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Thrombolytic actions of reptilase

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ABSTRACT In thrombolytic model *in vitro*, reptilase (Rep, defibrase) did not show appreciable thrombolytic actions on red and white thrombi. After daily iv infusion of Rep 0.25 IU for 10 d, the time of 50% lysis of euglobulin ($ELT_{1/2}$) was shortened from 9.3± 0.8 to 6.7± 1.0 h ($P<0.01$), alteplase activity was increased from 1.9± 0.7 to 3.7± 0.9 IU · ml^{-1}, and plasminogen inactivator (PI) activity reduced from 4.3± 0.6 to 1.8± 0.9 AU · ml^{-1} (all $P<0.01$). The findings indicate that the thrombolytic action of Rep shown *in vivo* may not be from the direct action on thrombi but from the influence on alteplase and PI activity.

KEY WORDS reptilase; fibrinolytic agents; snake venoms; alteplase; plasminogen inactivators; urokinase

Reptilase (Rep, defibrase) isolated from venoms of adders is a thrombin-like, basic-amino acid hydrolytic enzyme which catalyzes the breakdown of arginine-glycine link within the fibrinogen molecules[1]. But unlike thrombin, Rep splits off only the A-chain leading to the formation of des-A-fibrin monomers which polymerize into non-crosslinked structures and develop into atypical fibrin clots. Physiologically, these clots while developing are readily lyzed by endogenous plasmin and eliminated by the reticuloendothelial system[2]. Animal experimental myocardial infarction size[3] and clinical situation[4] hinted that Rep may be a potentially thrombolytic agent and even better than urokinase[3]. But the evidence of the thrombolytic effect of Rep has been far from convincing. In this study, we attempted to evaluate whether Rep possesses thrombolytic activity and to probe its probable mechanism.

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MATERIALS AND METHODS

Subjects The experiments were conducted on blood from 62 apparently healthy and nonsmoking people, 40 M and 22 F, aged 32± *s* 10 a. They claimed not to have taken any drugs during 14 d before blood collection. Those who were chosen to take part in the tests of euglobulin lysis time (ELT), alteplase activity and plasminogen inactivator (PI) activity were normal in laboratory tests except the increased level of plasma fibrinogen which was the reason for them to accept the trial therapy with Rep.

Materials Bovine thrombin (Sigma); urokinase (The First Pharmaceutical Factory of Suzhou); Rep (Changbeishan Pharmaceutical Factory of Shenzhen); alteplase and PI activity diagnostic kits (Research Section for Molecular Genetics, Shanghai Medical University).

Formation and lysis of red thrombi The artificial thrombi full of red blood cells were prepared[5]. The lytic test of red thrombi was conducted in a system designed on our own. The core of the system was a quartz cell containing 2 ml of platelet-poor plasma (PPP). Cells were cultivated at 37℃. The medium was agitated by a mini-magnetic-stirrer (Model 333, Hellma, Germany). On being formed and weighed, the thrombi were transferred into PPP, the thrombolytic medium. Urokinase or Rep was added before agitation. After incubation for 30 min, the residual thrombi were weighed again. Thrombolytic rates were calculated.

Formation and lysis of white thrombi The white thrombi full of platelets and fibrin were formed in platelet-rich-plasma (PRP)[6], and lyzed in defibrinogenated PPP. Bovin thrombin 5 IU was used instead of collagen to induce the formation of thrombi. PPP was defibrinogenated by adding

thrombin 100 IU · ml^{-1} and centrifuging at 1000 × *g* before thrombolysis. Changes in thrombus weight and absorbance of the medium were employed to indicate the thrombolysis.

Euglobulin lysis time (ELT) The preparation of euglobulin fraction and ELT were tested[7]. $ELT_{1/2}$ was utilized to represent the time of 50% lysis of the euglobulin clots.

Alteplase and plasminogen inactivator (PI) activity Alteplase and PI activity was assayed according to reference[8].

Statistical methods The *t* test and one way analysis of variance were used.

RESULTS

Lysis of red thrombi The artificial red thrombi had a weight of 65 ± 26 mg. Saline (control, $n=10$) led a rate of 26 ± 4% reduction in the thrombus weight. Urokinase (500–5000 IU · ml^{-1}) produced a concentration-related thrombolysis, and its action reached the maximum at 2000 IU · ml^{-1}. Rep 1.25×10^{-3} and 6.25×10^{-3} IU · ml^{-1} reduced the thrombus weight slightly but not significantly different ($P>0.05$) from the control (saline). Nevertheless, at the level of 1.25×10^{-2} and 2.5×10^{-2} IU · ml^{-1} it increased the weight of the thrombi (Tab 1).

After depleting the fibrinogen in the medium by thrombin, the action of urokinase was almost the same as that in PPP. Rep, although failed to increase the weight of thrombi, still displayed no significantly thrombolytic property in comparison with saline (Tab 1).

Lysis of white thrombi The weight of the white thrombi ($n=20$) was 22 ± 2 mg. In the control ($n=10$), the weight of the residual thrombi decreased by 25 ± 4% after incubation for 30 min. But the absorbance appeared almost unchanged.

Urokinase (2000 IU · ml^{-1}) caused the reduction of residual weight of white thrombi and absorbance at a rate of 71 ± 5% and 22 ± 3%, respectively (both $P<0.01$ *vs* saline). Rep 1.25×10^{-3}– 2.5×10^{-2} IU · ml^{-1} did not bring about appreciable decrease in thrombus weight. The change of absorbance was not noteable although it was significant in statistically *vs* saline (Tab 1).

Tab 1. Changes of weight of thrombi and absorbance after incubation with reptilase for 30 min. $n=10$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

Reptilase / $\times10^{6}$ IU · ml^{-1}	Thrombus weight / mg Pre– treatment	Post– treatment	Absorbance / %
Red thrombi in platelet–poor plasma			
0	65 ± 21	51 ± 20	
1 250	69 ± 18*	54 ± 13*	
6 250	64 ± 25*	59 ± 13*	
12 500	70 ± 12*	77 ± 13**	
25 000	59 ± 20*	83 ± 18***	
Red thrombi in defibrinogenated platelet–poor plasma			
0	66 ± 16	49 ± 11	
1 250	64 ± 10*	47 ± 10*	
6 250	65 ± 16*	44 ± 11*	
12 500	66 ± 14*	47 ± 12*	
Urokinase 2000 IU · ml^{-1}	64 ± 13*	21 ± 11***	
White thrombi in defibrinogenated platelet–poor plasma			
0	20.3 ± 1.4	15.1 ± 1.2	−3.0 ± 1.4
1 250	21.4 ± 1.1*	16.3 ± 1.3*	1.4 ± 1.3**
6 250	19.8 ± 1.2*	15.4 ± 1.5*	1.5 ± 1.3**
12 500	20.6 ± 1.6*	15.8 ± 1.4*	2.5 ± 1.3***
25 000	19.6 ± 1.4*	14.7 ± 1.1*	1.9 ± 1.4**

ELT After daily iv infusion of Rep 0.25 IU in 500 ml of 10% glucose saline for 10 d, $ELT_{1/2}$ was shortened from 9.3 ± 0.8 to 6.7 ± 1.0 h ($n=10$, $P<0.01$).

Alteplase and PI activity After iv infusion of Rep for 10 d, human alteplase activity increased from 1.9 ± 0.7 to 3.7 ± 0.9 IU · ml^{-1} and PI activity decreased from 4.3 ± 0.6 to 1.8 ± 0.9 AU · ml^{-1}, respectively (both $P<0.01$).

DISCUSSION

After iv infusion of Rep, the thrombolytic action in circulating blood was

enhanced. But the mechanism remains unidentified. Our *in vitro* approaches did not indicate any direct lytic feature of Rep on red and white thrombi. As alteplase and PI were considered to play an important role in the regulation of fibrinolytic system, the increase in alteplase activity and decrease in PI activity after medication may be the bases that underlie the thrombolytic effects of Rep observed *in vivo*. The rheological changes in blood may be another mechanism to comprehand the action shown *in vivo*[2]. The precise mechanism of Rep on alteplase and PI activity remains unclear. Fibrin is capable of suppressing the release of PI from endothelial cells[9]. Whether the reduction in PI activity is the result of the increased production of fibrin by the thrombin-like action of Rep which inhibits the release of PI from endothelial cells is to be explored.

REFERENCES

1 Esnouf MP, Tunnah GW. The isolation and properties of the thrombin like activity from *Agkistrodon rhodostoma*. *Br J Haematol* 1967; **13** : 581-90.

2 Ernst E. Hemorheological treatment. In: Chien S, Dormandy J, Ernst E, Matrai A, editors. *Clinical hemorheology — Applications in cardiovascular and hematological disease, diabetes, surgery and gynecology*. Dordrecht: Martinus Nijhoff, 1987 : 329-73.

3 Huang DX, Song AL, Yang XS, Wang SR, Li WH. Comparison of the efficacy of intravenous urokinase and defibrase on 6-hour-old canine coronary thrombosis. *Chin J Cardiol* 1988; **16** : 363-5.

4 Li TD, Gao LY, Wang SR, Yang ST, Zhi G, Du LS, *et al*. Coronary thrombolysis with defibrase. *Chin J Intern Med* 1990; **29** : 88-90

5 Liao CL, Zou QJ, Li JM, Cui H, Yuan NR. Antithrombotic activity of verapamil. *Acta Pharmacol Sin* 1992; **12** : 28-30.

6 Terres W, Beythien C, Kupper W, Bleifeld W. Effects of aspirin and prostaglandin E_1 on *in vitro* thrombolysis with urokinase——Evidence for a possible role of inhibiting platelet activity in thrombolysis. *Circulation* 1989; **79** : 1309-14.

7 Takada A, Takada Y, Urano K, Sakakibara K, Rydzewski A. Fluctuations of euglobulin lysis time, tissue plasminogen activator, and free and total plasminogn activator inhibitor levels in plasma in daytime. *Thromb Res* 1990; **57** : 13-20.

8 Wang JY, Jia HY, Song HY. Determination of Tissue-type plasminogen activator and plasminogen activator inhibitor in human plasma. *Chin J Med Lab Technol* 1989; **12** : 163-6.

9 Fukao H, Ueshima S, Tanaka N, Okada K, Matsuo O. Supression of plasminogen activator inhibitor 1 release by fibrin from human umbilical vein endothelial cells. *Thromb Res* 1990; **Suppl 10** : 11-20.

蛇毒凝血酶的溶栓作用

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提要 蛇毒凝血酶在体外对红、白血栓均无明显作用。iv 该药 0.25 IU · d^{-1} 1 周后，人优球蛋白溶解 50%的时间，组织纤维蛋白溶酶原激活剂(alteplase)活性及血纤维蛋白溶酶原失活剂(PI)活性均呈明显改变。结果显示体内所观察到的溶栓作用并非蛇凝血酶对血栓的直接作用，而是通过影响 alteplase 和 PI 继而使体内的纤溶活性增加。

关键词 蛇毒凝血酶；纤维蛋白溶解剂；蛇毒；组织纤维蛋白溶酶原激活剂；血纤维蛋白溶酶原失活剂；尿激酶类