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Comparative Study of a Mutant Tissue-type Plasminogen Activator, YM866, with a Tissue-type Plasminogen Activator in a Canine Model of Femoral Arterial Thrombosis

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Abstract

Because tissue-type plasminogen activator (tPA), used to treat myocardial infarction, has several disadvantages thought to be connected with its low half-life, mutants of tPA have been prepared with longer half-lives. We have compared the thrombolytic effect of such a mutant, YM866, with that of tPA in copper-coil-induced femoral arterial thrombosis in dogs.

One hour after thrombus formation, YM866 was administered by intravenous bolus injection, while tPA was given by the same method or by 60-min infusion under adequate heparinization. Both agents exhibited dose-dependent thrombolysis without systemic fibrinogenolysis. The recanalization rate and recanalization time of YM866 by bolus at 0·2 mg kg^{-1} were, however, equivalent to those of tPA by infusion at 0·4 mg kg^{-1} (total dose), whereas the recanalization rate of tPA by bolus was low (0·4 mg kg^{-1}). No significant difference in reocclusion rate, reocclusion time, or patency status after successful thrombolysis was seen.

These results suggest that YM866 administered at a lower dose by intravenous bolus injection exerted a thrombolytic effect equivalent to that of tPA by infusion, and that heparin could not prevent reocclusion after successful thrombolysis even under adequate anticoagulation.

Tissue-type plasminogen activator (tPA) has high affinity for fibrin, and its beneficial effects in thrombolytic therapy for myocardial infarction are well established (Collen et al 1988). Despite its widespread use, some significant limitations have been reported, including resistance to reperfusion in approximately 25% of patients (Rentrop 1985), occurrence of acute coronary reocclusion in 5–25% (Johns et al 1988), prolonged time (average 45 min) to restoration of coronary flow (Simoons et al 1988), and a tendency to bleeding, with frequency of intracranial bleeding of approximately 0·5% (The International Study Group 1990). Various means of overcoming these shortcomings are currently under investigation in laboratory studies or clinical trials. Some of the shortcomings are thought to be attributable to the extremely short half-life of tPA (Gold et al 1986). Attempts have been made to modify the tPA molecule by genetic engineering techniques, and several mutants of tPA that have prolonged plasma half-life and that can be administered by intravenous bolus injection have been reported (Suzuki et al 1991; Martin et al 1992).

YM866 is a mutant tPA with deletion of the kringle-1 domain and with point mutation at the site of the kringle-2 domain linkage to the light chain (del. 92-173, ^{275}Arg-Glu; Kawauchi et al 1991). It has been shown in-vitro to possess pronounced affinity for fibrin, and to retain the same specific activity of tPA (Katoh et al 1991). Further, the inhibition of YM866 and tPA activities by plasminogen activator-I (PAI-1) is comparable (Katoh et al, unpublished data). A remarkably sustained plasma concentration of YM866 has been demonstrated in several animals and in man. Antigenicity studies of the preparation in chimpanzees have shown no antigenic potential of any toxicological concern (Oohata et al, unpublished data). Clinical trials of YM866 in patients with acute myocardial infarction are currently in progress, and some expected results have been obtained, namely high reperfusion rates by intravenous bolus injection and a shortened time to restoration of coronary flow.

We previously reported that the thrombolytic activity of YM866 by intravenous bolus injection was 2 to 4 times greater than that of tPA by intravenous bolus injection or intravenous infusion in some models of thrombosis (Kawasaki et al 1993, 1994a, 1994b), and that the incidence of reocclusion during a limited, short time (1 h) after successful thrombolysis was lower than that of tPA by intravenous bolus injection at dosages attaining satisfactory recanalization rates (Kawasaki et al 1993). We have not, however, quantitatively investigated the effect of YM866 on recanalization, reocclusion or patency status after successful thrombolysis.

In this study we have evaluated the effect of YM866 on recanalization and reocclusion after successful thrombolysis in comparison with that of tPA in a canine model of femoral arterial thrombosis.

Materials and Methods

Thrombolytic agents

YM866 is a recombinant tissue-plasminogen activator analogue which contains a finger domain, growth factor domain, kringle-2 domain, serine protease domain, and a point mutation at the kringle-2-serine protease linkage site (Mr = 55 000, del. 92–173, ^{275}Arg-Glu; Kawauchi et al 1991). Preparations of YM866 used in this study contained more than 98% of the single-chain form. Lyophilized preparations of YM866 and

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tPA (Mw = 65 000, Activase, 50 mg vial^{-1}; Genentech Inc., USA) were dissolved in distilled water for injection and were diluted with saline. The specific activities of YM866 and tPA as determined by fibrin clot lysis assay calibrated with international standard (83/157) were 570 000 and 600 000 IU mg^{-1}, respectively (Katoh et al 1991). For the control group, a lyophilized preparation (placebo) with the same constituents as those above but without YM866 was dissolved and diluted in the same manner.

Induction of thrombosis

Forty adult mongrel dogs, approximately 11 kg, of both sexes were used. The animals were anaesthetized by intramuscular injection of xylazine (Ceractal; Bayer Japan) at 10 mg kg^{-1} and by intravenous injection of sodium pentobarbital (20–30 mg kg^{-1}), incubated, and artificially ventilated with room air. Catheters were placed in the cephalic and femoral vein for injection of test drugs and collection of blood samples, respectively. Femoral arterial thrombosis was induced by a copper coil method modified from that of Bush et al (1989). Left carotid artery and left femoral artery were gently isolated and exposed. Before insertion of the coil, baseline flow of the femoral artery was measured with an electromagnetic flow probe (2- to 3-mm diameter) placed in contact with the exposed artery and connected to an electromagnetic blood flow-meter (MVF-2100; Nihon Kohden, Tokyo, Japan) for recording on a polygraph. Animals which had a baseline flow of more than 20 mL min^{-1} were used. An 8F Sones catheter (USCI, Billerica, MA, USA) was inserted into the left carotid artery and advanced to the left femoral artery under fluoroscopy. A Teflon-coated guide wire (Terumo Co., Tokyo, Japan) was then passed through the hollow catheter so that its tip extended several centimetres beyond the end of the hollow catheter. A copper coil (8 mm long, 2·5 mm in diameter) was placed over the guide wire, followed by the hollow catheter. The hollow catheter was used to push the copper coil to the femoral artery where it was secured 2 to 3 cm distal to the bifurcation of the deep femoral artery. To prevent the coil from moving with formation of the occlusive thrombus, the coil was secured with a silk string. The guide wire was quickly removed. Before withdrawing the hollow catheter, the femoral segment containing the copper coil was flushed with 2 to 3 mL of saline. The electromagnetic flow probe was then repositioned approximately 3 cm distal to the copper coil.

Experimental protocols

The experimental protocol is shown in Fig. 1. Femoral arterial blood flow began to fall from several minutes after the insertion of the copper coil, and stabilized at the zero level. The time from insertion of the coil to a 50% decrease in blood flow level was designated the thrombosis time. One hour after the induction of thrombus, YM866 was administered by intravenous bolus injection, and tPA was given by the same method or by 60-min infusion using a constant-rate infusion pump (STC-523; Terumo, Tokyo, Japan). Heparin (Novo Heparin; Kodama, Tokyo, Japan) at 200 IU kg^{-1} (1 mL kg^{-1}) was given to all animals by intravenous bolus injection 50 min after thrombus formation and at hourly intervals (50 IU kg^{-1}) thereafter. Recanalization as assessed by blood flow was observed for 2 h after intravenous bolus injection or for 3 h after the start of 60-min infusion. Animals showing no evidence of successful thrombolysis at the end of observation were considered to have failed to attain thrombolysis. Even when recanalization occurred, blood flow was continuously monitored for the same observation period to assess patency status and reocclusion after successful thrombolysis. The patency status from intravenous bolus injection or the start of infusion of test drug to the end of the observation period was schematically represented in each animal. To obtain a parametric score, femoral arterial patency status was expressed according to the classification: 1, persistent occlusion (PO)-no reflow; 2, cyclic reflow (CR)-cyclic reflow and reocclusion after initial reflow; 3, persistent patency (PP)-persistent flow without reocclusion after initial reflow.

Measurement of fibrinolytic system parameters

Citrated blood samples (5 mL) were collected periodically in 1 mM PPACK (D-Phe-Pro-Arg-chloromethylketone; Calbiochem, San Diego, CA, USA; Tiefenbrunn et al 1985) for measurement of fibrinogen, plasminogen, and α_2-plasmin inhibitor. Plasma samples were stored frozen at -70°C until assay. Fibrinogen was determined by a thrombin time method (Fibrinogen B-Test; Wako Pure Chemicals, Osaka, Japan; Ratnoff & Menzie 1951) and plasminogen and α_2-plasmin inhibitor were measured by the use of synthetic substrates (Testzym PLG-2 kit and Testzym APL-2 kit; Daiichi Pure Chemicals, Tokyo, Japan; Wohl et al 1983).

Determination of YM866 and tPA concentration in plasma

Plasma antigen concentrations of YM866 and tPA were determined in PPACK-added plasma samples by enzyme-linked immunosorbent assay (Guesdon et al 1979) standardized against YM866 and tPA, respectively. These assays have been proved to have no significant cross-reactivity with urokinase.

Measurement of coagulation parameters

Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in citrated plasma samples collected without PPACK at 37°C using a coagulometer (KC4A; Amelung, Lehbringweg, Germany) and appropriate reagents (Ortho Diagnostic Systems, Tokyo, Japan).

Statistical analysis

The experiments were performed on groups of 5 animals. Data are expressed as mean ± s.e.m. Comparison among groups was performed using the Fisher's exact test for the recanalization rate and reocclusion rate, and Tukey's multiple comparison test for recanalization time and reocclusion time. Changes in plasma fibrinogen, plasminogen, α_2-plasmin inhibitor, and coagulation times (PT and APTT) were analyzed by the Dunnett multiple range test. A P value of less than 0·05 was considered significant.

Results

Thrombolytic activity of YM866 and tPA

In all animals, femoral arterial blood flow decreased gradually after insertion of the copper coil and reached a stable zero level. Group mean values were 5 min 35 s to 11 min 30 s, showing no significant intergroup difference in thrombosis time (one-way analysis of variance, data not shown). Occlusive

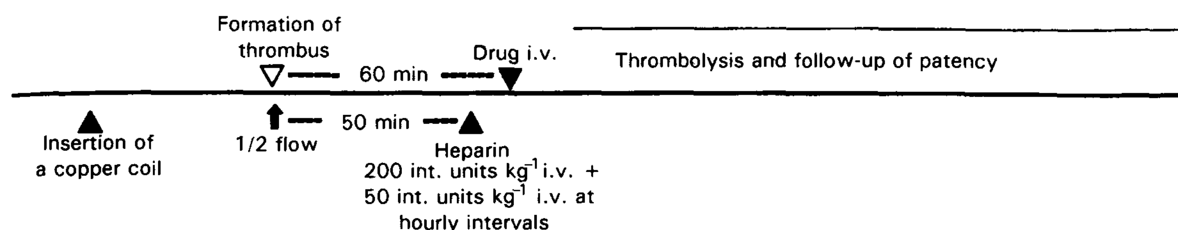

FIG. 1. Experimental protocol. Sixty minutes after the induction of thrombus, test drug was administered intravenously by bolus or continuous infusion over 60 min. Fifty minutes after the formation of thrombus, heparin was administered intravenously by a single bolus injection at 200 IU kg^{-1} and followed by 50 IU kg^{-1} at hourly intervals. Femoral arterial patency status was monitored with an electromagnetic blood flowmeter continuously for up to 2 h after injection in the bolus group or 3 h after the start of administration in the infusion groups.

thrombus formation and subsequent thrombolysis were clearly seen. Thrombolytic activities of YM866 and tPA are summarized in Table 1. No recanalization occurred in the placebo group. In contrast, administration of both YM866 and tPA resulted in dose-dependent thrombolysis. Recanalization rate and recanalization time in the YM866 bolus groups at 0·1 mg kg^{-1} and at 0·2 mg kg^{-1} were comparable with those in the tPA infusion groups at 0·2 mg kg^{-1} and at 0·4 mg kg^{-1}, respectively. Recanalization occurred earlier in the tPA bolus groups than in the YM866 bolus or tPA infusion groups, but the recanalization rate with tPA bolus was low. Recanalization time was shortened in a dose-dependent manner in animals given YM866 by bolus and tPA by infusion, while no distinct dose-dependency was observed with tPA by bolus.

Effect of YM866 and tPA on reocclusion

Reocclusion rate and reocclusion time determined by blood flow in each animal are summarized in Table 1. Total reocclusion rates after successful thrombolysis with YM866 and tPA by bolus and tPA by infusion were 70% (7/10), 100% (3/3) and 88% (7/8), respectively, showing no significant difference among these groups (Fisher's exact test). When compared at doses producing an equal recanalization rate, reocclusion rate and reocclusion time were also comparable; YM866 by bolus at 0·2 mg kg^{-1}, 60% (3/5) and 64 ± 21 min, and tPA by infusion at 0·4 mg kg^{-1}, 80% (4/5) and 60 ± 13 min, respectively.

Femoral arterial patency status

Femoral arterial patency status, categorized as persistent occlusion, cyclic reflow and reocclusion, and persistent patency, are summarized in Table 1. The patency status in individual animals is also represented schematically in Fig. 2. One of 5 animals given tPA by bolus at 0·2 mg kg^{-1} showed rapid restoration of blood flow, but soon experienced cyclical flow reductions followed by reocclusion. Two animals given tPA by bolus at 0·4 mg kg^{-1} showed rapid restoration of blood flow, but reocclusion after the initial reflow at 38 and 88 min, respectively. In the YM866-treated groups, 2 animals at 0·05 mg kg^{-1} and 2 of 3 animals attained recanalization at 0·1 mg kg^{-1}, and 1 of 5 animals at 0·2 mg kg^{-1} showed cyclical flow reductions. The incidence of cyclic reflow was dose-dependently reduced, whereas that of persistent patency was increased. Two of 5 animals given tPA by infusion at 0·2 mg kg^{-1} showed recanalization during the 60-min infusion period, whereas 1 of 5 animals attained recanalization 63 min after the cessation of infusion. All 5 animals given tPA by infusion at 0·4 mg kg^{-1} attained recanalization 7·2 to 18 min after the start of infusion, but 4 of 5 animals showed reocclusion 38 min to 96 min after the cessation of infusion.

Changes in femoral arterial blood flow after drug injection of YM866 and tPA

Changes in femoral arterial blood flow 2 h after the injection of YM866 by bolus are shown in Fig. 3. Blood flow in the YM866 groups was restored in a dose-dependent manner. There was, however, no significant difference in restoration of

Table 1. Effects of YM866 and tPA on femoral artery recanalization, reocclusion and patency status after thrombolysis.

Agent	Dose (mg kg^{-1})	Protocol	n	Recanalization		Reocclusion		Patency status		
				Total	Time (min)	Total	Time (min)	PO	CR	PP
Placebo	–	i.v. bolus	5	0/5	–	–	–	5	0	0
YM866	0·05	i.v. bolus	5	2/5	78 (mean)	2/2	11 (mean)	3	2	0
YM866	0·1	i.v. bolus	5	3/5	26 ± 16	2/3	33 (mean)	2	2	1
YM866	0·2	i.v. bolus	5	5/5	13 ± 3·3	3/5	64 ± 21	0	3	2
tPA	0·2	i.v. bolus	5	1/5	3·0	1/1	9·5	4	1	0
tPA	0·4	i.v. bolus	5	2/5	3.3 (mean)	2/2	63 (mean)	3	2	0
tPA	0·2	i.v. infusion	5	3/5	63 ± 31	2/3	74 (mean)	2	2	1
tPA	0·4	i.v. infusion	5	5/5	13 ± 2·1	4/5	60 ± 13	0	4	1

The data represent mean ± s.e.m. Recanalization time indicates the time to reflow in animals that achieved successful thrombolysis. Reocclusion time indicates the time to reocclusion in animals that attained thrombolysis. PO, persistent occlusion; CR, cyclic reflow and reocclusion after initial reflow; PP, persistent patency.

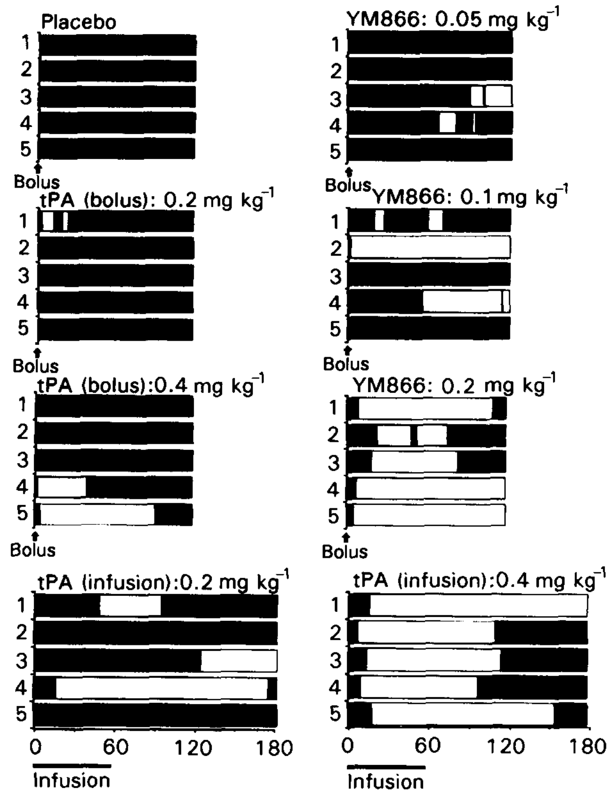

FIG. 2. Schematic representation of the femoral arterial patency status in each animal: bolus groups, for 2 h after intravenous bolus injection; infusion groups, for 3 h after the start of infusion. Open bars, patency; solid bars, occlusion of the femoral artery. Each horizontal bar represents one animal.

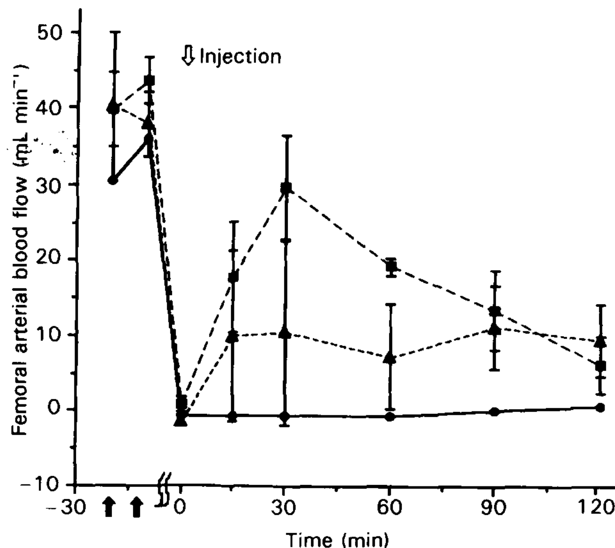

FIG. 3. Changes in femoral arterial blood flow 2 h after intravenous bolus injections of YM866 at doses of 0·05, 0·1 and 0·2 mg kg^{-1}. Values are means from 2 animals at 0·05 mg kg^{-1} or mean ± s.e.m. from 3 and 5 animals at 0·1 and 0·2 mg kg^{-1}, respectively. ● 0·05 mg kg^{-1}, ▲ 0·1 mg kg^{-1}, ■ 0·2 mg kg^{-1}. The dark arrows indicate baseline value and values after the insertion of the coil.

blood flow between YM866 by bolus at 0·2 mg kg^{-1} and tPA by infusion at 0·4 mg kg^{-1} (Student's *t*-test, data not shown).

Changes in fibrinolytic system parameters

Changes in plasma levels of fibrinogen, plasminogen, and α_2-plasmin inhibitor before and after drug injection are shown in Fig. 4. No significant change in fibrinogen and α_2-plasmin inhibitor was observed in the placebo group, whereas plasminogen levels showed a slight but significant decrease (Dunnett multiple comparison test). No significant change in fibrinogen level was observed in any group (compared with baseline value, Dunnett multiple comparison test), whereas plasminogen and α_2-plasmin inhibitor levels showed a significant decrease in all groups. In particular, α_2-plasmin inhibitor level with YM866 by bolus at 0·2 mg kg^{-1} showed a significant decrease to 35% of baseline values 60 min after intravenous injection (compared with placebo group, Dunnett multiple comparison test).

Plasma antigen levels of YM866 and tPA

Fig. 5 shows the time-course of plasma YM866 and tPA antigen levels for 3 h after bolus injection of YM866 and tPA, and from the start of the 60-min infusion of tPA. Plasma YM866 antigen levels after a bolus dose were markedly sustained compared with those of plasma tPA. At the same dose of 0·2 mg kg^{-1}, the mean antigen levels of YM866 and tPA 10, 30 and 60 min after bolus injection were, respectively, 67%, 42% and 19% (YM866) and 15%, 1·8% and 0·95% (tPA) of the values at 30 s after the bolus injection. With intravenous infusion of tPA, plasma antigen reached a plateau within about 15 min of infusion and fell rapidly after the end of infusion. The plasma antigen levels 60 min after the intravenous bolus injection of YM866 at 0·2 mg kg^{-1} and the start of infusion of tPA at 0·4 mg kg^{-1} were closely similar (197 ± 35 ng mL^{-1} and 228 ± 26 ng mL^{-1}, respectively), but 120 min after the intravenous bolus injection of YM866 at 0·2 mg kg^{-1} and the start of infusion of tPA at 0·4 mg kg^{-1} the levels were markedly different (51 ± 94 ng mL^{-1} and 6·2 ± 0·7 ng mL^{-1}, respectively).

Changes in coagulation parameters

Table 2 shows the changes of APTT. In all groups, APTT was markedly and significantly prolonged to 7·2 to 9·8 times the baseline value immediately after the initial bolus injection of heparin at 200 IU kg^{-1}, and was maintained at 3·5 to 5·2 times baseline 30–150 min after initial injection, showing no significant inter-group difference (Dunnett multiple comparison test). The extent of change in PT values was smaller than that in APTT values (data not shown).

Discussion

We previously evaluated the thrombolytic activity of YM866 in a canine model of copper-coil-induced coronary artery thrombosis (Kawasaki et al 1993). In the model used, however, determination of the exact timing of complete occlusion and thrombolysis, and quantification by angiography of blood flow restoration after successful thrombolysis was limited. In this study, therefore, we quantitatively evaluated the thrombolytic effect of YM866 using a thrombogenic copper-coil-induced femoral arterial thrombosis model.

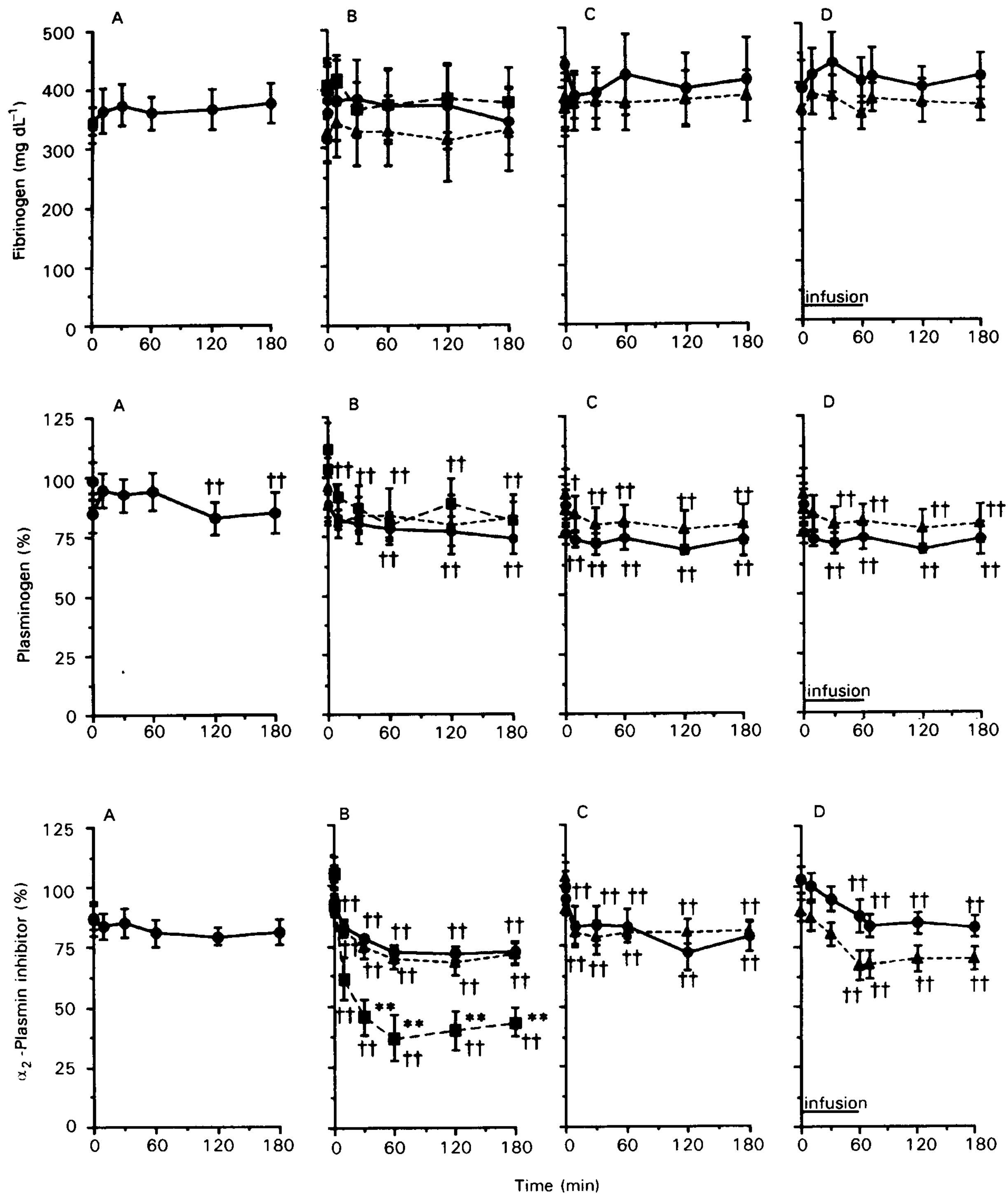

FIG. 4. Changes in plasma fibrinogen, plasminogen, and α_2-plasmin inhibitor levels after intravenous bolus injection of placebo (A), YM866 (B), tPA (bolus) (C) and tPA (infusion) (D). Values are means ± s.e.m. from 5 animals. $*P < 0.05$, $**P < 0.01$ compared with the baseline value; $††P < 0.01$ compared with the placebo group by Dunnett multiple comparison test. YM866: ● 0·05; ▲ 0·1; ■ 0·2 mg kg^{-1}; tPA (bolus): ● 0·1; ▲ 0·2 mg kg^{-1}; tPA (infusion): ● 0·2; ▲ 0·4 mg kg^{-1}.

Recanalization rate and recanalization time of YM866 by bolus at 0·2 mg kg^{-1} were equivalent to those of tPA by infusion at 0·4 mg kg^{-1}. The recanalization rate of tPA by bolus injection was, however, low. This suggests that YM866 by intravenous bolus injection has equivalent thrombolytic potency to tPA by intravenous infusion. Several clinical trials of intravenous bolus injection of tPA aimed at early recanalization of the occluded coronary vessel have been reported (Khan et al 1990). These studies obtained recanalization rates comparable with those obtained by intravenous infusion with

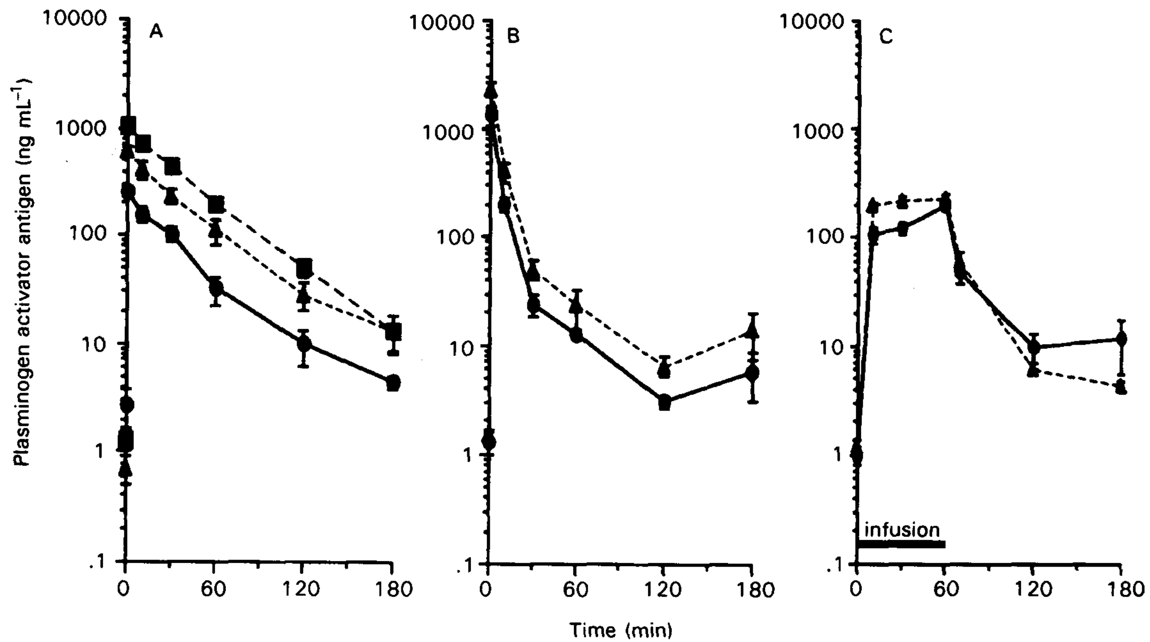

FIG. 5. Plasma antigen concentrations of YM866 (A), tPA (bolus) (B), and tPA (infusion) (C): bolus groups (A, B), during the 3-h period after bolus injection; infusion group (C), during the 1 h infusion period and 2 h after the end of infusion. Values are means ± s.e.m. from 5 animals.

bolus doses of tPA (Tebbe et al 1989). In this study, however, bolus injections of tPA produced recanalization rates obviously lower than those produced by infusion but dosages producing sufficient recanalization (100%) in this study were higher than those in a copper-coil-induced coronary artery thrombosis study (Kawasaki et al 1993). There are two possible reasons for this. Firstly, the size of the copper coil used in this study was bigger (1·6-fold greater volume) than that used in coronary thrombosis. Secondly, it is thought that there is a difference in the distribution volume of the drug between the coronary and femoral arteries.

Recanalization time for tPA administered in bolus doses showed no obvious dose-dependence, occurring in all animals within 10 min of intravenous bolus injection. In contrast, the time for YM866 decreased in a dose-dependent manner, and occurred even later than 30 min after the bolus injection. This finding seems to reflect the plasma half-lives of these drugs; the lack of dose-dependency in recanalization time observed with tPA by bolus in this study is consistent with our previous findings of a lack of such dependency in thrombolysis time after intravenous bolus injection in experimental thromboses (Kawasaki et al 1993, 1994b).

Reocclusion rate and patency status of YM866 by bolus at 0·2 mg kg^{-1} were the same as those of tPA by infusion at 0·4 mg kg^{-1}. The reocclusion rate tended to decrease with increasing dose. Improvement in femoral arterial patency status was seen at higher doses. These findings suggest that the occurrence of acute reocclusion and patency status are closely related to the thrombolytic activity of the drug. Clinically, a relationship between coronary reocclusion and residual thrombus after thrombolytic therapy has been described (Badimon et al 1988). Thrombus in the present model was mainly constituted from fibrin, platelets and red blood cells (Bush et al 1989), and it is considered natural that thrombolytic reocclusion as a result of reduced plasma concentration of the drug would occur unless the thrombogenic copper coil was

Table 2. Changes in activated partial thromboplastin time before and after heparinization in each group.

Agent	Dose (mg kg^{-1})	Protocol		Activated partial thromboplastin time (s) after:			
			Baseline	0 min	30 min	90 min	150 min
Placebo	–	i.v. bolus	18 ± 0·7	152 ± 16*	81 ± 11*	72 ± 7·5*	69 ± 9·0*
YM866	0·05	i.v. bolus	18 ± 0·2	176 ± 2·3*	90 ± 11*	77 ± 7·1*	71 ± 7·1*
YM866	0·1	i.v. bolus	19 ± 0·9	186 ± 8·5*	96 ± 8·7*	73 ± 3·6*	86 ± 9·2*
YM866	0·2	i.v. bolus	19 ± 0·7	136 ± 22*	92 ± 15*	72 ± 8·9*	78 ± 9·4*
tPA	0·2	i.v. bolus	18 ± 0·8	170 ± 12*	78 ± 6·9*	76 ± 8·8*	72 ± 9·3*
tPA	0·4	i.v. bolus	19 ± 0·7	159 ± 18*	82 ± 8·0*	71 ± 9·0*	66 ± 9·4*
tPA	0·2	i.v. infusion	20 ± 0·7	195 ± 10*	99 ± 11*	86 ± 6·7*	104 ± 17*
tPA	0·4	i.v. infusion	19 ± 1·1	179 ± 15*	95 ± 14*	83 ± 13*	82 ± 14*

The data represent mean ± s.e.m. from 5 animals. Blood samples were collected before (pre-value), immediately after (0 min), and 30, 60, 90 and 150 min after the initial intravenous bolus injection of heparin (200 IU kg^{-1}). Additional injections of heparin (50 IU kg^{-1}) were given at hourly intervals after the initial injection. Coagulation times greater than 10-fold the baseline value of each animal are given as 10-fold value for calculation of mean ± s.e.m. *$P < 0·01$ (Dunnett multiple comparison test) compared with baseline values.

removed from the femoral artery. In view of this, additive infusion with a low dose of thrombolytic agent (Johns et al 1988) or double bolus injection of mutant tPA at an interval (Grunewald et al 1995) might be effective in preventing reocclusion after successful thrombolysis.

Although the necessity of heparin in thrombolytic therapy has been previously established (Hans et al 1991), the unfractionated heparin used to maintain adequate anticoagulation in the present study did not exert a preventive effect on reocclusion after successful thrombolysis, irrespective of thrombolytic agents used. Explanations for the limited potency of heparin include the insensitivity of clot-bound thrombin to heparin (Weitz et al 1990) and inhibition of heparin by a fibrin monomer (Hogg & Jackson 1989). Adjunctive use of new antiplatelet agents such as anti-GPIIb/IIIa monoclonal antibody (Coller et al 1991; Kaku et al 1995), low molecular GPIIb/IIIa antagonist (Nicholson et al 1995), inhibitors of GPIb-vWF interaction (Zahger et al 1995), and platelet thrombin receptor antagonist (Cook et al 1995), or anticoagulants such as thrombin inhibitors (Mellot et al 1990) and Xa inhibitors (Hara et al 1995) can be expected to maintain vascular patency and prevent reocclusion.

Blood flow in the YM866 group was improved dose-dependently, but no significant difference in blood flow between YM866 by bolus at 0·2 mg kg^{-1} and tPA by infusion at 0·4 mg kg^{-1} was seen. Saito et al (1994) reported that a mutant tPA (E6010) with improved plasma half-life produced a more gradual increase in coronary blood flow than tPA and urokinase, and that the frequency of reperfusion arrhythmia and the mortality rate of E6010 were significantly lower than those of tPA and urokinase, irrespective of whether the recanalization time was the same. Although no reasonable explanation of this was given in their paper, the degree and speed of restoration of blood flow after administration of YM866 were the same as those of tPA by infusion in the present study.

Levels of α_2-plasmin inhibitor in plasma after injection of YM866 at 0·2 mg kg^{-1} showed a significant decrease compared with those in the placebo group, but no significant change in plasma fibrinogen was detected with either agent, suggesting the higher affinity of YM866 and tPA for fibrin.

In conclusion, YM866 administered by intravenous bolus injection showed equivalent thrombolytic activity at a lower dose than by infusion. Heparin could not prevent reocclusion after successful thrombolysis even under adequate anticoagulation, suggesting the necessity of more effective adjunctive agents.

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