

New Enzyme Complex Isolated from Earthworms is Potent Fibrinolytic

Posted By Administration, Friday, April 10, 2009

Updated: Friday, April 18, 2014

Lumbrokinase Has Anti-Platelet, Anti-Thrombotic Activity

BY EDWIN COOPER, PH.D., SC.D., DISTINGUISHED PROFESSOR, LABORATORY OF COMPARATIVE IMMUNOLOGY AT UCLA, EDITOR-IN-CHIEF OF ECAM, AN OXFORD UNIVERSITY PRESS JOURNAL

When the rains surge through southern California, a confetti of earthworms is washed out of the soil. I lift the worms onto grass so they can find their way home--these creatures whose potent medicinal properties I have spent forty years studying.

The earthworm's antioxidant, immune-boosting, and clot-dissolving "medicine chest" is as powerful as that of any plant and even many pharmaceuticals. Earthworms have managed to survive for millions of years despite the constant threat of extinction by microbial pathogens. If we can begin to understand their remarkable capacities, we might design similar strategies to assist our own survival.

I have often wondered if earthworms are the creatures who first demonstrated a functional dichotomy in evolution: they evolved to be able to clean up the battlefield after having killed foreign invaders. They have cells that, much to my wondering eyes, look very much like human natural killer cells and neutrophils when examined with cytofluorimetric analysis and microscopy. I may sound a little eccentric when I tell you that my excitement over my beloved creatures is immense--I believe they hold healing treasures for us all.

In research I did in Modena in the late 1990's, I discovered that earthworm leukocytes can recognize human cancer cells as foreign and then kill them. Electron microscopy showed the astonishing "cinematography" of earthworm cells becoming incredibly active, throwing out "pseudopodia", and literally tearing apart cancer cell membranes from a human cell cancer line named K562. In fact, in all the time I've studied earthworms, I've never once been able to induce cancer in them. I could irritate them only to the point that they formed inflammatory lesions.

As Charles Darwin once wrote, "It may be doubted whether there are many other animals which have played so important a part in the history of the world."

Earthworms: Ancient Medicine, New Science

The last ten years have been a busy time for scientists exploring the medicinal treasures of earthworms. Laboratory, animal and clinical human studies have isolated enzymes and compounds that have proven to be potent fibrinolytics.

In healthy human volunteers, an enzyme complex isolated from earthworms increased levels of tissue plasminogen activator (t-PA) and consequently, fibrinolytic activity--without harmful side effects. In a study in 2000 the complex was found to be beneficial for ischemic stroke, without increasing the risk of excessive bleeding as other anticoagulants can. Using spectrofluorimeter and flow cytometry, a third study found that this complex has both anti-platelet activity (by reducing calcium release), anti-thrombotic activity (by reducing intercellular adhesion molecule-1) and anti-apoptotic activity (by inhibiting a specific pathway). All these activities, the researchers conclude, were “remarkably regulated.”

Earthworms have a long history in folk medicine--as far back as the 1300's. In ancient Burma and Laos, smallpox victims bathed in water where earthworms had been soaked. Worms were boiled in water with salt and onions and the broth given to women with postpartum weakness or difficulty nursing. In Iran dried earthworms were prescribed to help treat jaundice, and American Cherokee Indians used earthworm poultices to draw out thorns. According to the most famous ancient Chinese materia medica, earthworms could treat hemiplegia (a condition where half of the body is paralysed), fever, and blood clots.

Worms produce unique and potent molecules. One of my first research papers proved that earthworms have an immune system powerful enough to destroy other earthworm allografts, xenografts, but never autografts (an autograft is your own body's graft; allograft is a graft of foreign material from your own species; and a xenograft is a graft from another species, such as a pig heart valve into a human). Earthworms can kill bacteria and lyse foreign cells; their body fluid contains leukocytes that are as varied as those of many vertebrates. This is in spite of the fact that, unlike us, earthworms have no adaptive immune system, and do not form antibodies.

Earthworms happily crawl and munch their way through garbage teeming with bacteria and fungi, and not only fight off infection but alter that garbage so that their nitrogen and mineral-rich castings transform it into fertile, oxygen-rich soil. And, as practically every curious child knows, you can slice some earthworms and they will regenerate.

In the last ten years a number of the earthworm's clot-dissolving, lytic and immune-boosting compounds have been isolated and tested in laboratory and clinical studies. In particular, research has focused on clot-dissolving molecules. Fibrinolytic enzymes have been purified and studied from several species of earthworm, including *Lumbricus rubellus* and *Eisenia fetida*, and been found to be both potent and safe. This is very good news, since according to a 2008 conference report from the American Society of Hematology, thromboembolism impacts over one million Americans a year and is responsible for more deaths annually than breast cancer, HIV and motor vehicle crashes combined!

The Key to Lumbrokinase: Active Only in the Presence of Fibrin

Lumbrokinase (LK) is a group of six, novel proteolytic enzymes derived from the earthworm *Lumbricus rubellus*. In a 1992 study, a crude extract of the worm was shown to have a potent thrombolytic effect. The heat-stable, purified enzymes were first isolated in 1992 by Japanese researchers. The enzymes have potent fibrin-dissolving properties (fibrin is a protein deposited to create a mesh around a wound), decrease fibrinogen (a protein produced by the liver

that is involved in the clotting cascade), lower blood viscosity and markedly reduce platelet aggregation.

Recent research suggests that LK may be effective in the treatment and prevention of ischemic heart disease, as well as myocardial infarction, thrombosis of the central vein of the retina, embolism of peripheral veins, and pulmonary embolisms.

One key, remarkable property of lumbrokinase is that, unlike the medications streptokinase and urokinase, it is only active in the presence of fibrin. Though it dissolves fibrinogen and fibrin very specifically, it hardly hydrolyzes other important blood proteins such as plasminogen or albumin. It has the profound advantage of not causing hemorrhage due to excessive fibrinolysis. In fact, its plasminogen activator is remarkably similar to the plasminogen activator in the tissues of other species. Toxicological experiments have found no negative effects of LK on nervous, cardiovascular, respiratory and blood systems of rats, rabbits and dogs. Long-term animal experiments show no damage to liver or kidney function, no negative influence on embryonic development, and no mutagenic effects in embryonic rats. LK has no negative effects on blood levels of glucose and lipids. And a 2001 study tested one of the six enzymes of LK to determine whether LK does indeed pass into the blood from the intestines while maintaining its biological activity. This research found that approximately 10% of the full-size enzyme could pass through the intestinal epithelium intact and into the blood. This is not surprising; research from The Hebrew University has shown that many peptides can pass intact and biologically active through the intestinal lumen into the blood.

In a laboratory experiment in 1994 from Seoul National University, lumbrokinase (the six enzymes) was extracted from the earthworm. LK was then immobilized onto a polyurethane surface to investigate its antithrombotic activity. Platelets adhered to the surface and then drastically decreased in number, suggesting that LK digested the fibrinogen and inhibited the ability of platelets to stick to the surface. Similar results were found with an experiment on a rabbit shunt in the laboratory; occlusion time was monitored and it was found that on shunts without LK, occlusion time was 32 and 42 minutes, respectively, but those with LK-immobilized polyurethane had an occlusion time of 140 minutes--as much as four times longer.

Such studies show the potential of immobilized-LK surfaces for eventual use in tissue transplantation. In one remarkable 1999 study, Lumbrokinase was tested on LK-immobilized polyurethane valves which were then fitted to total artificial hearts in three healthy lambs. In the control lamb, the valves were untreated; in the second lamb, only valves on the right were treated, and in the third lamb, only valves on the left were treated. Implants were left in for up to three days. In the control lamb, thrombi were observed in the inlet parts of the valves. In the other two lambs, thrombi formed only on untreated control valves. Similarly, fibrinolytic activity was observed only in treated valves, and the proteolytic activity of the treated valves was three times higher than that of untreated valves.

A Potent Clot-Dissolver

Animal studies have demonstrated that LK is a potent clot-dissolver. A study in rabbits looked at LK's ability to dissolve an embolism in the pulmonary artery. The embolism was radioactively tagged, and blood radioactivity was tested 30 minutes, one hour, two hours, three hours, and five hours after LK had been administered. Radioactivity increased markedly at three and five hours, indicating that LK had begun to dissolve the embolism and disperse it into the bloodstream. In

another study rectal administration of LK reduced the size of a thrombus in the inferior vena cava in rats. And in yet another 1998 study, freeze dried *Lumbricus rubellus* was given to rats orally, and then plasmin activity in the blood was measured. At half a gram of LK per kilogram of weight a day, the activity doubled; at one gram, it quintupled. These results suggest that earthworm powder alone is valuable for thrombotic conditions. Finally, grafts treated with LK and inserted into the inferior vena cava of rabbits were compared to those not treated with LK, at five hours, 1, 2 and 4 weeks after implantation of the graft. Non-treated grafts were totally occluded with thrombus only five hours after implantation. LK treated graft were clear one week later, and those treated with a special covalent bonding method were clear four weeks later. Researchers concluded LK has potential antithrombotic effects in vascular prosthesis.

Lumbrokinase may help protect against myocardial ischemia and heart attack. A 2006 study in rats from Harbin Medical University in China induced heart attack in rats by permanently clamping shut the left anterior descending coronary artery. Lumbrokinase decreased the size of the infarct in a dose-dependent manner.

Human Studies Demonstrate Potency and Efficacy

Clinical trials in humans have been equally impressive. Research has found LK safe and effective as a thrombolytic in human volunteers. A hundred and twenty milligrams of freeze-dried earthworm powder was given orally to seven healthy volunteers aged 28-52 years old, three times a day for seventeen days. Blood was withdrawn before the trial to establish a baseline, and then at days 1, 2, 3, 8, 11 and 17. Fibrin degradation products, tissue plasminogen activator (t-PA) levels and activity were measured in the blood. The t-PA levels gradually increased through the entire experiment. Fibrinolytic activity also increased.

In an even more significant study from Shanghai Medical University in 2000, LK was used in patients who had suffered a stroke. Fifty-one stroke victims were randomly divided into a treatment group (31) and a control group (20). The Chinese stroke score was used to evaluate the effect of LK. Several measures of blood viscosity were used--prothrombin time, fibrinogen content, tissue plasminogen activator (t-PA) activity, D-dimer level, and more. In the treatment group, t-PA activity and D-dimer level increased, while fibrinogen decreased significantly. Plasminogen activator inhibitor activity and prothrombin time were unchanged. Lumbrokinase inhibits the coagulation pathway and activates fibrinolysis by increasing t-PA activity. This suggests that LK is not only beneficial for ischemic stroke, but that it may not increase the risk of excessive bleeding as anticoagulants can.

This stroke study is backed up by a 2008 study from Harbin Medical University in China. Researchers wondered how LK might have an anti-ischemic action in the brain. Using spectrofluorimeter and flow cytometry, they found that LK has both anti-platelet activity (by reducing calcium release), anti-thrombotic activity (by reducing intercellular adhesion molecule-1) and anti-apoptotic activity (by inhibiting a specific pathway). All these activities, the researchers conclude, were "remarkably regulated by LK."

Future Directions: A New Antimicrobial?

Do earthworms hold other treasures for us? We know that plasmin has been implicated in wound healing, pathogen invasion, cancer invasion and metastasis. Might earthworms like *Lumbricus rubellus* also have antimicrobial and anti-cancer potential?

Preliminary research is intriguing. Lumbricin I is an antimicrobial peptide that has been isolated from *Lumbricus rubellus*. It exhibits antimicrobial activity against both Gram negative and Gram positive bacteria as well as fungi, yet without hemolytic activity against human blood cells. Lumbricin I is rich in proline and actually shares characteristics with peptides found in insects and fruit flies.

What about cancer? Earthworms are able to lyse and destroy foreign cells. As I mentioned at the beginning of this research review, I have been unable to provoke my earthworms into getting cancer. When earthworms are examined by electron microscopy their fabulous complexity is revealed. Researchers from Japan, Korea, China and Croatia have been studying how earthworm peptides may inhibit the growth of spontaneous tumors since the 1990's. One "killer" glycolipoprotein extract called G-90 retards tumor growth in mice. Lombricine, from *Lumbricus terrestris*, was purified by Japanese researchers in 1991, and was shown to inhibit mammary tumors in mice. Daily subcutaneous injections markedly slowed the growth of tumors. Lombricine given orally as part of the diet also slowed the growth of tumors, though to a lesser degree than injection.

In addition, LK may help degrade and lyse fibrin clots from the venous blood of patients with malignant tumors. We know that cancer patients are at greater risk of clotting disorders, especially during treatment. According to research, malignant tumors secrete molecules that inhibit plasminogen activators and protect tumors. Might earthworm-derived enzymes like LK combat a tumor's protective mechanisms, and render it more vulnerable to treatment and to the innate immune system?

The Future of Earthworms as Medicine

We now know that earthworm enzymes and peptides may provide us with novel, potent and safe approaches to the treatment of thrombosis. Since thrombosis remains the main cause of death in America despite available drugs, the potential of LK is enormous. I think back to my boyhood, when I refused to fish, so I would not have to inadvertently kill earthworms by using them as lure. But I never knew that my commitment to developmental biology and comparative immunology would lead me to study these simple, profound creatures.

References available at:

<http://www.allergyresearchgroup.com/Mar-2009-Focus-Newsletter-Earthworm-References-sp-93.html>