



A Review: Oral Lumbrokinase Potential Agent Prevents Myocardial Reinfarction Post-Reperfusion in ST-Elevation Myocardial Infarction Patients

Muhammad Mufaiduddin^{1*}, Innelya¹, Alfi Rahmatika¹

¹Faculty of Medicine, Diponegoro University, Semarang, 50275, Indonesia

*Corresponding Author: muhammad_mufaiduddin@gmail.com

ARTICLE INFO

Article history:

Received 27 December 2023
Revised 17 February 2024
Accepted 19 February 2024
Available online 29 February 2024

E-ISSN: 2686-0864
P-ISSN: 2088-8686

How to cite:

Mufaiduddin M, Innelya and Rahmatika A. A Review: Oral Lumbrokinase Potential Agent Prevents Myocardial Reinfarction Post-Reperfusion in ST-Elevation Myocardial Infarction Patients. SCRIPTA SCORE Sci Med J. 2024 Feb 29;5(2):121-31

ABSTRACT

Background: Coronary Heart Disease (CHD) is a major cause of death and disability worldwide and its prevalence is expected to continue to increase in the coming years. Myocardial infarction is the largest and most dangerous manifestation of CHD, including in ST-Elevation Myocardial Infarction (STEMI). However, early therapy of STEMI cannot prevent the risk of myocardium reinfarction. Reinfarction post-STEMI reperfusion therapy has poor consequences and prognosis. Therefore, prevention of myocardial reinfarction is important. **Objectives:** The author has an innovation to use Lumbrokinase from *Lumbricus rubellus* extract in an oral dosage form as an alternative therapy to prevent reinfarction after reperfusion therapy in STEMI. **Methods:** Our article reviews the effect of lumbrokinase in preventing myocardial reinfarction using a non-systematic review method. **Results and Discussion:** Lumbrokinase can prevent myocardial reinfarction by several mechanisms, including its thrombolytic effect by activating plasminogen and degrading fibrin, its anti-inflammatory and antioxidant effects, and can prevent further myocardial apoptosis. Lumbrokinase can prevent apoptosis by increasing the activity of Silent Information Regulator 1 (Sirt1) and decreasing the pro-caspase-3 pathway. Lumbrokinase can prevent further inflammation by reducing levels of Nuclear Factor kappa-B (NF-kB) and the Toll-like Receptor 4 (TLR4) pathway, as well as preventing oxidative damage by deacetylating Forkhead Box-O (FoxO). The effect of Lumbrokinase plays an important role in preventing myocardial reinfarction. Oral lumbrokinase is made in capsule form. **Conclusion:** Oral lumbrokinase can potentially prevent myocardial reinfarction post-reperfusion in STEMI patients. **Keyword:** *Lumbricus rubellus*, Lumbrokinase, Myocardial reinfarction, Post-reperfusion STEMI

ABSTRAK

Latar Belakang: Penyakit Jantung Koroner (PJK) merupakan penyebab utama kematian dan kecacatan di seluruh dunia dan prevalensinya diperkirakan akan terus meningkat di tahun mendatang. Tingginya angka mortalitas PJK, maka PJK menjadi penyakit yang patut diwaspadai, terutama untuk negara-negara berkembang. Infark miokardium adalah manifestasi terbesar dan paling berbahaya dari PJK, termasuk STEMI. Namun, terapi awal dari STEMI tidak dapat mencegah resiko reinfark. Reinfark yang terjadi setelah terapi reperfusi memiliki konsekuensi dan prognosis yang buruk. Oleh karena itu, pencegahan reinfark miokardium setelah terapi STEMI menjadi penting. **Tujuan:** Penulis memiliki inovasi untuk menggunakan Lumbrokinase dari ekstrak *Lumbricus rubellus* dalam bentuk sediaan oral sebagai upaya terapi alternatif pencegahan reinfark setelah terapi reperfusi pada STEMI. **Metode:** Tinjauan pustaka ini bertujuan untuk meninjau efek dari lumbrokinase dalam mencegah reinfark miokardium setelah terapi STEMI dengan menggunakan metode *non-systematic review*. **Pembahasan:** Lumbrokinase dapat mencegah reinfark miokardium dengan beberapa mekanisme, termasuk efek trombolitiknya dengan mengaktifasi plasminogen dan mendegradasi fibrin, efek anti inflamasi dan anti oksidannya, serta dapat mencegah apoptosis miokardium lanjutan. Lumbrokinase dapat mencegah terjadinya apoptosis dengan meningkatkan aktivitas *silent information regulator 1* (Sirt1) dan



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.
<https://doi.org/10.32734/scripta.v5i2.15156>

menurunkan jalur *pro-caspase-3*. Lumbrokinase juga dapat mencegah inflamasi lanjutan dengan menurunkan level *nuclear factor kappa B* (NF-Kb) dan jalur *toll-like receptor 4* (TLR4), serta mencegah kerusakan oksidatif dengan deasetilasi *Forkhead Box O* (FoxO). Efek dari Lumbrokinase tersebut berperan penting dalam pencegahan reinfark miokardium setelah terapi STEMI. Penggunaan lumbrokinase secara oral dibuat dalam bentuk kapsul. **Kesimpulan:** Lumbrokinase oral memiliki potensi dalam mencegah reinfark miokardium setelah terapi reperfusi pada STEMI.

Kata Kunci: *Lumbricus rubellus*, Lumbrokinase, Reinfark miokardium, STEMI pasca reperfusi

1. Introduction

Coronary Heart Disease (CHD) is the main cause of death worldwide.^[1] CHD is a disorder caused by narrowing of the coronary arteries.^[2] CHD is classified into 3, namely: Silent Ischemia, Angina Pectoris, and Acute Myocardial Infarction (AMI). A study stated that AMI was the biggest manifestation of CHD. AMI is usually caused by blockage of the coronary arteries which results in tissue death. AMI is divided into two types based on electrocardiogram (ECG) changes, namely ST Elevation Myocardial Infarction (STEMI) and Non-ST Elevation Myocardial Infarction (NSTEMI). STEMI is caused by total occlusion of a thrombus which results in stopping blood flow (perfusion) to the myocardial tissue. The pathogenesis of STEMI involves impaired platelet aggregation and the formation of a thrombus (blood clots) in the intracoronary arteries which completely block them.^[3]

ST Elevation Myocardial Infarction (STEMI) is a cardiac emergency that must be treated as soon as possible.^[3] The treatment goal of STEMI is generally to restore oxygenation and metabolic supply due to persistent complete blockage in the coronary arteries. In general, initial therapy for STEMI involves restoring perfusion as quickly as possible to save as much of the myocardium as is at risk of tissue damage. Restoration of perfusion can be done by using thrombolytic drugs or Percutaneous Coronary Intervention (PCI) and Coronary Artery Bypass Graft (CABG) surgery.^[3,4]

Reperfusion treatment after the first STEMI attack does not prevent subsequent myocardial reinfarction. A cohort study that conducted a 3-year follow-up on STEMI patients showed that 264 reinfarctions occurred in 221 patients who had received PCI treatment.^[5] The greatest risk of myocardial reinfarction is within 30 days after PCI and increases steadily over the next 3 years myocardial reinfarction that occurs in the first 3 years after a STEMI attack is also associated with other adverse outcomes, such as ischemia-driven target vessel revascularization, stroke, and bleeding.^[5,6] Based on this, preventing reinfarction and bleeding complications after initial STEMI therapy can increase patient survival rates.

Prevention of reinfarction can be done by using thrombolytic agents. Thrombolytics help destroy thrombus.^[7] Presently available thrombolytic agents include streptokinase, alteplase, reteplase, tenecteplase, and urokinase. But this thrombolytic agents have several complications and side effect.^[8,9] The complications and side effects with thrombolytic drugs are mostly due to molecules in the drug that are not specific only for fibrin in thrombi, so they also degrade plasma proteins in the circulation which these proteins should be used to maintain homeostasis. Its use is less efficient because it can only be administered intravenously, so it must be carried out by competent medical personnel. Due to the many side effects and complications of currently available thrombolytic drugs and their less efficient use, it is necessary to carry out progressive research to find effective thrombolytic agents with minimum side effects and easy to use.

Some literature showed that there was a thrombolytic enzyme extracted from earthworms (*Lumbricus rubellus*) called Lumbrokinase (LK).^[10] The extract has a potent thrombolytic effect.^[11] Several studies have found that LK could work as a Plasminogen Activator and Fibrin Specific Serine Protease.^[12] The thrombolytic of LK are specific for fibrin and fibrinogen which play a role in thrombus formation. Apart from having powerful thrombolytic properties, other studies also found that LK had other properties, including anti-apoptotic, anti-inflammatory, and anti-oxidant effects.^[13]

Due to the various advantages and benefits of the LK enzyme, in this review the author has the innovation to use the LK enzyme as an alternative thrombolytic therapy to prevent post-STEMI myocardial re-infarction. The advantages of LK make this enzyme not only useful for preventing myocardial reinfarction, but also for protecting the myocardium from ischemic injury, apoptosis, and inflammation post-reperfusion. Previously, a study was conducted on the LK enzyme as a post-ischemic treatment by Wang YS et.al in 2018 by looking at

the activation of the Sirt1 molecule.^[13] Another study conducted by Wang YH et.al in 2022 also showed that post-stroke treatment with lumbrokinase protects against ischemic stroke, thereby regulating Endoplasmic Reticulum (ER) stress through the collective inhibitory effect of the Inositol-requiring Enzyme-1 (IRE1) signalling pathways to decrease apoptosis, autophagy, and inflammatory responses.^[14] In this review, we conducted a further review regarding the effectiveness of LK in the prevention of post-STEMI myocardial reinfarction by also reviewing other indicators, such as Forkhead Box-O (FoxO), Nuclear Factor kappa-B (NF-kB) signalling, Succinate Dehydrogenase (SDH) activity and Cytochrome c Oxidase (CcO), expression of Cyclooxygenase-2 (COX-2), Inducible Nitric Oxygenase (iNOS), and Matrix Metalloproteinase-9 (MMP-9), activation of pro-caspase-3, and activation of signalling in the Toll-like Receptor 4 (TLR4) pathway all play a role in myocardial reinfarction. Oral preparations of LK enzyme as a thrombolytic agent in preventing post-STEMI myocardial re-infarction can be made in capsule form. Earthworms (*Lumbricus rubellus*) are extracted into powder form and then put into a capsule shell made from gelatin.

This review focuses on the potential of the LK enzyme as a thrombolytic agent in preventing myocardial reinfarction post-reperfusion which can later help reduce the mortality rate due to STEMI attacks. Apart from that, the use of LK from the *Lumbricus rubellus* earthworm is also an effort to utilize Indonesia's abundant natural resources.

2. Methods

We used a non-systematic review method for this review. A computer-based literature search was performed to identify relevant articles published from 2010 to 2019 in Google Scholar, Pubmed, Science Direct, and Research Gate. The main search terms using medical subject headings (MeSH) to create subgroup terms: “*Lumbrokinase*”, “*Myocardial Reinfarction*”, “*Lumbrokinase*”, “*Post-reperfusion STEMI*”, and “*Lumbricus rubellus*”.

The inclusion and exclusion criteria were determined before the search. The included studies fulfilled the following inclusion criteria: (1) the study was published during 2010 – 2019; (2) the study had relevant topics and conformity between the research objectives and the journal's conclusions; (3) the study was available in full text. The exclusion criteria included: (1) the study was published less than 2010; (2) the study had irrelevant topics for this review and did not match the research objectives with the research conclusions.

We identified 102 articles matching the search criteria. We extracted 86 articles after reading the title and removing duplicate publications. After reading the abstract, 72 articles were assessed and analyzed to this review.

3. Results and Discussion

3.1 The Pathophysiology of Myocardial Reinfarction Post-Reperfusion

CHD is a condition caused by the narrowing of the lumen coronary arteries. Total narrowing of the lumen causes a heart attack or Myocardial Infarction.^[1,2] The main etiopathogenetic of CHD is atherosclerosis. Atherosclerotic plaque consists of inflammatory cells, cellular debris, smooth muscle cells, and varying amounts of cholesterol. This lipid core forms in some plaques under the fibrous cap which consists of collagen, smooth muscle cells, and elastin. The accumulation of inflammatory cells originating from foam cells (macrophages containing fat) can weaken and thin the fibrous cap. These plaques are called thin-capped fibroatheromas (TCFA). These processes can ultimately cause tearing of the fibrous cap, exposing the thrombogenic lipid core beneath the cap to flowing blood, leading to the formation of an intraluminal coronary thrombus.^[15]

Thrombi that form in ruptured atherosclerotic plaques are rich in small platelets, fibrin, and few leukocytes and erythrocytes. When an atherosclerotic plaque ruptures, platelets initially circulating in the blood are rapidly recruited to the injury site, through the interaction of specific platelet cell surface receptors with collagen and von Willebrand factor.^[16] After adhesion to the blood vessels, receptors on platelets will mediate the fast binding of other platelets called platelet aggregation, which will result in the formation of a larger thrombus. Apart from undergoing aggregation, at this stage, platelets are also activated. The main pathway for platelet activation is cleavage, which results in the activation of the platelet receptor PAR-1 (Protease Activated Receptor-1) or Thrombin Receptor by Thrombin Protease (Factor II) which is activated by the blood clotting cascade. Activated platelets will release granules which then increase platelet recruitment, attachment, aggregation, and activation.^[16,17]

Thrombin activates platelets by cleaving and activating PAR-1 and PAR-4. These receptors activate G proteins leading to stimulation of phosphoinositide hydrolysis, increasing cytosolic Ca^{2+} concentrations, and decreasing intracellular Cyclic Adenosine Mono Phosphate (cAMP) levels. cAMP is a control molecule in platelets that interrupts several signalling pathways and has an important role in down-regulating platelet activation. The synthesis of cAMP in platelets is stimulated by the binding of mediators such as prostacyclin and adenosine to cell surface receptors in combination with G proteins (GTP) binding proteins. Increased cAMP levels can inhibit platelet activity and the release of substances for platelet aggregation. A significant decrease in cAMP levels in CHD causes platelet hyperactivity and continued platelet aggregation.^[18] Continuous thrombus formation can cause total blockage of the arterial lumen. If total blockage occurs, it will stop the blood supply to the heart and cause ischemia and even MI. With severe and prolonged MI, dead heart muscle tissue is replaced with scar tissue that lacks myocardial contractility. In other words, the myocardium permanently loses contractility.

STEMI is a clinical syndrome resulting from myocardial ischemia associated with ECG ST segment elevation and the release of myocardial necrosis biomarkers.^[19] In STEMI, transmural myocardial injury or death occurs.^[4] The etiology of this condition is a total and permanent blockage of one or more coronary arteries that supply blood to the heart by a thrombus.^[20] Management of STEMI to restore oxygenation and the supply of metabolic substrates due to total thrombotic occlusion in the coronary arteries with tissue reperfusion as quickly as possible to save as much of the myocardium. Restoration of perfusion can be done through the use of thrombolytic drugs, through Percutaneous Coronary Intervention (PCI), or Coronary Artery Bypass Graft (CABG) surgery.^[7,21]

Reperfusion treatment after the first STEMI attack does not prevent subsequent myocardial reinfarction. Reinfarction is a serious complication after acute myocardial infarction. The greatest risk of reinfarction occurs within 30 days after PCI and increases steadily over the next 3 years with a higher risk of death. Myocardial reinfarction in the first 3 years after a STEMI attack is also associated with other adverse outcomes, such as ischemia-driven target vessel revascularization, stroke, and bleeding.^[22] Post-STEMI treatment myocardial reinfarction can cause various complications such as ventricular tachycardia, ventricular fibrillation, atrial fibrillation, high-grade Atrio-ventricular (AV) block, acute intraventricular conduction defects, heart failure, and death.^[5,6] Prevention of myocardial reinfarction can be done by using thrombolytic agents.^[22] Presently available thrombolytic agents include streptokinase, alteplase, reteplase, tenecteplase, and urokinase. But this thrombolytic agents have several complications and side effect.^[8,9] Some of these include internal bleeding, bruising or bleeding at the entrance, blood vessel damage, migration of blood clots to other blood vessels, kidney damage in patients with diabetes or previous kidney disease, allergic reactions, and intracranial bleeding (rare).^[8] Concurrently, these thrombolytic drugs also have many contraindications and are not safe for pregnant women.^[23] The reason for complications and side effects with thrombolytic drugs is mostly due to molecules in the drug which are not specific only for fibrin in thrombus, so they also degrade plasma proteins in the circulation which these proteins should be used to maintain homeostasis. Another therapy, namely PCI, is known to reduce infarct size, maintain left ventricular systolic function, and increase the survival rate in STEMI patients. However, PCI has side effects that can induce myocardial injury and post-treatment heart cell death, also known as the “Myocardial ischemia-reperfusion injury” phenomenon.^[18]

The pathophysiology of Myocardial ischemia-reperfusion injury is very complex. As a result of coronary artery occlusion, reduced myocardial oxygen supply shifts cellular metabolism to anaerobic respiration, resulting in a decrease in intracellular pH, primarily due to lactate production. A decrease in intracellular pH induces activation of the Na^+/H^+ exchanger, which extrudes H^+ to produce excess intracellular Na^+ . This condition results in an acidic condition in the cells. Decreasing intracellular pH results in inhibited activation of several enzymes and contractile devices which ultimately causes cardiomyocyte hypercontracting in the early stages of reperfusion.^[24]

Sudden reperfusion also induces oxidative stress due to the influx of oxygenated blood and activation of inflammatory cells. Reactive oxygen species (ROS) cause lipid peroxidation in cell membranes and damage various intracellular enzymes and Deoxyribonucleic Acid (DNA). It causes myocardial death through apoptosis or necrosis. Another mechanism that can induce ROS overproduction is related to electron transport chain (ECT) deficiency. Mitochondrial ECT is conventionally recognized as a major source of *Reactive Oxygen Species* (ROS).^[25]

Important components of myocardial ischemia-reperfusion injury that can ultimately lead to reinfarction of the myocardium also include infiltration of inflammatory cells, swelling of endothelial cells, decreased endothelial function, and rapid micro thrombosis, which leads to the “no-reflow” phenomenon.^[26] Activation of the nuclear factor kappa B (NF- κ B) signalling pathway is known to play a central role in the inflammatory process.^[13] TLR4 signalling also plays an important role in myocardial ischemia-reperfusion injury. Activation of the TLR4 pathway can directly impair cardiomyocyte contractility and also increase the expression of some molecules such as COX2, iNOS, and MMP-9 which can induce more severe ischemic injury to the myocardium.^[27]

The heart has cardioprotective molecules that can reduce the risk of post-reperfusion treatment ischemic injury and myocardial reinfarction. The molecule is Sirt1. Sirt1 can reduce oxidative damage in myocardial ischemia-reperfusion injury and reduce the rate of cardiomyocyte apoptosis. However, in post-STEMI conditions, there is a downregulation of Sirt1.^[13]

3.2 The Role of Lumbrokinase in Myocardial Reinfarction Post-Reperfusion in STEMI Patients

Earthworm (*Lumbricus rubellus*) belongs to the phylum *Annelida* and class *Oligochaeta*.^[28] These worms are widely cultivated in Indonesia because they can reproduce in media that are low in nutrients and have high reproductive power.^[29]

There are some studies about *Lumbricus rubellus* because it has properties that are useful as therapeutic agents. The *Lumbricus rubellus*' coelomic fluid contains more than 40 proteins that play a role in several biological activities, such as cytolytic, proteolytic, antimicrobial, hemolytic, hemagglutination, and mitogenic activities.^[29] These worms contain 9 essential amino acids, 4 non-essential amino acids, phosphorus, and calcium.^[30] Other research found that *Lumbricus rubellus* extract showed the activity of various enzymes on certain substrates, including protease, alpha-amylase, lipase, amyloglucosidase, chitinase, and cellulase. Studies on *Lumbricus rubellus* extract showed that the extract had anti-platelet aggregation effects and lyses blood clots so it can be a safe and promising oral thrombolytic drug.^[31] The thrombolytic effect arises due to the presence of the lumbrokinase (LK) enzyme in *Lumbricus rubellus* extract.^[12] Other substances found in the body of *L. rubellus* include lumbrofebrin which can reduce body temperature due to infection and lumbricin which acts as an anti-bacterial.^[32]

LK is a group of serine proteases with strong thrombolytic and antithrombotic effects, which are usually used as a treatment for stroke and cardiovascular disease.^[33,34] LK which is the result of extraction from *Lumbricus rubellus* was identified in the early 1990s as a group bioactive proteolytic enzyme with a molecular weight of 25 to 32 kDa. The mechanism of LK as a thrombolytic agent is by activating the conversion of plasminogen to plasmin which will degrade fibrin and fibrinogen in the thrombus until it dissolves.^[12,35]

The potential of LK to prevent ischemic injury and myocardial reinfarction post reperfusion treatment is achieved through four properties of LK, including protective effects on the myocardium from ischemic injury post reperfusion treatment, anti-inflammatory effects, antioxidant effects which can prevent oxidative damage post reperfusion treatment, and prevent apoptosis and necrosis of the myocardium. The risk of apoptosis in the myocardium begins immediately after the onset of myocardial infarction and increases significantly during reperfusion.^[36]

Lumbrokinase was able to significantly increase levels of silent information regulator 1 (Sirt1) (Figure 1).^[13] Sirt1 is a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase that activation can protect against myocardial ischemia-reperfusion injury.^[37-39] A recent study showed that increasing Sirt1 in the heart can improve cardiac function and reduce infarct size after myocardial injury, and these phenomena are associated with a decrease in serum creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and cardiomyocyte apoptosis.^[39,40] Sirt1 stimulates the expression of pro-survival molecules and negatively regulates the survival of pro-apoptotic molecules through deacetylation of Forkhead Box O (FoxO). It will reduce oxidative damage and apoptosis in myocardial ischemia-reperfusion injury.^[41,42] The oxidative damage that occurs due to the presence of ROS molecules can trigger inflammation by activating polymorphonuclear neutrophils (PMNs), causing endothelial dysfunction and severe heart damage.^[43,44] LK can also reduce the activity of succinate dehydrogenase (SDH) and Cytochrome c Oxidase (CcO). Some studies showed that inhibition of SDH (complex II) or CcO (complex IV) in mitochondria can reduce ROS generation.^[45-47]

Decreasing Sirt1 is also able to reduce NF-κB levels, which activation of the NF-κB signalling pathway plays a central role in the inflammatory process that occurs during post-reperfusion ischemic injury to the myocardium.^[48] Sirt1 activation can increase the expression of COX-2 and iNOS which triggers inflammation in the myocardium (Figure 1).^[13] Inflammation in post-reperfusion treatment ischemic injury can result in cell death through apoptosis and necrosis.^[25,49,50] Reducing NF-κB in STEMI can prevent inflammation and protect existing myocardial cells from undergoing apoptosis or necrosis. Apoptosis after infarction can also occur due to the activation of pro-caspase-3. Caspases are a class of protease enzymes that are important mediators of programmed cell death.^[51]

Lumbrokinase specifically inhibits the Sirt1 inhibitor, EX527.^[13] LK protects the presence of Sirt1 which is involved in post-ischemic cardioprotective effects such as reducing myocardial injury through anti-inflammatory activity, increasing autophagy, preventing oxidative events, and protecting the myocardium from apoptosis and necrosis.^[13]

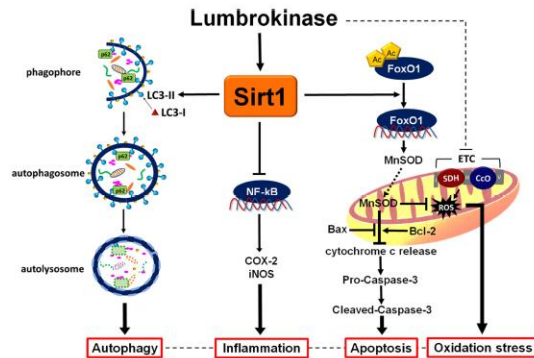


Figure 1. Post-ischemic lumbrokinase treatment reduces myocardial injury through activation of the Sirt1 signalling pathway^[13]

Another study demonstrated the cardioprotective effect of LK derived from the inhibition of COX-2, iNOS, and matrix MMP-9 expression induced by myocardial ischemia-reperfusion injury, which was mediated by TLR-4 signalling through the JNK and NF-κB pathways (Figure 2).^[52] TLR4 signalling plays an important role in post-reperfusion myocardial ischemic injury. Activation of TLR4 can directly damage cardiomyocyte contractility.^[27,53] TLR4 expression can induce an inflammatory process by increasing COX-2, iNOS, and MMP-9 and increase lymphocyte cell infiltration in post-reperfusion myocardial ischemic injury.^[53] Modulation of TLR4 signalling is also a potential therapeutic strategy in post-reperfusion myocardial ischemic injury.^[54]

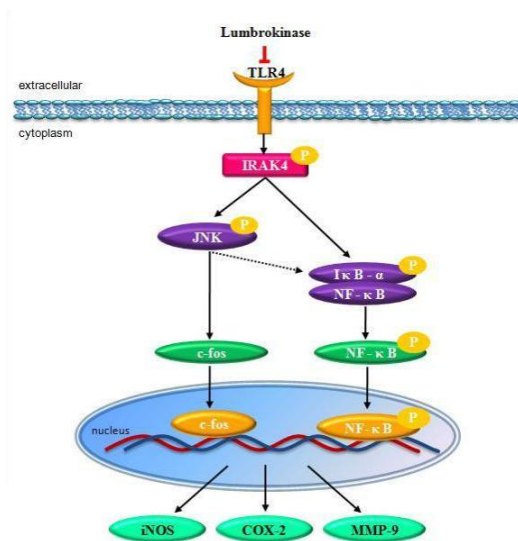


Figure 2. The mechanism of lumbrokinase in protecting the myocardium from ischemia-reperfusion injury is through the TLR4 signalling pathway^[52]

A study stated that LK was effective in reducing the effects of myocardial fibrosis in passive smoking-induced by SHS.^[55] SHS causes the activation of ERK1/2 signalling which will induce connective tissue growth factor (CTGF) and it is closely related to the occurrence of cardiac fibrosis and changes in heart shape. In this case, lumbrokinase can reduce the effects of myocardial fibrosis by regulating signalling in p-ERK1/2, SP1, CTGF expression, and p-ERK1/2, uPA, and Matrix Metalloproteinase-2 (MMP-2) (Figure 3).^[55] Another study also implied that lack of expression of plasminogen activator inhibitor-1 (PAI-1), a potent inhibitor of urokinase-type plasminogen activator (uPA) and tissue plasminogen activator (tPA), played a role in the pathogenesis of tissue fibrosis.^[56] PAI-1, a serine protease inhibitor, is a major physiological inhibitor of plasminogen activation through inactivation of the plasminogen activators uPA and tPA. Inactivation of the plasminogen activator causes a decrease in levels of the main fibrinolytic enzyme-plasmin. The transcription factor specificity protein1 (SP1), in fibroblasts is known to mediate this activation.^[57]

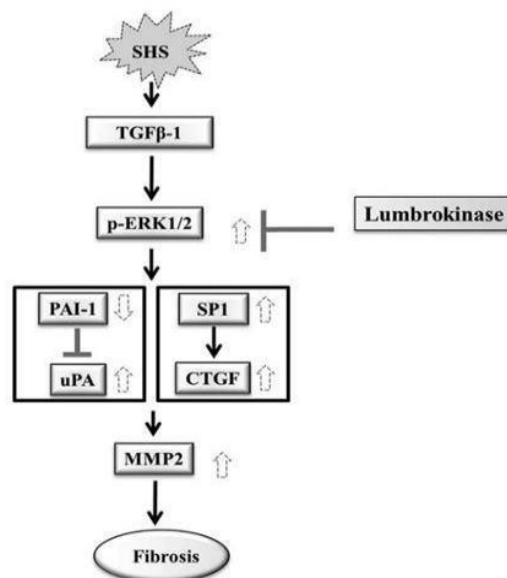


Figure 3. The effect of lumbrokinase on SHS-induced fibrosis^[55]

Another reason why LK can be used to prevent myocardial reinfarction after reperfusion treatment is the characteristics of LK. LK is relatively stable over a wide pH range and relatively more resistant to thermal denaturation when compared with other thrombolytic agents. LK can be given orally because it will not be damaged by gastric pH and its absorption is intact in the intestinal epithelium.^[11,58,59] It makes LK more efficient for patient use compared to other thrombolytic agents which are still limited to intravenous injection. The mechanism of LK as a specific thrombolytic for fibrin and thrombus fibrinogen makes LK have minimal side effects. It is different from other thrombolytic agents in that the mechanism of action is not specific so it also degrades plasma proteins in the blood circulation. It makes the use of other thrombolytic agents vulnerable to the risk of side effects and complications.

3.3 Oral Lumbrokinase

Lumbrokinase has the potential to prevent myocardial reinfarction post-STEMI reperfusion treatment due to the mechanism of action plays an important role in preventing ischemic injury and reinfarction in the myocardium post reperfusion treatment, has minimal side effects, and can be given orally which makes it more efficient for patients at home. The dosage of LK in therapy to prevent myocardial reinfarction can be made in capsules because the manufacturing cost is relatively cheap and easy to make. The stage of making LK capsules begins with making the capsule contents in the form of *Lumbricus rubellus* extract powder. Worms are cleaned from the growth medium and other adhering dirt and then washed with running water. The worms are stored in the refrigerator at 400C for 12 hours. Next, the worms are mixed with a formic acid solution (80%) of 3 ml per 100 grams of worm weight and ground into a paste. The paste is then dried in the oven at 50 - 7000C for 4-6 hours. After drying, it is ground to a particle size of ± 40 mesh.^[60]

After the contents of the capsule are formed, put the worm meal into the capsule shell. In making LK capsules, gelatin is used as a hard capsule shell. Gelatin is a mixture of water and water-soluble proteins (8-15%). Gelatin is a pure protein obtained from animal collagen with stability of pH 4 to 7. In the last step, put the extracted flour from *Lumbricus rubellus* into gelatin capsules.^[61]

4. Conclusion

Lumbrokinase capsules containing the thrombolytic enzyme powder, Lumbrokinase, are extracted from earthworms (*Lumbricus rubellus*). *Lumbricus rubellus*' extract has the enzyme Lumbrokinase which has specific thrombolytic properties against fibrin and fibrinogen and plays a role in thrombus formation, anti-apoptosis, anti-inflammatory, and anti-oxidant. Lumbrokinase has advantages compared to thrombolytic agents in general which is relatively stable over a wide pH range and more resistant to thermal denaturation. Lumbrokinase works by increasing Sirt1, decreasing the activity of SDH, and CcO which can reduce ROS generation. Lumbrokinase also has a cardioprotective effect. Due to its characteristics, we can conclude that lumbrokinase can be a promising innovation as an alternative therapy to prevent myocardial reinfection in post-STEMI reperfusion treatment.

5. Recommendations

There are no recommendations in this article.

References

- [1] Elosua R, Sayols-Baixeras S, Lluís-Ganella C, Lucas G. Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. *Appl Clin Genet* [Internet]. 2014 Jan;7:15. Available from: <http://www.dovepress.com/pathogenesis-of-coronary-artery-disease-focus-on-genetic-risk-factors--peer-reviewed-article-TACG>
- [2] InformedHealth.org. Coronary artery disease: Overview [Internet]. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2017. Available from: <https://www.nhs.uk/conditions/coronary-heart-disease/>
- [3] Heusch G, Gersh BJ. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. *Eur Heart J* [Internet]. 2016 Jun 26;38(11):ehw224. Available from: <http://eurheartj.oxfordjournals.org/lookup/doi/10.1093/eurheartj/ehw224>
- [4] Hina Akbar, Foth C, Kahloon RA, Mountfort S. Acute ST-Elevation Myocardial Infarction [Internet]. StatPearls, editor. Treasure Island (FL): StatPearls Publishing; 2023. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK532281/>
- [5] Stone SG, Serrao GW, Mehran R, Tomey MI, Witzenbichler B, Guagliumi G, et al. Incidence, Predictors, and Implications of Reinfarction After Primary Percutaneous Coronary Intervention in ST-Segment–Elevation Myocardial Infarction. *Circ Cardiovasc Interv* [Internet]. 2014 Aug;7(4):543–51. Available from: <https://www.ahajournals.org/doi/10.1161/CIRCINTERVENTIONS.114.001360>
- [6] White HD, Reynolds HR, Carvalho AC, Pearte CA, Liu L, Martin CE, et al. Reinfarction after percutaneous coronary intervention or medical management using the universal definition in patients with total occlusion after myocardial infarction: Results from long-term follow-up of the Occluded Artery Trial (OAT) cohort. *Am Heart J* [Internet]. 2012 Apr;163(4):563–71. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0002870312000634>
- [7] O’Gara PT, Kushner FG, Ascheim DD, Casey DE, Chung MK, de Lemos JA, et al. 2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction. *Circulation* [Internet]. 2013 Jan 29;127(4):362–425. Available from: <https://www.ahajournals.org/doi/10.1161/CIR.0b013e3182742cf6>
- [8] Aslanabadi N, Safaie N, Talebi F, Dousti S, Entezari-Maleki T. The Streptokinase Therapy Complications and its Associated Risk Factors in Patients with Acute ST Elevation Myocardial Infarction. *Iran J Pharm Res IJPR* [Internet]. 2018;17(Suppl):53–63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29796029>
- [9] Chusna S. Pola Penggunaan Obat pada Pasien Penyakit Jantung Koroner Rawat Inap di Rumah Sakit Umum Daerah Kudus Tahun 2012. Muhammadiyah Surakarta; 2012.
- [10] Hwang C-M, Kim D-I, Huh S-H, Min B-G, Park J-H, Han J-S, et al. In vivo evaluation of lumbrokinase, a fibrinolytic enzyme extracted from *Lumbricus rubellus*, in a prosthetic vascular graft. *J Cardiovasc Surg (Torino)* [Internet]. 2002 Dec;43(6):891–4. Available from: <https://pubmed.ncbi.nlm.nih.gov/12483186/>
- [11] Wang KY, Tull L, Cooper E, Wang N, Liu D. Recombinant Protein Production of Earthworm Lumbrokinase for Potential Antithrombotic Application. *Evidence-Based Complement Altern Med* [Internet]. 2013;2013:1–8. Available from: <http://www.hindawi.com/journals/ecam/2013/783971/>
- [12] Verma MK, Pulicherla KK. Lumbrokinase - a potent and stable fibrin-specific plasminogen activator. *Int J Bio-Science Bio-Technology*. 2011;3(2):57–70.
- [13] Wang Y-H, Li S-A, Huang C-H, Su H-H, Chen Y-H, Chang JT, et al. Sirt1 Activation by Post-ischemic Treatment With Lumbrokinase Protects Against Myocardial Ischemia-Reperfusion Injury. *Front Pharmacol* [Internet]. 2018 Jun 15;9(JUN):1–14. Available from: <https://www.frontiersin.org/article/10.3389/fphar.2018.00636/full>

- [14] Wang Y-H, Liao J-M, Chen K-M, Su H-H, Liu P-H, Chen Y-H, et al. Lumbrokinase regulates endoplasmic reticulum stress to improve neurological deficits in ischemic stroke. *Neuropharmacology* [Internet]. 2022 Dec;221:109277. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0028390822003367>
- [15] Ambrose JA, Singh M. Pathophysiology of coronary artery disease leading to acute coronary syndromes. *F1000Prime Rep* [Internet]. 2015 Jan 14;7(January):1–5. Available from: <https://facultyopinions.com/prime/reports/m/7/8/>
- [16] Ho K-J. Cardiovascular Diseases. In: *Nutritional Aspects Of Aging*. 1st ed. CRC Press; 1986. p. 26.
- [17] Meneguz-Moreno RA, Costa RA, A A, Ribamar Costa J, Abizaid A. Thrombus aspiration in ST-segment elevation myocardial infarction. *Minerva Cardioangiol* [Internet]. 2015 Dec;63(6):563–75. Available from: <https://pubmed.ncbi.nlm.nih.gov/26603617/>
- [18] Otsuka F, Yasuda S, Noguchi T, Ishibashi-Ueda H. Pathology of coronary atherosclerosis and thrombosis. *Cardiovasc Diagn Ther* [Internet]. 2016 Aug;6(4):396–408. Available from: <http://cdt.amegroups.com/article/view/10916/11563>
- [19] Hwang C, Levis JT. ECG Diagnosis: ST-Elevation Myocardial Infarction. *Perm J* [Internet]. 2014 Jun;18(2):e133. Available from: <http://www.thepermanentejournal.org/doi/10.7812/TPP/13-127>
- [20] Lippi G, Franchini M, Targher G. Arterial thrombus formation in cardiovascular disease. *Nat Rev Cardiol* [Internet]. 2011 Sep 5;8(9):502–12. Available from: <http://dx.doi.org/10.1038/nrcardio.2011.91>
- [21] Steg PG, James SK, Atar D, Badano LP, Lundqvist CB, Borger MA, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J* [Internet]. 2012 Oct 1;33(20):2569–619. Available from: <https://academic.oup.com/eurheartj/article/33/20/2569/447818>
- [22] Kumar A, Cannon CP. Acute Coronary Syndromes: Diagnosis and Management, Part I. *Mayo Clin Proc* [Internet]. 2009 Oct;84(10):917–38. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19797781%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2755812>
- [23] Kalyanasundaram A, Lincoff AM. Managing adverse effects and drug–drug interactions of antiplatelet agents. *Nat Rev Cardiol* [Internet]. 2011 Oct 13;8(10):592–600. Available from: <http://dx.doi.org/10.1038/nrcardio.2011.128>
- [24] Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest* [Internet]. 2013 Jan 2;123(1):92–100. Available from: <http://www.jci.org/articles/view/62874>
- [25] Brown DI, Griendling KK. Regulation of Signal Transduction by Reactive Oxygen Species in the Cardiovascular System. *Circ Res* [Internet]. 2015 Jan 30;116(3):531–49. Available from: <https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.116.303584>
- [26] Araszkiwicz A, Grygier M, Lesiak M, Grajek S. The impact of ischemia-reperfusion injury on the effectiveness of primary angioplasty in ST-segment elevation myocardial infarction. *Adv Interv Cardiol* [Internet]. 2013;3(3):275–81. Available from: <http://www.termedia.pl/doi/10.5114/pwki.2013.37509>
- [27] Fallach R, Shainberg A, Avlas O, Fainblut M, Chepurko Y, Porat E, et al. Cardiomyocyte Toll-like receptor 4 is involved in heart dysfunction following septic shock or myocardial ischemia. *J Mol Cell Cardiol* [Internet]. 2010 Jun;48(6):1236–44. Available from: <http://dx.doi.org/10.1016/j.yjmcc.2010.02.020>
- [28] Anderson C, Cunha L, Sechi P, Kille P, Spurgeon D. Genetic variation in populations of the earthworm, *Lumbricus rubellus*, across contaminated mine sites. *BMC Genet* [Internet]. 2017 Dec 17;18(1):97. Available from: <https://bmcbgenet.biomedcentral.com/articles/10.1186/s12863-017-0557-8>
- [29] Shazari PA, Kurniawan B. The Benefit of Fibrinolytic Protease Enzyme Earthworms (*Lumbricus Rubellus*) for Ischemic Stroke Patient. *Majority*. 2016;5(5).
- [30] Ovianto E. Uji Aktivitas Fibrinolitik Tepung Cacing Tanah (*Lumbricus rubellus*) Secara in vitro dan Evaluasi Pengaruhnya Terhadap Beberapa Parameter Aterosklerosis pada Monyet Ekor Panjang *Macaca fascicularis* Sehat. *IPB Repos*. 2010;
- [31] Trisina J, Sunardi F, Suhartono MT, Tjandrawinata RR. DLBS1033, A Protein Extract from *Lumbricus rubellus*, Possesses Antithrombotic and Thrombolytic Activities. *J Biomed Biotechnol* [Internet]. 2011;2011:1–7. Available from: <http://www.hindawi.com/journals/bmri/2011/519652/>
- [32] Rinanda T, Sakdiah, Oktavia R, Da Fhonsa M, Fitra M. Broad Spectrum Antimicrobial Activity of *Lumbricus*. *4th Annu Int Conf Syiah Kuala Univ (AIC Unsyiah) 2014*. 2014;4(2):113–8.
- [33] Liao J, Ren X, Yang B, Li H, Zhang Y, Yin Z. Targeted thrombolysis by using c-RGD-modified N,N,N-Trimethyl Chitosan nanoparticles loaded with lumbrokinase. *Drug Dev Ind Pharm* [Internet]. 2019 Jan 2;45(1):88–95. Available from: <http://dx.doi.org/10.1080/03639045.2018.1522324>

- [34] Verma MK, Pulicherla KK. Broad substrate affinity and catalytic diversity of fibrinolytic enzyme from *Pheretima posthumous*—Purification and molecular characterization study. *Int J Biol Macromol* [Internet]. 2017 Feb;95:1011–21. Available from: <http://dx.doi.org/10.1016/j.ijbiomac.2016.10.090>
- [35] Cooper EL, Balamurugan M. Unearthing a source of medicinal molecules. *Drug Discov Today* [Internet]. 2010 Nov;15(21–22):966–72. Available from: <http://dx.doi.org/10.1016/j.drudis.2010.09.004>
- [36] Gu J, Fan Y, Liu X, Zhou L, Cheng J, Cai R, et al. SENP1 protects against myocardial ischaemia/reperfusion injury via a HIF1 α -dependent pathway. *Cardiovasc Res* [Internet]. 2014 Oct 1;104(1):83–92. Available from: <https://academic.oup.com/cardiovascres/article-lookup/doi/10.1093/cvr/cvu177>
- [37] Guarente L. Sirtuins, Aging, and Medicine. *N Engl J Med* [Internet]. 2011 Jun 9;364(23):2235–44. Available from: <http://www.nejm.org/doi/abs/10.1056/NEJMra1100831>
- [38] Candilio L, Malik A, Hausenloy DJ. Protection of organs other than the heart by remote ischemic conditioning. *J Cardiovasc Med* [Internet]. 2013 Mar;14(3):193–205. Available from: <http://journals.lww.com/01244665-201303000-00005>
- [39] Ding M, Lei J, Han H, Li W, Qu Y, Fu E, et al. SIRT1 protects against myocardial ischemia–reperfusion injury via activating eNOS in diabetic rats. *Cardiovasc Diabetol* [Internet]. 2015 Dec 21;14(1):143. Available from: <http://www.cardiab.com/content/14/1/143>
- [40] Yamamoto T, Tamaki K, Shirakawa K, Ito K, Yan X, Katsumata Y, et al. Cardiac Sirt1 mediates the cardioprotective effect of caloric restriction by suppressing local complement system activation after ischemia-reperfusion. *Am J Physiol Circ Physiol* [Internet]. 2016 Apr 15;310(8):H1003–14. Available from: <https://www.physiology.org/doi/10.1152/ajpheart.00676.2015>
- [41] Yu L, Li Q, Yu B, Yang Y, Jin Z, Duan W, et al. Berberine Attenuates Myocardial Ischemia/Reperfusion Injury by Reducing Oxidative Stress and Inflammation Response: Role of Silent Information Regulator 1. *Oxid Med Cell Longev* [Internet]. 2016;2016:1–16. Available from: <http://www.hindawi.com/journals/omcl/2016/1689602/>
- [42] Hsu C-P, Zhai P, Yamamoto T, Maejima Y, Matsushima S, Hariharan N, et al. Silent Information Regulator 1 Protects the Heart From Ischemia/Reperfusion. *Circulation* [Internet]. 2010 Nov 23;122(21):2170–82. Available from: <https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.110.958033>
- [43] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive Oxygen Species in Inflammation and Tissue Injury. *Antioxid Redox Signal* [Internet]. 2014 Mar;20(7):1126–67. Available from: <http://www.liebertpub.com/doi/10.1089/ars.2012.5149>
- [44] Valle Raleigh J, Mauro AG, Devarakonda T, Marchetti C, He J, Kim E, et al. Reperfusion therapy with recombinant human relaxin-2 (Serelaxin) attenuates myocardial infarct size and NLRP3 inflammasome following ischemia/reperfusion injury via eNOS-dependent mechanism. *Cardiovasc Res* [Internet]. 2017 Jan 10;113(6):cvw246. Available from: <https://academic.oup.com/cardiovascres/article-lookup/doi/10.1093/cvr/cvw246>
- [45] Dröse S, Bleier L, Brandt U. A Common Mechanism Links Differently Acting Complex II Inhibitors to Cardioprotection: Modulation of Mitochondrial Reactive Oxygen Species Production. *Mol Pharmacol* [Internet]. 2011 May;79(5):814–22. Available from: <http://molpharm.aspetjournals.org/lookup/doi/10.1124/mol.110.070342>
- [46] Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* [Internet]. 2014 Nov 20;515(7527):431–5. Available from: <https://www.nature.com/articles/nature13909>
- [47] Yang Z, Duan Z, Yu T, Xu J, Liu L. Inhibiting Cytochrome C Oxidase Leads to Alleviated Ischemia Reperfusion Injury. *Korean Circ J* [Internet]. 2017;47(2):193. Available from: <https://e-kcj.org/DOIx.php?id=10.4070/kcj.2016.0137>
- [48] Kitada M, Ogura Y, Koya D. The protective role of Sirt1 in vascular tissue: its relationship to vascular aging and atherosclerosis. *Aging (Albany NY)* [Internet]. 2016 Oct 15;8(10):2290–307. Available from: www.aging-us.com
- [49] Padfield GJ, Din JN, Koushiappi E, Mills NL, Robinson SD, Cruden NLM, et al. Cardiovascular effects of tumour necrosis factor α antagonism in patients with acute myocardial infarction: a first in human study. *Heart* [Internet]. 2013 Sep 15;99(18):1330–5. Available from: <https://heart.bmj.com/lookup/doi/10.1136/heartjnl-2013-303648>
- [50] Yang Y, Duan W, Lin Y, Yi W, Liang Z, Yan J, et al. SIRT1 activation by curcumin pretreatment attenuates mitochondrial oxidative damage induced by myocardial ischemia reperfusion injury. *Free Radic Biol Med* [Internet]. 2013 Dec;65:667–79. Available from: <http://dx.doi.org/10.1016/j.freeradbiomed.2013.07.007>

- [51] Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* [Internet]. 2010 Mar 19;10(3):210–5. Available from: <http://dx.doi.org/10.1038/nri2725>
- [52] Wang Y-H, Chen K-M, Chiu P-S, Lai S-C, Su H-H, Jan M-S, et al. Lumbrokinase attenuates myocardial ischemia-reperfusion injury by inhibiting TLR4 signaling. *J Mol Cell Cardiol* [Internet]. 2016 Oct;99:113–22. Available from: <http://dx.doi.org/10.1016/j.yjmcc.2016.08.004>
- [53] Avlas O, Fallach R, Shainberg A, Porat E, Hochhauser E. Toll-Like Receptor 4 Stimulation Initiates an Inflammatory Response That Decreases Cardiomyocyte Contractility. *Antioxid Redox Signal* [Internet]. 2011 Oct;15(7):1895–909. Available from: <http://www.liebertpub.com/doi/10.1089/ars.2010.3728>
- [54] Arslan F, Smeets MB, O'Neill LAJ, Keogh B, McGuirk P, Timmers L, et al. Myocardial Ischemia/Reperfusion Injury Is Mediated by Leukocytic Toll-Like Receptor-2 and Reduced by Systemic Administration of a Novel Anti-Toll-Like Receptor-2 Antibody. *Circulation* [Internet]. 2010 Jan 5;121(1):80–90. Available from: <https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.109.880187>
- [55] Lai C-H, Han C-K, Shibu MA, Pai PY, Ho T-J, Day CH, et al. Lumbrokinase from earthworm extract ameliorates second-hand smoke-induced cardiac fibrosis. *Environ Toxicol* [Internet]. 2015 Sep;30(10):1216–25. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/tox.21993>
- [56] Ghosh AK, Bradham WS, Gleaves LA, De Taeye B, Murphy SB, Covington JW, et al. Genetic Deficiency of Plasminogen Activator Inhibitor-1 Promotes Cardiac Fibrosis in Aged Mice. *Circulation* [Internet]. 2010 Sep 21;122(12):1200–9. Available from: <https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.110.955245>
- [57] Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol* [Internet]. 2012 Feb 23;227(2):493–507. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/jcp.22783>
- [58] Yan XM, Kim C-H, Lee CK, Shin JS, Cho IH, Sohn UD. Intestinal Absorption of Fibrinolytic and Proteolytic Lumbrokinase Extracted from Earthworm, *Eisenia andrei*. *Korean J Physiol Pharmacol* [Internet]. 2010;14(2):71. Available from: <https://synapse.koreamed.org/DOIx.php?id=10.4196/kjpp.2010.14.2.71>
- [59] Zhou J, Guo D, Zhang Y, Wu W, Ran H, Wang Z. Construction and Evaluation of Fe₃O₄-Based PLGA Nanoparticles Carrying rtPA Used in the Detection of Thrombosis and in Targeted Thrombolysis. *ACS Appl Mater Interfaces* [Internet]. 2014 Apr 23;6(8):5566–76. Available from: <https://pubs.acs.org/doi/10.1021/am406008k>
- [60] Sanjaya IGM, Samik S, Koestiari TK. Pelatihan Pembuatan Tepung Cacing Tanah Sebagai Bahan Tambahan Berpro Tinggi dalam Upaya Memperkuat Ekonomi Masyarakat. *J ABDI* [Internet]. 2016 Oct 28;1(2):150. Available from: <https://journal.unesa.ac.id/index.php/abdi/article/view/106>
- [61] Foox M, Zilberman M. Drug delivery from gelatin-based systems. *Expert Opin Drug Deliv* [Internet]. 2015 Sep 2;12(9):1547–63. Available from: <http://www.tandfonline.com/doi/full/10.1517/17425247.2015.1037272>