

Thrombolysis effect with F II_a from *Agkistrodon acutus* venom in different thrombosis model¹

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KEY WORDS thrombolysis; snake venoms; plasmin

ABSTRACT

AIM: A fibrinolytic enzyme from *Agkistrodon acutus* venom, called F II_a, was tested for thrombolytic activity in animals. **METHODS:** Carotid thrombosis model in rats and rabbits and middle cerebral artery (MCA) thrombosis model in rats were used. **RESULTS:** Intravenous administration of F II_a, at a dosage of 0.625 mg/kg, resulted in thrombolysis of carotid thrombi. However, in middle cerebral artery thrombosis, the effective thrombolysis dose was 1.25 mg/kg. When the dosage of F II_a increased, the thrombolytic effect was stronger. Histological examination of kidney, liver, heart, and lung tissue showed no hemorrhage. **CONCLUSION:** It shows that F II_a from *Agkistrodon acutus* venom is able to solubilize thrombus *in vivo* without hemorrhage at an effective dose for thrombolysis.

INTRODUCTION

It is known that snake venoms can affect blood coagulation. Ever since the thrombin-like enzyme (TLE) isolated from the venom of *Agkistrodon rhodostoma*⁽¹⁾ called anrod is being used in clinic, the utilization of snake venom is being attached importance to. Thrombin-like enzymes are able to decrease the fibrinogen levels in plasma rapidly.

Fibrinolytic enzymes have been purified from some snake venoms⁽²⁻⁴⁾. They can directly solubilize fibrin *in vitro* and hence are different from TLE. A variety of proteases have been isolated from *Agkistrodon acutus* venom. These include TLE and fibrinolytic en-

zyme^(5,6). But little action of the thrombolytic activities of the fibrinolytic enzyme from *Agkistrodon acutus* venom has been determined in experimental animals.

In this paper, we examined the thrombolytic activity of F II_a, a fibrinolytic enzyme isolated from *Agkistrodon acutus* venom. Three different artery models of thrombosis were used and the hemorrhagic activity was also observed.

MATERIALS AND METHODS

F I_a *Agkistrodon acutus* venom lyophilized was purchased from Qimen snake farm, Anhui, China. Column chromatography was used for the isolation and characterization as described before⁽⁷⁾. Fractionation was done until homogeneity was achieved. All other chemicals and solvents used were of analytical grade from commercial sources.

Venom fractionation Isolation procedures were performed by the method previously described⁽⁷⁾. The procedure comprised of three steps including ion-exchange chromatography on DEAE-Sephadex A-50 (Pharmacia, Sweden) and gel filtration on Sephadex G-50 (Pharmacia, Sweden) twice. The final product was homogenous as established by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Carotid thrombosis model in rabbit The carotid thrombosis model in rabbit was carried out according to the method of Wang *et al*⁽⁸⁾. Twenty-four male New Zealand white rabbits (2.2 kg ± 0.2 kg, obtained from Experimental Animal Center of Sun Yat-Sen University of Medical Sciences, Certificate No 26-98A018) were divided into four groups. The left carotid artery was separated from surrounding tissue after anesthesia. Two artery clips were applied on the artery at 4-cm distance. A thread was drawn into the vessel by a needle. Two hours after the removal of clips, single dosages of F II_a of either 0.625, 1.25, or 2.5 mg/kg body weight were injected through marginal ear vein into three separate

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groups of rabbits. The control group was given single injection of saline. After 1 h, the thread with thrombus in the control and the treated group was removed and weighed.

Carotid thrombosis model in rat A rat model with artificial thrombus in the extracorporeal shunt carotid artery of Wistar rat was performed by the method of Chen and Liang^[9]. Twenty-four male Wistar rats (220 g ± 20 g, obtained from Experimental Animal Center of Sun Yat-Sen University of Medical Sciences, Certificate No 98A001) were divided into four groups. The left carotid artery was separated from surrounding tissue after anesthesia. Two ends of the tube with artificial thrombus were inserted in between the cut ends of carotid artery to form blood passage. Single dosages of F II_a of either 0.625, 1.25, or 2.5 mg/kg body weight were injected through hypoglossal vein into three separate groups of rats. The control group was given single injection of saline. After 1 h of injection, thrombus was taken out, the decrease in weight of thrombus was recorded.

Middle cerebral artery thrombosis model in rat A middle cerebral artery (MCA) thrombosis model in Wistar rat was performed by the method of Liu and Xu^[10]. Twenty-four male Wistar rats (220 g ± 20 g, obtained from Experimental Animal Center of Sun Yat-Sen University of Medical Sciences, Certificate No 98A001) were divided into four groups. A section of the left MCA was exposed after anesthesia. The filter paper with 50 % FeCl₃ solution was put on this section for 30 min. Single dosages of F II_a of either 1.25, 2.5, or 5 mg/kg body weight were injected through hypoglossal vein into three separate groups of rats. The control group was given single injection of saline. Then the in-

cision was sewed up. After 24 h, the rat was sacrificed and the brain was quickly removed and sliced and subjected to TTC (triphenyltetrazolium chloride) staining. The weight percent of infarction was estimated.

Histological examination Histological examination was performed on rats of MCA thrombosis model after sacrificing the animals. Tissue section from kidney, liver, heart, and lung were fixed with 10 % formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Each tissue was examined for hemorrhage.

Statistical analysis Data are presented as $\bar{x} \pm s$ and compared with *t*-test. A level of significance less than 0.05 was taken as a minimum.

RESULTS

Thrombolysis was observed to occur at a dosage of 0.625 mg/kg body weight of F II_a in carotid artery thrombosis in rabbit. At dosages of 1.25, 2.5 mg/kg body weight, the thrombolytic effect of F II_a increased (Tab 1). The difference was significant statistically when compared with the saline group ($P < 0.01$, $n = 6$). The same results were obtained in carotid thrombosis in rat (Tab 2). However, administration of F II_a 0.625 mg/kg body weight did not result in any detectable thrombolysis in MCA thrombosis model in rat. By increasing the dosage of F II_a to 1.25 mg/kg body weight, marked thrombolysis was observed. F II_a also lysed thrombus in MCA in a dose-dependent manner (Tab 3).

Microscopic examination of tissue sections from kidney, liver, heart, and lung from MCA thrombosis rat showed no hemorrhage. No other morphological changes were observed between administration of F II_a and saline.

Tab 1. Thrombolytic effect of F II_a in rabbit's carotid artery thrombosis model. $n = 6$. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs saline group.

Dose/mg·kg ⁻¹	0.625	1.25	2.50	Saline
Thrombi weight/mg	13.6 ± 2.9 ^b	10 ± 3 ^c	5.1 ± 2.4 ^c	17 ± 4

Tab 2. Thrombolytic effect of F II_a in rat's carotid artery thrombosis model. $n = 6$. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs saline group.

Dose/mg·kg ⁻¹	0.625	1.25	2.50	Saline
Thrombi decreased weight/mg	1.9 ± 0.6 ^b	4.8 ± 0.9 ^c	7.0 ± 0.8 ^c	0.63 ± 0.21

Tab 3. Thrombolytic effect of FII_a in rat's MCA thrombosis model. $n = 6$. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs saline group. MCA: middle cerebral artery.

Dose/mg·kg ⁻¹	1.25	2.50	5.00	Saline
Infarcted area / %	6.0 ± 0.5 ^b	4.1 ± 0.3 ^c	1.4 ± 0.4 ^c	9.7 ± 0.4

DISCUSSION

In vivo, thrombin-like enzymes, such as ancrod and batroxobin, are effective in fibrinogen removal, discontinuation of fibrin accretion in an established thrombus, and moderate reduction of blood viscosity^[11]. Application of thrombin-like enzymes do not directly result in thrombolysis. Fibrinolytic enzymes from snake venoms which directly dissolve the fibrin can, theoretically, dissolve thrombi *in vivo*. It has been reported that atroxase and VIF (fibrinolytic enzyme from *Vipera lebetina* venom and *Crotalus atrox*, respectively) have thrombolytic activities *in vivo*^[12,13].

Three thrombolysis models were used for determination of the thrombolytic activities of FII_a *in vivo*. This is the first study reporting the thrombolytic activity of FII_a from *Agkistrodon acutus* venom *in vivo*. The results showed that at the dosage of 1.25 mg/kg the thrombi in the three models were all lysed as compared to saline group ($P < 0.05$). At the highest dosage of 5 mg/kg, hemorrhage was not observed by microscopic examination of tissue sections from kidney, liver, heart, and lung. It is thus indicated that FII_a was a thrombolytic agent which does not cause hemorrhage at an effective dose for thrombolysis. It has the potential to become a new thrombolytic agent for thrombosis diseases.

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尖吻蝮蛇毒纤维蛋白溶解酶 FII_a 对不同血栓模型的溶解作用¹

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关键词 血栓溶解; 蛇毒; 纤溶酶

目的: 本文研究尖吻蝮蛇毒纤维蛋白溶解酶 FII_a 体内的溶栓作用。方法: 采用兔颈动脉血栓模型, 大鼠颈动脉血栓模型和大鼠大脑中动脉血栓模型三种动脉血栓模型, 观察 FII_a 的体内溶栓活性。结果: FII_a 剂量为 0.625 mg/kg 时, 对兔颈动脉血栓和大鼠颈动脉血栓即有溶解作用; 而在 1.25 mg/kg 时, 对大鼠大脑中动脉血栓才有溶解作用。随着剂量的增加, 对三种血栓的溶解作用相应增强。大鼠的肾、肝、心、肺组织学检查没有发现出血。结论: FII_a 在体内能够溶解血栓, 并且在有效的溶栓剂量下不会引起出血反应。

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