Journal of Korean Medical Science
Vol. 8, No. 2, 117-120 April 1993

The Thrombolytic Effect of Lumbrokinase Is Not as potent as Urokinase in a Rabbit Cerebral Embolism Model

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The purpose of the present study is to determine whether lumbrokinase has an in vivo thrombolytic effect in a rabbit cerebral embolism model. In our previous studies, we found that lumbrokinase, an extract from Korean earth worms, has a strong in vitro fibrinolytic effect without the presence of plasminogen and significant in vivo thromobolytic effects of lumbrokinase in a rat human-clot-induced cerebral embolism model. We established the cerebral embolism model in rabbits by injecting a piece of human clot into the internal carotid artery via the external carotid artery and confirmed the occlusion with angiography. Twenty one rabbits were divided into three groups and 5cc of saline, urokinase of 50,000 u/ml, and equipotent LK were injected intraarterially for 30 minutes into each group of 7 animals. Ten minutes after the end of infusion, an angiogram was performed to confirm the recanalization.
Clot lysis occurred in one, six, and one animals in the saline, urokinase and lumbrokinase treated groups respectively. With regard to its in vitro effect, lumbrokinase is not as potent in vivo. Further investigation should be performed to determine the cause of its weakened in vivo effect and to develop a method to potentiate it.

Key Words: *Lumbrokinase, thrombolytic effect, cerebral embolic model*

INTRODUCTION

Recently, considerable attention has been given to thrombolytic therapy for ischemic stroke. The use of thrombolytics such as streptokinase or urokinase is controvertial (Clarke & Cliffton, 1960; Herdon et al., 1960; Meyer et al, 1963; Atkin et al., 1964; Meyer et al., 1965; Fletcher et al., 1976; Hanaway et al., 1976) since these agents produce a significant hemorrhagic tendency. Tissue plasminogen activator (tPA), a thrombus specific plasminogen activator, seems to be more advantageous but much more studies need to be done to prove its efficacy (Hennerici et al., 1991).

In oriental medicine, earthworm has long been used as a therapeutic agent for thrombotic disorders such as stroke. Recently, Mihara et al. (1983) extracted a fibrinolytic protein from Lumbricus rubellus, a species of Korean earth worm, and named it lumbrokinase. Subsequent investigations showed LK can be further divided into 6 fragments, four of which showed a strong fibrinolytic effect even without the presence of plasminogen (Mihara et al., 1989; Park et al., 1989). If LK has fibrinolytic effects without activating plasminogen, it would not induce systemic bleeding tendency, and may theoretically be a better thrombolytic agent. We found increased blood d-dimer in human beings who orally ingested earch worm powder without evidence of bleeding (Park et al., 1989). When LK was added to thrombin it had a significant thrombolytic effect and reduced the fatality rate in a thrombin-

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This work was supported by a Grant from the Asan Institute for Life sciences (1990).

induced lung thrombosis model in mice (Lee et al., 1990). Furthermore, we also found that when human clot mixed with Tc-99m sulfur colloid was used as embolus, intraarterial infusion of LK significantly reduced the size of the clot measured by count in the rat cerebral embolism model, indicating a desirable in vivo effect (Kim et al., 1992). However, its effect was much less potent compared with its in vitro effect. The present experiment was carried out to determine the in vivo effect of lumbrokinase in rabbit cerebral embolism model using angiogram.

MATERIALS AND METHODS

LK was extracted from Korean earth worms as previously described, and six fractions were identified. In this experiment, we used fragment 1-III. Before the animal experiment, we made a fibrin plate as previously described and tested the in vitro fibrinolytic effects of LK. When applied to the surface of the fibrin plate, fragment 1-III showed significant fibrinolytic effect which was equivalent to urokinase of 400,000 units/ml. In this experiment, we used 10,000 units/ml of urokinase as a control. Therefore, we diluted the LK so that it was equipotent with the urokinase.

We used New Zealand white rabbits weighing 2.5 to 3.0kg. The animals were anesthetized with ketamine hydrochloride 5ml/kg IM initially and 1-2 ml IM at every 30 minutes thereafter. Animals underwent midline neck dissection to expose the right common, external and internal carotid arteries. The common carotid artery is temporarily occluded with a bull-dog clip and a polyethylene tube (PE50) was inserted into one of the branches of the external carotid artery so that the tip of the tube was situated about 5mm away from the lumen of the internal carotid artery. After releasing the bull-dog clip, all the external carotid branches were tightly ligated, and an angiogram was done to confirm the occlusion of all vessels except the internal carotid artery (Fig. 1).

As a thrombus material we used human venous blood drawn in the thrombin excess tube three to four hours before the experiment. 1mm^3 of the blood clot was taken and pushed via the polyethylene tube followed by flushing with 2cc of saline.

Twenty one animals were divided into three groups. In each group (7 animals), 5cc of saline, urokinase (50,000 units/5cc), or equipotent LK were injected intraarterially beginning 10 minutes after embolization via polyethylene tube for 30 minutes. Ten minutes after infusion, an angiogram was performed to confirm the recanalization. The angiogram was repeated 30 minutes later if not recanalized. Seven days after the

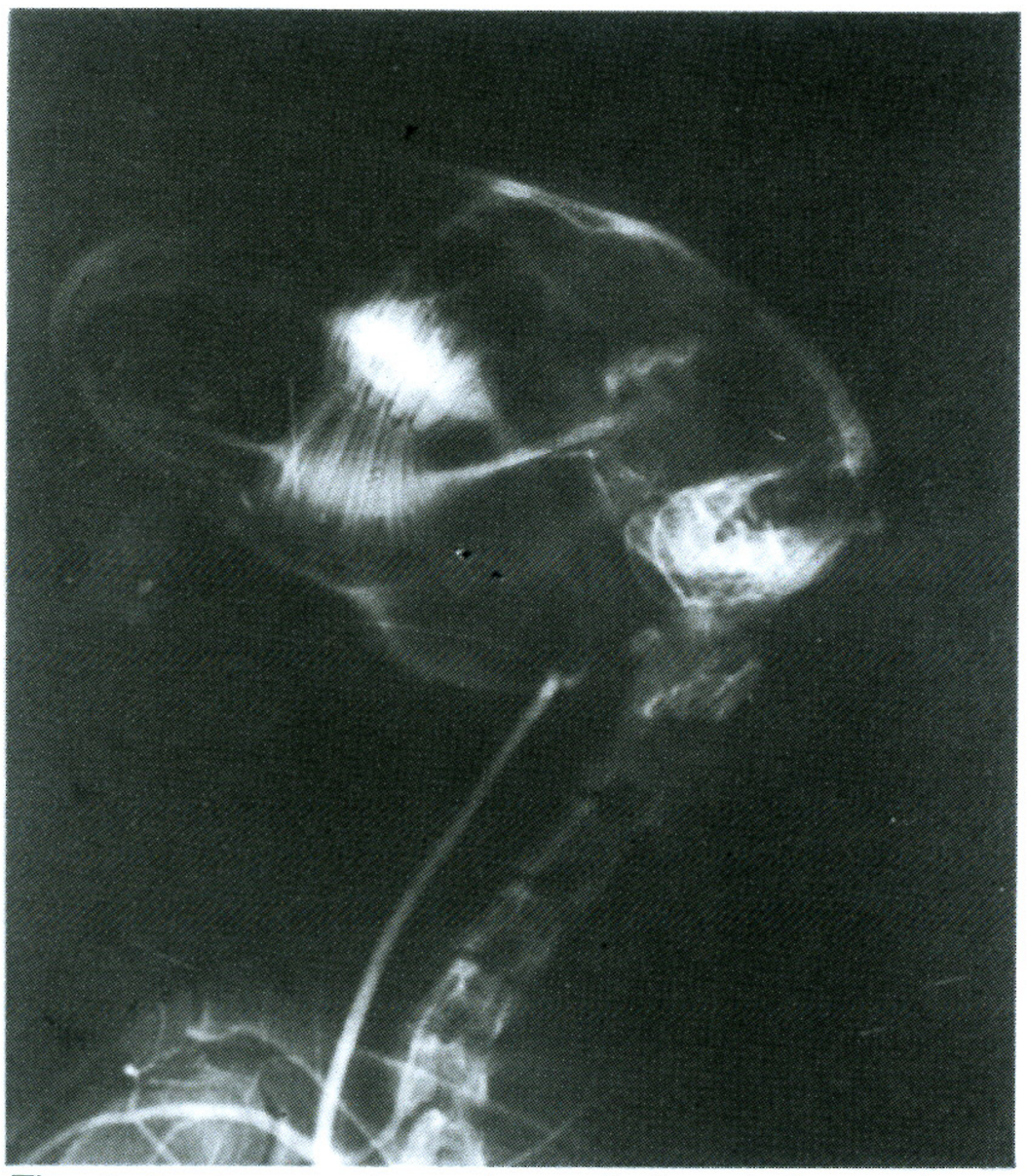

Fig. 1. Angiogram shows the occlusion of all vessels except the internal carotid artery.

experiments, the animals were sacrificed by intravenous pentobarbital injection. The brains were removed and infarcted areas were evaluated with H & E stain.

RESULTS

Reperfusion of the internal carotid artery was demonstrated in 1, 6 and 1 animals of the 7 animals in the saline, urokinase, and LK treated groups, respectively (Fig. 2) In each group, 2, 3 and 2 animals died before pathological examination. Pathological examination showed no gross bleeding in any of the brains. Animals with recanalized vessels did not show infarction whereas non-recanalized animals showed infarction of various sizes (Fig. 3) in the distribution of the internal carotid artery.

DISCUSSION

Despite numerous investigations, fibrinolytic therapy for acute ischemic stroke is still unsatisfactory. In an effort to reduce the side effect of bleeding, tissue specific fibrinolytics such as tPA and its analogues have been tried. Lumbrokinase, an extract from Korean earth worms, may be a potentially useful agent in that it has a strong in vitro fibrinolytic effect even without the presence of plasminogen.

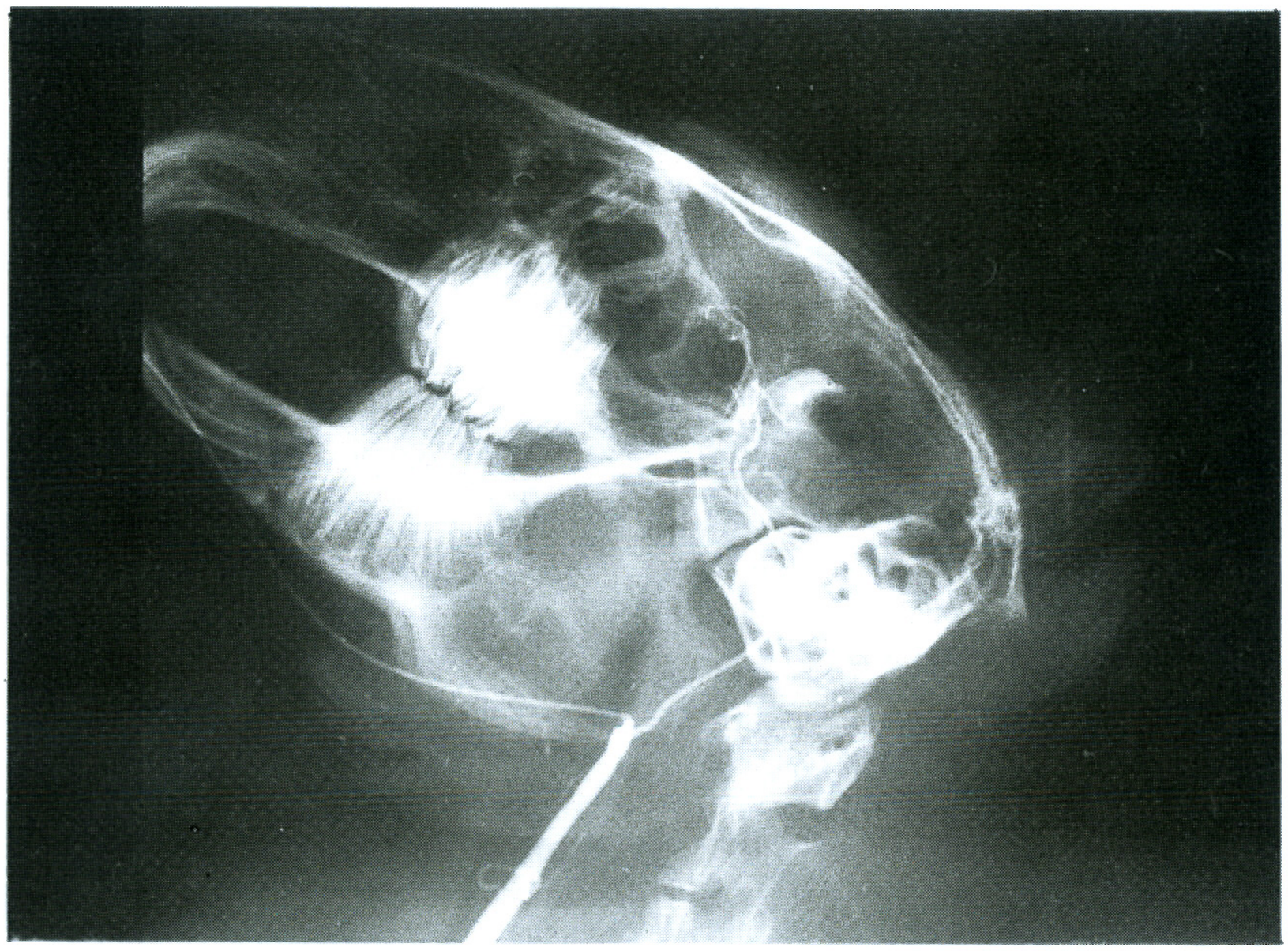

Fig. 2. Angiogram shows reperfusion of the internal carotid artery.

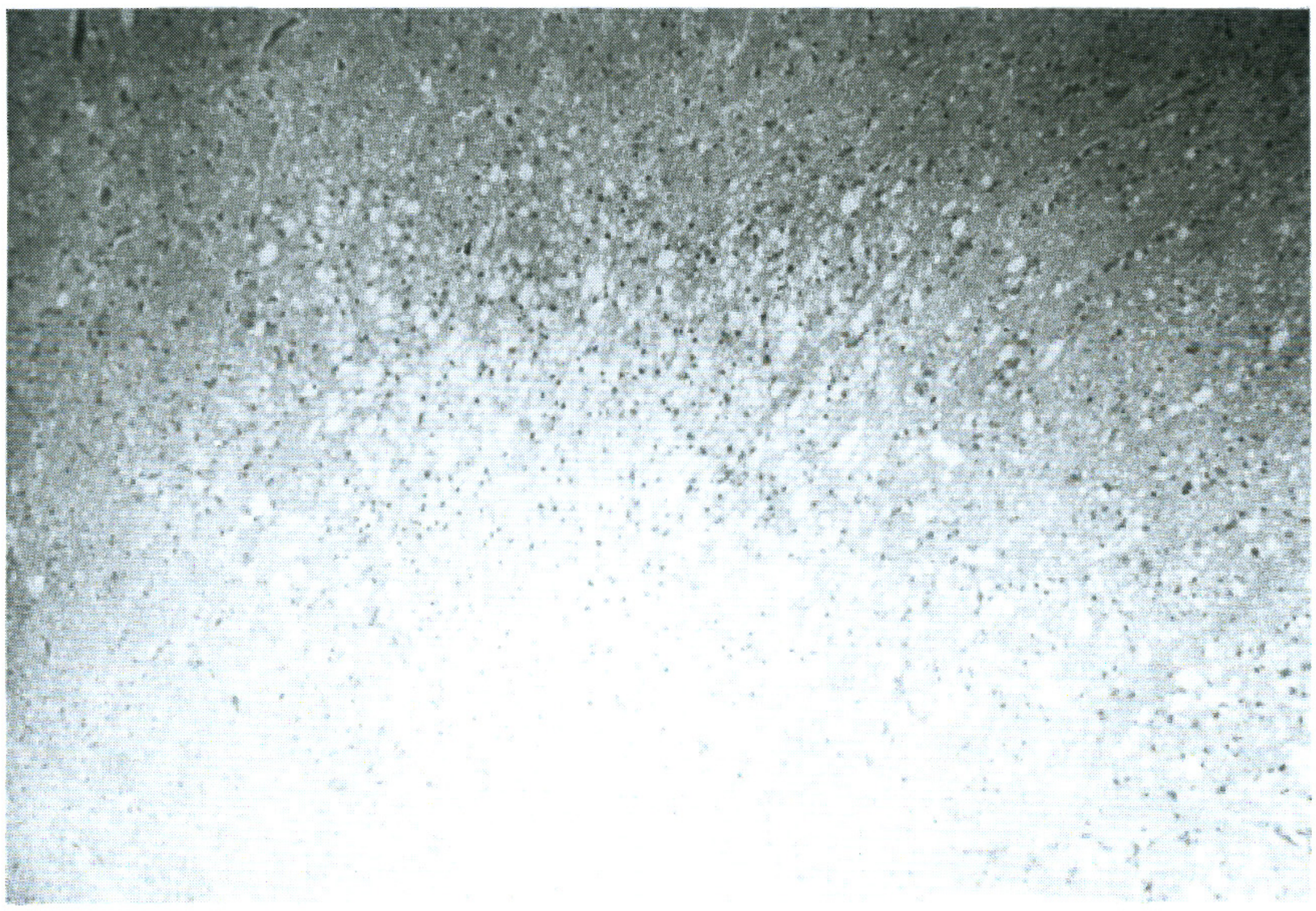

Fig. 3. The figure demonstrates the localized infarcted area in non-canalized animals.

However, contrary to our expectations, the in vivo thromobolytic effect of LK was not as significant as that of equipotent urokinase. Although LK has been known as a strong in vitro fibrinolytic, many things are still unknown including its pharmacokinetics and protein chemistry. In our previous study, LK was effective in thrombolysis in a rat cerebral embolism model. However in that experiment we used a far greater dose of LK compared with urokinase, but the thrombolytic effect was similar in the two groups. Possibly, lumbrokinase may be an enzyme not specific to fibrin. If this is true, many substrates in the blood may weaken its fibrinolytic effect. Some inhibitors or neutralizers of LK may be present in the rabbit blood, or there may

be a strong binding protein for LK which may inactivate it. Further investigations should be performed to determine its weakened in vivo effect and to develop means to potentiate it.

Finally, in our experiment, pathologic studies showed normal findings in the brains of recanalized animals wherease they revealed infarction in the non-recanalized brains. Therefore our model of rabbit cerebral embolism can be used in future investigations for thrombolysis. In the t-PA studies of Phillips et al. (1988) infarcted areas remained despite angiographically recanalized vessels probably due to the late recanalization (180 minutes).

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