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Venous Thrombolysis by Tissue-Type Plasminogen Activator in Conjunction with the Urokinase-Fibrinogen Covalent Conjugate

Effects of Potentiation and Faster Action in Dogs

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ABSTRACT

Conjunctive administration of the tissue-type plasminogen activator (t-PA) and the urokinase-fibrinogen covalent conjugate (UK-Fbg) was studied by the example of venous thrombosis in dogs. Comparing the effect of separate use of the two components, we observed the potentiation of thrombolytic effect induced by an iv bolus infusion administration of the tissue-type plasminogen activator (1 and 4 mg, respectively) combined with a bolus administration 15 min after the first injection of the 25,000 IU UK-Fbg. Faster-action and potentiation effects of thromboysis were observed with the same administration scheme when the t-PA was used as bolus infusion (1 and 1 mg, respectively) combined wiht a bolus of the 250,000 IU fibrinogen-modified urokinase. The findings indicate an approach to the development of efficient thrombolytic compositions.

Index Entries: Tissue-type plasminogen activator; urokinase-fibrinogen covalent conjugate; venous thrombosis; conjunctive thrombolytic action; potentiating effect.

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INTRODUCTION

Plasminogen activators catalyze the conversion of plasma protein plasminogen into the plasmin enzyme in the living organisms (*1*). Plasmin exhibits proteolytic activity with respect to fibrin. The proteolysis of fibrin is responsible for dissolving thrombus (*2*). The blood flow is restored in a thrombosed vessel, and cardiovascular injuries become less crucial.

New preparations of plasminogen activators are under development to enhance the thrombolytic efficacy (*3*). Chemical (*4*) and biological syntheses (*5*) are employed, and the search for new plasminogen activators from other sources is in progress (*6*). Earlier, we conjugated urokinase with fibrinogen (*7*). This preparation exhibited a pronounced and prolonged thrombolytic effect in vivo (*8*). However, other approaches exist for increasing the efficacy of thrombolysis (*6*). One of such is a conjunctive administration of various plasminogen activator preparations (*9*). The efficacy of this approach is determined by different action mechanisms of plasminogen activators on the fibrin surface (*10*). It is suggested that a combination of the trigger action of the tissue-type plasminogen activator (t-PA) (the later results in the exposure of new plasminogen binding sites of the second type on the lysed fibrin clot *[11]*) and prolonged action of another activator that sustains thrombolysis (*6,12*) will be advantageous for the conjunctive thrombolytic therapy (*9*).

This work pursued an aim of testing this approach by the example of venous thrombosis in dogs using the t-PA as a trigger and a prolonged form of the urokinase-fibrinogen covalent conjugate (UK-Fbg) as a supporting agent for combined thrombolysis.

METHODS

Preparations of the recombinant t-PA (Karl Thomae GmbH, Germany) and the UK-Fbg obtained as described earlier (*7*) from native urokinase (Japan Chemical Research Co., Ltd., Japan) and human fibrinogen (Sigma, USA) were used in the study.

Thrombolytic efficacy of the preparations was assayed by the example of venous thrombosis in dogs as described earlier (*13*). Mongrel dogs of body wt 10–21 kg (mean wt 16 kg) were used. The initial level of blood radioactivity was determined on a "Compugamma" counter (LKB, Sweden) in 5 min after removal of the ligature from the vein with thrombus formed by interacting ^{131}I-fibrinogen and blood fibrinogen with thrombin. Blood samples were also taken after 15, 30, 60, 90, 120, 150, 180, and 240 min after the thrombolytic was administered. Changes in the blood radioactivity level (% from background) indicated the dynamics of thrombolysis in vivo (*13*). Each group included three to four dogs. Administration modes and doses administered are listed in Table 1. The general scheme of experment is represented in Fig. 1.

Table 1
Animal Groups

Group of animals, no.	Administration regime of plasminogen activators: t-PA administration — The first injection of t-PA, mg	t-PA administration — 2-h infusion, mg	Administration of UK-Fbg iv as a bolus 15 min after the first t-PA bolus, IU	Number of dogs in the group, *n*
1	NaCl isotonic solution, 10 mL, Control			4
2	—	—	25,000	4
3	2.5	—	—	3
4	1	1	25,000	4
5	1	4	25,000	4
6	1	1	250,000	4
7	—	—	250,000	3

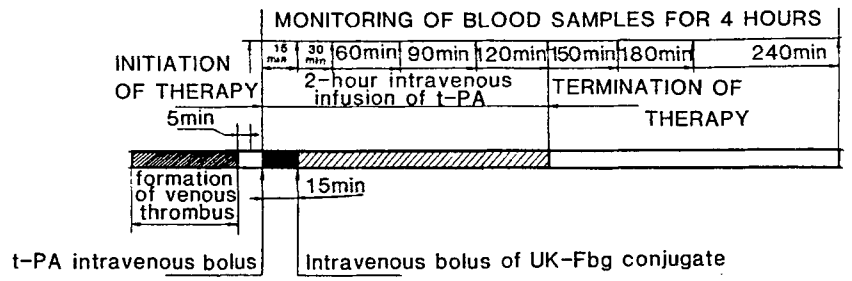

Fig. 1. General scheme of the experiment. Conventional depiction of the tested administration regime of t-PA and the UK-Fbg.

Results are given as the mean values with the standard deviations. The statistical analysis was performed by using a Kwikstat 2.11® statistical program (*14*).

RESULTS AND DISCUSSION

An iv bolus administration of 25,000 IU of the UK-Fbg was reported (*13*) to prolong the thrombolytic effect of the preparation. No significant difference was observed in the separate action of this dose and t-PA administered intravenously as a bolus (2.5 mg dose, groups 2 and 3 in Table 2). The conjunctive administration of t-PA and the UK-Fbg enhanced the thrombolytic effect. In particular, we performed a 2-h infusion of t-PA (in 50 mL saline) after bolus administration of 1 mg t-PA (in 10 mL saline) followed by UK-Fbg (in 10 mL saline) as an iv bolus 15 min after the first bolus

Table 2
Blood Samples' Radioactivity in Dogs
(% of the Background Level) After Administration of Plasminogen Activators

Group of animals, no.[a]	Blood sample radioactivity after the first administration, 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 ± 10
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}		*b*	*b*	*b*	*b*	*b*	*b*	*b*
P_{6-3}		*b*	*b*	*b*	*b*	*b*	*b*	*b*
P_{6-7}		*b*	*b*	*b*	*b*	*b*	*b*	*b*
P_{6-5}		*b*	*b*	*b*	*b*	*b*	*b*	*b*
P_{5-1}				*b*	*b*	*b*	*b*	*b*
P_{5-2}				*b*	*b*	*b*	*b*	*b*
P_{5-4}				*b*	*b*	*b*	*b*	*b*
P_{2-3}								*b*
P_{3-4}								*b*

[a]Designation of the animal groups according to Table 1.
[b]Significant differences in the values of the compared groups; significance level $P < 0.05$ (*t*-test and ANOVA).

(Table 1). Different ratios of plasminogen activators were tested in this administration procedure (Fig. 1, Table 1). The bolus infusion administration in 1 mg t-PA and the bolus administration of 25,000 IU UK-Fbg showed virtually no significant difference compared with the independent action of similar doses (groups 2–4, Table 2). Taking into account that the highest thrombolytic effect in dogs was achieved with the bolus infusion scheme of urokinase administration at the dose ratio of 1:3 (*15*) and the fact that t-PA has a short half-life in the blood flow (*2,3,6,10*), we initiated the bolus administration of 1 mg t-PA and infusion of 4 mg t-PA combined with 25,000 IU of Uk-Fbg. Such a combination increased the thrombolysis considerably, especially when the t-PA infusion was finished (group 5, Table 2). However, the highest rate of thrombolysis was achieved with the increased dose of UK-Fbg. The conjugate possesses the prolonged thrombolytic action (*8,13*). A bolus dose of UK-Fbg was raised to 250,000 IU during the bolus infusion administration in 1 mg t-PA (group 6, Table 2), which provided a significant faster-acting effect of the composition (Fig. 2). The bolus administration of UK-Fbg alone (group 7, Table 2) gave a significantly different result (group 6, Table 2). This suggests that the thrombolytic potential of UK-Fbg is realized either in a combination with mean t-PA doses infused (group 5, Table 2) or in the presence of small t-PA doses in combination with a bolus of large UK-Fbg doses (group 6, Table 2). Likely, such dose regimes and ratios trigger t-PA effectively to

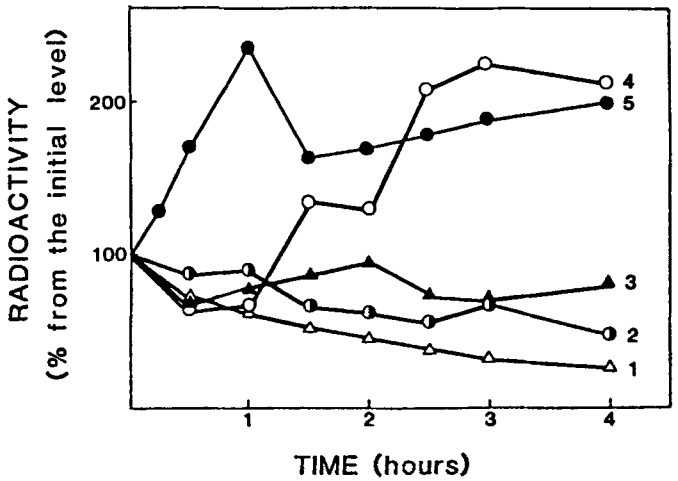

Fig. 2. Mean level of radioactivity of blood samples in dogs against time of taking blood samples after administration: 1—NaCl isotonic solution, a bolus of 10 mL (control); 2—2.5-mg bolus of t-PA; 3—1-mg bolus of t-PA, 1-mg infusion of t-PA, and 25,000 IU of UK-Fbg as a bolus; 4—1-mg bolus of t-PA, 4-mg infusion of t-PA, and 25,000 IU of UK-Fbg as a bolus; 5—1-mg bolus of t-PA, 1-mg infusion of t-PA, and 250,000 IU of UK-Fbg as a bolus.

enhance the thrombolytic effect of UK-Fbg. The conjunctive administration of the thrombolytics does potentiate their thrombolytic action (Fig. 2).

In conclusion, the conjunctive administration of t-PA and UK-Fbg appears to be promising in developing efficient thrombolytic compositions for urgent therapy.

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