropped out of the study due to generalized weakness. The patients enrolled in the study were typical of the Veteran population with a high prevalence of cardiac risk factors including hypertension, hyperlipidemia, tobacco use, and diabetes mellitus. There were no study related adverse events and no coronary events, hospitalizations or revascularizations.

Overall, the patients adhered to the HACR program very well. They found the program to be user-friendly and were motivated to perform their scheduled exercises. Aerobic exercise was performed an average of three days/week for an average duration of 20 minutes. Resistance exercise was performed an

Figure 1. MacNew Heart Disease Health-related Quality of Life Results

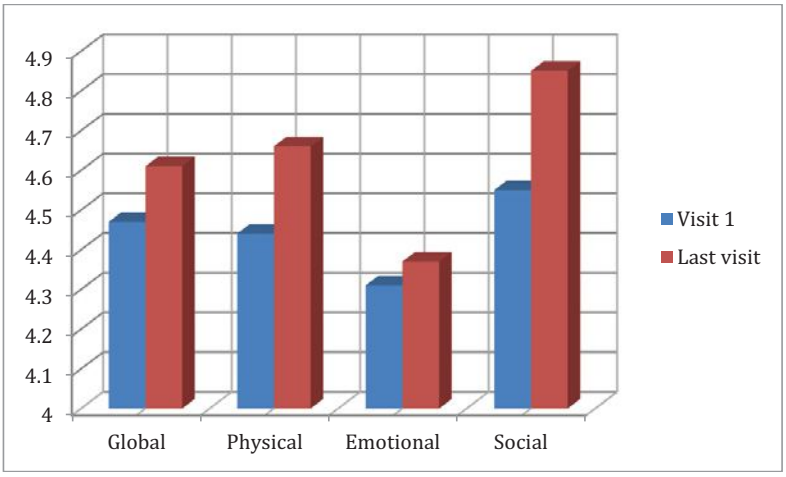
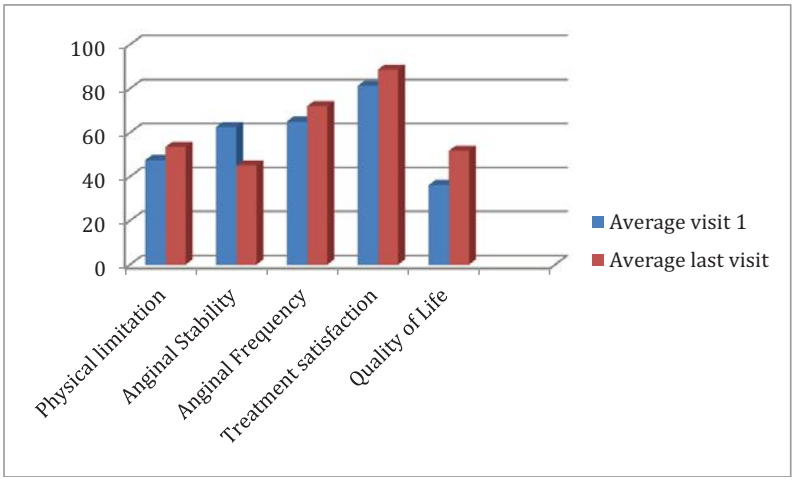


Figure 2. Seattle Angina Questionnaire Results



average of three days/week also for about 20 minutes. The participants raised no significant concerns with the program and were satisfied with the HA CR program and with their results, as evidenced by the questionnaires.

Participants showed some promising trends in perception of their functional status and quality of life. On both the MacNew and SAQ questionnaires, overall improvement was noted. There was, however, some expected variation in perceived improvement from subject to subject enrolled in this study. There was a perceived improvement in heart disease-related global, physical, emotional, and social well-being by the MacNew questionnaire (Figure1). In regards to the SAQ results, on average there was a decrease in anginal stability, but an improvement in physical limitation, anginal frequency, treatment satisfaction and overall quality of life after HACR (Figure 2).

The clinical and hemodynamic changes after HACR are shown in Table 1. There was a decrease in average blood pressure and a slight increase in average weight. Patients were able to exercise for an average of seven minutes longer and workload was increased by eight Watts after 12 weeks of HACR. Laboratory markers were not substantially changed from initial values following HACR (Table 2).

Discussion

Veterans with disabilities and walking limitations eagerly participated in HACR. The home exercise program was well tolerated with no adverse events despite numerous co-morbidities in this high-risk cohort. Veterans adhered to the exercise regimen of arm ergometry and upper body weight training for the three-month study period with a high HACR completion rate of 85%, despite this CR program being offered only to sicker Veterans ineligible for traditional CR due to comorbidities. The willingness of Veterans to participate and their motivation to complete the program is an advantage of this program.

Quality of life appears to improve with HACR participation by use of validated questionnaires to measure perceived functional capacity and heart disease related quality of life. Angina scores were mixed and this may reflect increased myocardial demand from increased physical activity levels or may be a limitation of the scoring system used.[29] In CR settings, where coronary anatomy is angiographically defined and appropriate revascularization completed, angina could represent achieving adequate exercise threshold to reduce systemic inflammation and stimulate collateral reserve.[30] We utilize this to help our cardiac patients deepen mind-body connection, explore meditative relaxation during exercise (exercise awareness smile experience, EASE), enter Flow state, gain self-confidence and genuinely enjoy exercise and sports.[31]

Table 1. Average Parameters During Arm Ergometer Stress Testing at Baseline and Post-HACR

	Resting Heart Rate (bpm)	Peak Heart Rate (bpm)	Resting Blood Pressure (mmHg)	Weight (lbs.)	BMI (kg/m^2)	Exercise time (min)	Max Workload (watts)
Baseline	71.16	105.66	141/81	230.2	31.4	18	43
Post- HACR	71.50	113.0	130/78	233.3	32.0	25	51

Table 2. Average Laboratory Values at Baseline and Post-HACR

	Hemoglobin (mg/dL)	Total Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)	BNP (pg/ml)	HbA1c (%)
Baseline	14.5	145	78	32	196	90	6.5
Post-HACR	14.88	161	88	36	208	82	6.5

Cardiac risk factors show mixed results with LDL levels worsening and HDL levels improving. BNP improved on average, although not all patients had a diagnosis of CHF in this study, and most BNP lab values were in the normal range. Weight increased despite completion of the HACR program. This may be related to more appetite due to increasing physical activity. Personal interactions with investigators also emphasized the crucial importance of behavioral interventions and frequent reinforcement to sustain lifestyle interventions. Importantly, functional capacity appears to improve with HACR as evidenced by improved workload, exercise time, and reduced dyspnea. This in turn may help Veterans regain their confidence to engage in more vigorous activities, gainful employment, and reduce their CAD-related disability.

This study does have limitations. It was a small cohort study without controls. As the HACR pilot group is comprised only of traditional phase II CR ineligible Veterans, we feel the results are encouraging as a proof of concept and demonstrate safety. A larger randomized study is needed to determine the ideal patient population, efficacy, reproducibility of the findings, and if cardiac risk factors and outcomes improve with this type of program. By increasing the study size, we would be able to determine if the results we achieved in this study are reproducible and statistically significant.

Conclusion

The home-based arm cardiac rehabilitation pilot program showed several encouraging findings. High risk disabled Veterans with medical comorbidities were able to accommodate HACR into their schedule, were pleased with the program and felt that it benefited them. Quality of life, blood pressure and functional capacity improved, while lipid profile and weight did not. Home-based tele-monitored individually-tailored cardiac rehabilitation is promising for disabled patients. Further studies with this structured program are needed in order to determine if tailored home arm CR programs would provide long-term benefits and reduce cardiac events.

References

1. Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the united states, 1970-2002. JAMA : The Journal of the American Medical Association. 2005;294:1255-1259

2. Wright SM, Petersen LA, Daley J. Availability of cardiac technology: Trends in procedure use and outcomes for patients with acute myocardial infarction. Medical Care Research and Review : MCRR. 1998;55:239-254
3. Leon AS, Franklin BA, Costa F, Balady GJ, Berra KA, Stewart KJ, Thompson PD, Williams MA, Lauer MS. Cardiac rehabilitation and secondary prevention of coronary heart disease: An american heart association scientific statement from the council on clinical cardiology (subcommittee on exercise, cardiac rehabilitation, and prevention) and the council on nutrition, physical activity, and metabolism (subcommittee on physical activity), in collaboration with the american association of cardiovascular and pulmonary rehabilitation. Circulation. 2005;111:369-376
4. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: The asteroid trial. JAMA : The Journal of the American Medical Association. 2006;295:1556-1565
5. Ballantyne CM, Raichlen JS, Nicholls SJ, Erbel R, Tardif JC, Brener SJ, Cain VA, Nissen SE. Effect of rosuvastatin therapy on coronary artery stenoses assessed by quantitative coronary angiography: A study to evaluate the effect of rosuvastatin on intravascular ultrasound-derived coronary atheroma burden. Circulation. 2008;117:2458-2466
6. Niebauer J, Hambrecht R, Velich T, Hauer K, Marburger C, Kalberer B, Weiss C, von Hodenberg E, Schlierf G, Schuler G, Zimmermann R, Kubler W. Attenuated progression of coronary artery disease after 6 years of multifactorial risk intervention: Role of physical exercise. Circulation. 1997;96:2534-2541
7. Sixt S, Beer S, Bluher M, Korff N, Peschel T, Sonnabend M, Teupser D, Thiery J, Adams V, Schuler G, Niebauer J. Long- but not short-term multifactorial intervention with focus on exercise training improves coronary endothelial dysfunction in diabetes mellitus type 2 and coronary artery disease. European Heart Journal. 2010;31:112-119
8. Taylor RS, Brown A, Ebrahim S, Jolliffe J, Noorani H, Rees K, Skidmore B, Stone JA, Thompson DR, Oldridge N. Exercise-based rehabilitation for patients with coronary heart disease: Systematic review and meta-analysis of randomized controlled trials. American Journal of Medicine. 2004;116:682-692
9. O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Leifer ES, Kraus WE, Kitzman DW, Blumenthal JA, Rendall DS, Miller NH, Fleg JL, Schulman KA, McKelvie RS, Zannad F, Pina IL. Efficacy and safety of exercise training in patients with chronic heart failure: Hf-action randomized controlled trial. JAMA : The Journal of the American Medical Association. 2009;301:1439-1450
10. Suaya JA, Shepard DS, Normand SL, Ades PA, Prottas J, Stason WB. Use of cardiac rehabilitation by medicare beneficiaries after myocardial infarction or coronary bypass surgery. Circulation. 2007;116:1653-1662
11. Ades PA. Cardiac rehabilitation and secondary prevention of coronary heart disease. New England Journal of Medicine. 2001;345:892-902
12. Witt BJ, Jacobsen SJ, Weston SA, Killian JM, Meverden RA, Allison TG, Reeder GS, Roger VL. Cardiac rehabilitation after myocardial infarction in the community. Journal of the American College of Cardiology. 2004;44:988-996
13. Allen JK, Scott LB, Stewart KJ, Young DR. Disparities in women's referral to and enrollment in outpatient cardiac rehabilitation. Journal of General Internal Medicine. 2004;19:747-753
14. Miller NH, Haskell WL, Berra K, DeBusk RF. Home versus group exercise training for increasing functional capacity after myocardial infarction. Circulation. 1984;70:645-649
15. Haskell WL. Cardiovascular disease prevention and lifestyle interventions: Effectiveness and efficacy. Journal of Cardiovascular Nursing. 2003;18:245-255
16. Harris DE, Record NB. Cardiac rehabilitation in community settings. Journal of Cardiopulmonary Rehabilitation. 2003;23:250-259
17. Southard BH, Southard DR, Nuckolls J. Clinical trial of an internet-based case management system for secondary prevention of heart disease. Journal of Cardiopulmonary Rehabilitation. 2003;23:341-348
18. Dalal HM, Zawada A, Jolly K, Moxham T, Taylor RS. Home based versus centre based cardiac rehabilitation: Cochrane systematic review and meta-analysis. BMJ. 2010;340:b5631
19. Franklin BA. Exercise testing, training and arm ergometry. Sports Medicine. 1985;2:100-119
20. Lewis S, Thompson P, Areskog NH, Vodak P, Marconyak M, DeBusk R, Mellen S, Haskell W. Transfer effects of endurance training to exercise with untrained limbs. European Journal of Applied Physiology and Occupational Physiology. 1980;44:25-34
21.Loftin M, Boileau RA, Massey BH, Lohman TG. Effect of arm training on central and peripheral circulatory function. Medicine and Science in Sports and Exercise. 1988;20:136-141
22. Rosler K, Hoppeler H, Conley KE, Claassen H, Gehr P, Howald H. Transfer effects in endurance exercise. Adaptations in trained and untrained muscles. European Journal of Applied Physiology and Occupational Physiology. 1985;54:355-362
23. Janaudis-Ferreira T, Hill K, Goldstein R, Wadell K, Brooks D. Arm exercise training in patients with chronic obstructive pulmonary disease: A systematic review. Journal of Cardiopulmonary Rehabilitation and Prevention. 2009;29:277-283
24. Treat-Jacobson D, Bronas UG, Leon AS. Efficacy of arm-ergometry versus treadmill exercise training to improve walking distance in patients with claudication. Vascular Medicine. 2009;14:203-213
25. Zwierska I, Walker RD, Choksy SA, Male JS, Pockley AG, Saxton JM. Upper- vs lower-limb aerobic exercise rehabilitation in patients with symptomatic peripheral arterial disease: A randomized controlled trial. Journal of Vascular Surgery. 2005;42:1122-1130
26. Spertus JA, Winder JA, Dewhurst TA, Deyo RA, Prodzinski J, McDonell M, Fihn SD. Development and evaluation of the seattle angina questionnaire: A new functional status measure for coronary artery disease. Journal of the American College of Cardiology. 1995;25:333-341
27. Lim LL, Valenti LA, Knapp JC, Dobson AJ, Plotnikoff R, Higginbotham N, Heller RF. A self-administered quality-of-life questionnaire after acute myocardial infarction. Journal of Clinical Epidemiology. 1993;46:1249-1256
28. Borg G. Borg's perceived exertion and pain scales. Champaign, IL: Human Kinetics; 1998.
29. Smith HJ, Taylor R, Mitchell A. A comparison of four quality of life instruments in cardiac patients: Sf-36, qli, qlmi, and seiqol. Heart. 2000;84:390-394
30. Degen A, Millenaar D, Schirmer SH. Therapeutic approaches in the stimulation of the coronary collateral circulation. Curr Cardiol Rev. 2014;10(1):65-72.
31. Chockalingam, A. Exercise Awareness Smile Therapy: Flow in Cardiac Rehabilitation and Implications for Sustainable Lifestyle Changes. AACVPR 35th Annual Virtual Meeting 2020. https://journals.lww.com/jcrjournal/Fulltext/2020/09000/AACVPR_35th_Annual_Meeting_Scientific_Abstract.13.aspx

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