Potentiation and Acceleration Effects in Combined Administration of Tissue Plasminogen Activator and Fibrinogen-Modified Urokinase in Dogs with Modeled Venous Thrombosis

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Combined administration of tissue plasminogen activator and a urokinase-fibrinogen covalent conjugate is studied using modeled venous thrombosis in dogs. In comparison with the effect of the individual preparations the thrombolytic effect was potentiated when intravenous bolus injection of 1 mg tissue plasminogen activator followed by a 2-hour infusion of 4 mg of this preparation was combined with bolus injection of 25,000 IU urokinase-fibrinogen covalent conjugate 15 min after the first bolus. Potentiation and acceleration of thrombolysis were attained with the same scheme when tissue plasminogen activator was administered in a dose of 1 mg for both bolus and infusion and combined with 250,000 IU of fibrinogen-modified urokinase.

Key Words: *tissue plasminogen activator; urokinase-fibrinogen covalent conjugate; venous thrombosis; combined thrombolytic activity; potentiation*

Plasminogen activators fulfill the function of a biocatalyzer, converting plasma protein plasminogen into plasmin [10]. The latter exhibits proteolytic activity and the substrate for this reaction is fibrin. The proteolytic cleavage of fibrin by plasmin is responsible for destruction of the thrombus [15]. This restores circulation in the obstructed vessel and reduces the severity of circulatory disorders.

New, more potent thrombolytic preparations of plasminogen activators for clinical use are now being developed [6] using methods of chemical [1] and biological [2] synthesis, and alternative sources of these preparations are being studied [4]. Previously we conjugated urokinase with fibrinogen [14] and such a modified urokinase preparation was found to possess pronounced and prolonged thrombolytic activity *in vivo* [13]. However, the development of new preparations is not the only way of enhancing the effecticiency of thrombolysis [4].

Combined administration of various plasminogen activators also boosts the efficiency of thrombolysis [3] due to their different mechanisms of action on the fibrin surface [9]. The major advantages of this combined thrombolytic therapy derive from the combination of the trigger effect of tissue plasminogen activator (creating new plasminogen binding sites of type II on the clot [8]) and the effect of another activator which sustains (prolongs) thrombolysis [4,11].

The present study was undertaken to test such an approach on a model of venous thrombosis in dogs using tissue plasminogen activator (TPA) as the trigger and a prolonged form of urokinase, a urokinase-fibrinogen (UK-FBG) covalent conjugate, as the sustaining agent.

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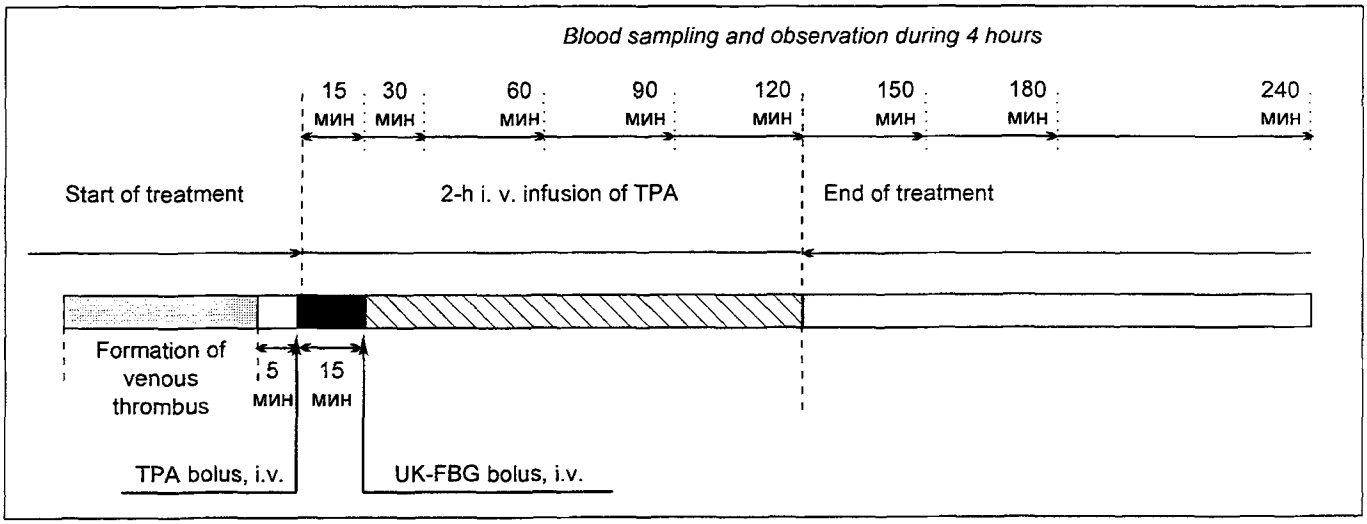

Fig. 1. General scheme of the experiment. Proposed regime of administration of TPA and UK-FBG covalent conjugate.

MATERIALS AND METHODS

Recombinant TPA (Dr. Karl Thomas) and a UK-FBG covalent conjugate synthesized from a commercial preparation of native urokinase (Jpn. Chem. Res.) and human fibrinogen (Sigma) as described previously [14] were used in the experiments.

The thrombolytic efficiency of plasminogen activators was evaluated using modeled venous thrombosis in dogs [5]. The experiments were carried out on mongrel dogs weighing 10-21 kg (16 kg on average). Blood samples were taken 5 min after the clamps were removed from the thrombosed vein (a result of interaction of ^{131}I-fibrinogen and serum fibrinogen with thrombin) and radioactivity was measured on a Compugamma (LKB) counter. The blood was also sampled 15, 30, 60, 90, 120, 150, 180, and 240 min after the first injection of the thrombolytic. Changes in the radioactivity of these samples (% of initial value) reflected the dynamics of thrombolysis *in vivo* [5]. Each experimental group comprised 3-4 dogs. The doses of the preparations and the mode of administration are presented in Table 1. The general scheme of the experiment is shown in Fig. 1.

The data are presented as means and standard deviations. Statistical processing of the results was performed using Kwikstat 2.11® software [7]. The differences were considered to be significant at $p<0.05$.

RESULTS

Intravenous bolus injection of UK-FBG conjugate (25,000 IU) has previously been noted to exhibit a prolonged thrombolytic effect [5]. This effect did not differ reliably from that produced by intravenous bolus injection of 2.5 mg TPA (group 2 and 3, Table 2). To enhance the thrombolytic effect, TPA and UK-FBG were administered in combination. For this, bolus injection of TPA (1 mg in 10 ml physiological saline) followed by a 2-hour infusion of a certain dose of TPA in 50 ml saline (Table 1) was combined with bolus injection of UK-FBG (in 10 ml saline, for dose see Table 1) 15 min after the first bolus (Fig. 1). This scheme was tested in various ratios of plasminogen activators (Table 1). It was found that the effect of bolus-infusion administration of TPA (1 mg for each mode) in combination with bolus injection of 25,000

TABLE 1. Experimental Groups of Animals

Group	No. of dogs *in group*	Mode of administration of plasminogen activators		
		TPA		UK-FBG conjugate, bolus 15 min after first TPA bolus, IU
		first injection, mg	2-hour infusion, mg	
1	4	Isotonic NaCl, 10 ml - control		
2	4	-	-	25,000
3	3	2.5	-	-
4	4	1	1	25,000
5	4	1	4	25,000
6	4	1	1	250,000
7	3	-	-	250,000

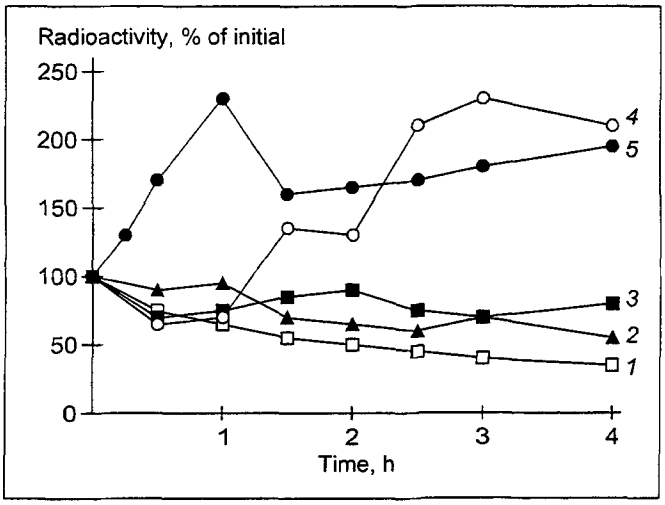

Fig. 2. Mean radioactivity of blood samples taken at different intervals after administration of: *1*) isotonic NaCl (10 ml, bolus); *2*) TPA (2.5 mg, bolus); *3*) TPA (1 mg, bolus+1 mg, infusion) and UK-FBG (25,000 IU, bolus); *4*) TPA (1 mg, bolus+4 mg, infusion) and UK-FBG (25,000 IU, bolus); *5*) TPA (1 mg, bolus+1 mg, infusion) and UK-FBG (250,000 IU, bolus).

IU UK-FBG conjugate did not differ reliably from the effect of the same doses of the individual components (groups 2-4, Table 2). Taking into account the fact that the bolus-infusion scheme of urokinase administration to dogs produced the maximal thrombolytic effect in a ratio of 1:3 [12] and tha TPA has a short half-life in the circulation [4,6,9,15], we injected 1 mg TPA in a bolus and infused 4 mg in combination with bolus injection of 25,000 IU UK-FBG conjugate. This combination markedly potentiated thrombolysis, especially after the TPA infusion was terminated (group 5, Table 2). However, the maximal potentiation of thrombolysis was attained when the dose of the prolonged agent UK-FBG conjugate was increased. When TPA was administered in a dose of 1 mg for each mode (bolus and infusion), the dose of UK-FBG conjugate was increased to 250,000 IU (group 6, Table 2), which markedly accelerated the effect of the compound (Fig. 2). Administration of UK-FBG conjugate alone (group 7, Table 2) in the same dose produced an effect reliably different from that of the above combination of thrombolytics (group 6, Table 2). It may be assumed that the thrombolytic capacity of UK-FBG conjugate manifests itself either in combination with infusion of medium doses of TPA (group 5, Table 2) or when low doses of TPA are combined with a bolus of high doses of UK-FBG conjugate (group 6, Table 2). This administration mode and dose ratio result in a strong trigger effect of TPA leading to a subsequent pronounced thrombolytic effect of UK-FBG conjugate. The combined administration of these agents potentiates their thrombolytic effects (Fig. 2.)

Thus, combined administration of TPA and UK-FBG covalent conjugate seems offer promise for the development of effective thrombolytic compounds for urgent care.

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TABLE 2. Radioactivity of Blood Samples (% of Initial) after Administration of Plasminogen Activators (*M±m*)

Group	Radioactivity after first injection, min							
	15	30	60	90	120	150	180	240
1	-	72±18	62±11	51±16	46±16	38±6	32±10	26±13
2	-	76±25	82±10	79±3	88±26	75±7	66±14	81±9
3	-	86±21	89±13	64±22	60±11	56±16	64±15	49±9
4	-	68±32	77±14	85±9	94±18	73±12	66±16	81±13
5	-	64±22	67±28	134±36	128±31	208±20	224±28	211±19
6	128±28	169±9	243±44	162±23	167±19	177±31	189±24	199±16
7	95±21	58±28	119±42	81±15	90±6	67±19	72±13	105±11
p_{6-1}		*	*	*	*	*	*	*
p_{6-3}		*	*	*	*	*	*	*
p_{6-7}		*	*	*	*	*	*	*
p_{6-5}		*	*		*	*	*	
p_{5-1}				*	*	*	*	*
p_{5-2}				*		*	*	*
p_{5-4}				*	*	*	*	*
p_{2-3}								*
p_{3-4}								*

Note. *Differences significant at $p<0.05$ (t test and ANOVA)

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