Antifibrinolytic effect of oral prescription of lumbrokinase in Mice

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Abstract

Antifibrinolytic effect of lumbrokinase in mice, in vivo, was studied. Liquid phase lumbrokinse from *Lumbricus rubellus* was purified by column chromatography method. Lumbrokinase was orally inserted to mice. Although oral dosage of lumbrokinase, to investigate whether lumbrokinase in mice causes antifibrinolytic effect, we have concentration of lumbrokinase varied, and detection of antifibrinolytic effect was carried out using a FDP (fibrin degradation product) test and euglobulin fibrinolytic activity test. FDP test and euglobulin fibrinolytic activity test were compared the data of PBS ingestion control with lumbrokinase ingestion. As a result, FDP was increased in 7.8mg, 26mg lumbrokinase concentrations after 25 hours succeeding oral prescription of lumbrokinase, and decreased after 49 hours, but 7.8mg lumbrokinase ingestion more increased than 26mg. Also, FDP of PBS ingestion control and 1.3mg lumbrokinase ingestion were not been observed nothing. Euglobulin fibrinolytic activity of PBS ingestion control and 1.3mg lumbrokinase ingestion were not been observed any clear zone after 8, 25, 48 hours, and 7.8mg and 26mg were observed the most largest clear zone after 25 hours and decreased after 48 hours. However, antifibrinolytic effect of oral prescription of lumbrokinase in mice was observed. Now we need to determine an efficient amount of lumbrokinase for antifibrinolytic effect.

Introduction

The incidence of thrombotic disorders, including cerebral stroke, mycardial infaction, and venous thromboembolism, is rapidly increasing in Korea. Various therapeutic agents are used for thrombotic disease, including anticoagulants and antiplatelet drugs, which may retard or prevent further progression of thrombotic agents, however, can lyse the thrombi, and currently available thrombolytic agents are used for various thrombotic disorders. In Oriental regions, a number of traditional treatment regimens have been used for thrombotic deseases, However, most natural products have not so far been evaluated in a scientific way. For the first time, a novel fibrinolytic enzyme called lumbrokinase was extracted from the earthwom (*Lumbricus rubelllus*) by Mihara, 1983 [1]. Recently, we purified lumbrokinase by a simpler method than Mihara's, obtaining a trypsin-like protease from the earthwom, a component of traditional Oriental antithrombotic drugs [2]. and to assess the antithrombotic activity of lumbrokinase immobilized on an artificial heart surface [3]. In this study, we present a animal test for antifibrinolytic effect of lumbrokinase ingestion [4].

Materials and Methods

Materials

Earthworm powder (species: *L.rubellus*) was purchased from Inovatech in Canada, Dialysis membrane (cutoff size: 10kDa) was Spectrum (Laguna Hills, CA, USA), diethylaminoethyl (DEAE) sephadex A-25, p-aminobenzamidine sepharose 6B were from Sigma (St.Louis, MO,USA), ICR mice were from Korea, Thrombo-Wellcotest was Murex (central road, temple hill, kartford, England).

Methods

Purification of fibrinolytic enzyme (LK)
Purification of fibrinolytic enzyme yme (LK) from earthworm (*L.rubellus*) was carried out by pretreatment and two-step column chromatography comprising DEAE-anion exchange and benzamidine affinity chromatography [2]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to prove that the purified protein formed a single band. Protein were quantitated by using the Lowry method. LK was dialyzed in PBS (pH 7.4).

Animal test
Purified liquid-phase lumbrokinase was prescribed into stomach to ICR mice of about 8 weeks at a time for 3 days. Control mice received PBS (pH7.4), and three groups of six mice individually received 1.3mg, 7.8mg, 26mg of lumbrokinase. To determine whether mice also have in vivo antifibrinolytic effect, we examined FDP test and euglobulin fibrinolytic effect after blood

collection.

FDP test
antisera are raised to highly purified preparations of fibrinogen fragments D and E. After solid-phase absorption to remove antibodies to all other serum proteins, the specific antibody globulins are extracted and used to coat by absorption a suspension of latex particles in glycine saline buffer. The sensitivity of the latex reagent is adjusted so that so that, in the presence of FDP concentrations of 2ug(fibrinogen equivalent) per ml or greater, the latex particles clump together giving macroscopic agglutination. This test is capable of detecting the presence of all the major breakdown products of fibrin or fibrinogen, and the levels measured by this latex test correlate well with results obtained with the established haemagglutination-inhibition immunoassay.

Euglobulin fibrinolytic activity test
Blood was taken from the portal vein of mice into a tube containing EDTA, and plasma was prepared by centrifugation at 3000rpm for 15minuites at 4. Euglobulin is obtained as dissolving in sodium borate buffer (pH9.0) plasma precipitation. Plasma precipitation was made by adding to 1% acetic acid for lowing pH. An aliquot of this solution was dropped in fibrin plate. Fibrinolytic activity was measured by scale (mm^2) of clear zone.

Results and Discussion

Recently, antifibrinolytic agents (t-PA, urokinase, and streptokinase) have been interested in as well as lysis of fibrin and thrombosis related disease (fibrinogenesis by inflammatory reaction and immun reacton), but one possible problem with the use of these agnents (t-PA, urokinase, and streptokinase) is that the clot over the bleeding source will dissolve and the internal bleeding will resume. Lumbrokinase became known to an anti-fibrinolytic enzyme which dissolve directly fibrin in a different mechanism of t-PA,urokinase and streptokinase which intermediate plasmin. Now we research antifibrinolytic effect of oral prescription of lumbrokinase but lumbrokinase may be digested in stomach. so, its activity may be lost. In this study, although digestion of LK in stomach we must determin an adequate amount of lumbrokinase for antifibrinolytic effect and how often prescribe to animal. By Fig.1 and Fig.2, every day oral dosage of 7.8mg lumbrokinase was most efficient for antifibrinolytic effect.

References

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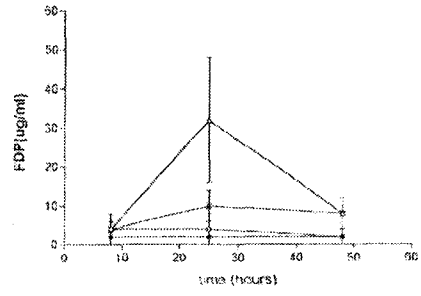

Fig.1. Fibrin Degradation Product (FDP) test
The closed squre is PBS ingestion control , the opend squre is 1.3mg lumbrokinase ingestion, the opened triangle is 7.8mg lumbrokinase ingestion, and the x is 26mg lumbrokinase ingestion

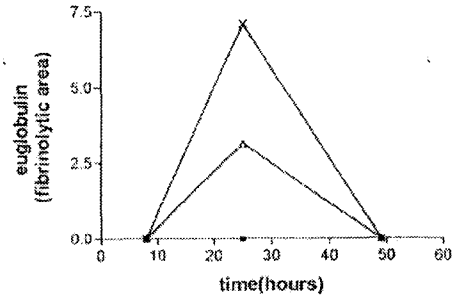

Fig.2. Euglobulin fibrinolytic activity test
The closed squre is PBS ingestion control , the opend squre is 1.3mg lumbrokinase ingestion, the opened triangle is 7.8mg lumbrokinase ingestion, and the x is 26mg lumbrokinase ingestion