Thrombolysis with Intracoronary Administration of YM866, a Novel Modified Tissue-Type Plasminogen Activator, in a Canine Model of Coronary Artery Thrombosis

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ABSTRACT—We compared the thrombolytic activity of YM866, a novel modified tissue-type plasminogen activator, with that of t-PA by intracoronary administration in a canine thrombosis model of copper coil-induced 6-hr-old thrombi. Either drug was administered by a single injection (10 min) or multiple injection (4 x 10 min) under heparinization (300 IU/kg, i.v.). The reperfusion rate of YM866 was 4 times higher than that of t-PA when administered by single injection. Time to reperfusion of YM866 by single injection was shorter than that of either agent by multiple injection. No group showed any decrease in plasma fibrinogen levels. No acute reocclusion was seen in animals with YM866 by single injection. The improved thrombolytic activity of YM866 by single injection correlated with the relatively higher antigen levels of this agent due to its prolonged biological half-life. These results suggest that single intracoronary administration of YM866 is a safe and effective thrombolytic method for rapid recanalization and lowered acute reocclusion without activation of systemic fibrinolysis.

Keywords: Tissue-type plasminogen activator (t-PA) (modified), Thrombolysis (intracoronary), Thrombi (aged)

Owing to its strict fibrin specificity, tissue-type plasminogen activator (t-PA) is a potent thrombolytic agent. However, due to the extremely short half-life of this agent (T½ < 5 min), administration must be by high-dosage infusion. This increases the risk of systemic bleeding, and results in a high incidence of acute reocclusion (1). To eliminate these shortcomings, attempts have been made to modify the t-PA molecule, and several mutants of t-PA that have prolonged plasma half-life and that can be administered by intravenous (i.v.) bolus injection have been reported (2–4).

YM866 is a novel modified t-PA with deletion of the K1 domain of the molecule and with a point mutation at the site of the K2 domain linkage to the L-chain (del 92–173, 275Arg → Glu) (5). It has been shown in vitro to possess a pronounced affinity for fibrin, and to retain the same specific activity as t-PA (6). The plasma clearance of YM866 calculated from pharmacokinetics in rats has been 7-fold slower than that of t-PA (6). We previously showed that YM866 administered by i.v. bolus injection produced a superior thrombolytic effect to t-PA by i.v. bolus injection or infusion in a canine model of copper coil-induced coronary artery thrombosis (7).

The benefit of thrombolysis is enhanced by early treatment and the re-establishment of coronary patency (8). This requirement makes widespread intracoronary (i.c.) thrombolysis impractical, and most clinical trials of t-PA have examined thrombolysis by the i.v. route. Against this trend, Gemmil et al. reported a regime in which additional i.c. t-PA was given to patients after i.v. bolus injection of the agent failed to clear the occluded coronary artery (9), while Pitney et al. used an i.c. infusion of t-PA to allow angioplasty in patients who had previously been unsuitable for the procedure (10).

In the present study, we compared the thrombolytic activity of YM866 with that of t-PA by intracoronary administration in a canine model of coronary artery thrombosis. As i.c. thrombolysis is generally undertaken later in the course of thrombus formation than thrombolysis by i.v. injection, we first prepared a thrombosis model of 6-
hr-old thrombi, and used this model to evaluate the thrombolytic activity of YM866 by i.c. administration in comparison with that of t-PA.

MATERIALS AND METHODS

Thrombolytic agents
YM866 is a recombinant tissue-type plasminogen activator analogue which contains a finger domain, growth factor domain, kringle-2 domain, and a serine protease domain, as well as a point mutation at the kringle-2-serine protease linkage site (del 92–173, 275Arg → Glu) (5). Preparations of YM866 used in this study contained more than 98% of the single-chain form. Lyophilized preparations of YM866 and t-PA (ACTIVASE®, 50 mg/vial; Genentech, Inc., South San Francisco, CA, USA) were dissolved in distilled water for injection and diluted with saline. The specific activities of YM866 and t-PA as determined by fibrin clot lysis assay calibrated with the international standard (83/157) were 570,000 and 600,000 IU/mg, respectively (6).

Preparation of aged thrombi and determination of thrombolytic activity
Sixty adult mongrel dogs of both sexes weighing approximately 15 kg were used. Animals were anesthetized with 20 mg/kg sodium pentobarbital and then intubated and artificially ventilated with room air. Catheters were placed in the cephalic vein, femoral vein, and femoral artery for maintenance of anesthesia, collection of blood samples, and monitoring of blood pressure/heart rate, respectively. Continuous monitoring of precordial leads was performed for the detection of arrhythmias. Coronary thrombosis was induced by placing a copper coil (length, 8 mm; diameter, 2 mm) over an intracoronary wire into the left anterior descending coronary artery (LAD) distal to the first diagonal branch under fluoroscopy, as described previously (11). The presence of an occlusive thrombus was confirmed angiographically. The thrombus was allowed to age for 6 hr before the administration of test drug. Heparin (Novo Heparin®; Novo BioLabs, Copenhagen, Denmark) was given to all animals by i.v. bolus at 300 IU/kg before administration of a thrombolytic agent. The experimental protocol is shown in Fig. 1. Test drug (YM866 or t-PA) was administered by a single injection (10 min) or multiple injections (4 × 10 min) into the LAD ostium through an angiography catheter using a constant-rate infusion pump (STC-523; Terumo Co., Ltd., Tokyo). Coronary angiograms were obtained to check the patency of the LAD. Animals showing no evidence of coronary reperfusion at 60 min were considered to have failed to attain reperfusion. Three further angiograms were obtained at 20-min intervals after the confirmation of reperfusion in the single injection group or after the 4th injection (at 60 min) in the multiple injection group. Reperfusion was defined as TIMI grade 2 or 3 and reocclusion as 0 or 1 (12). The reperfusion rate, time to reperfusion, and reocclusion rate calculated from coronary angiography served as parameters for the thrombolytic activity of the test drug. Lidocaine (Xylocaine®; Fujisawa-Astra Co., Ltd., Osaka) was used to prevent the development of arrhythmias.
mias during the occlusion and reperfusion processes.

**Measurement of fibrinolytic parameters**

Citrated blood samples were collected periodically in 1 μM PPACK (D-Phe-Pro-Arg-chloromethylketone; Calbiochem Co., Ltd., San Diego, CA, USA) (13) for measurement of fibrinogen, plasminogen, and α2-plasmin inhibitor. Plasma samples were preserved frozen at −70°C until assay. Fibrinogen was measured by the thrombin time method (14) (Fibrinogen B Test®; Wako Pure Chemical Co., Ltd., Osaka), and plasminogen and α2-plasmin inhibitor were determined by the use of synthetic substrates (15) (Testzym PLG Kit® and Testzym APL Kit®; Daiichi Pure Chemicals Co., Ltd., Tokyo).

**Measurement of YM866 and t-PA concentrations in plasma**

Plasma antigen levels of YM866 and t-PA were determined in PPACK-added plasma samples by enzyme-linked immunosorbent assay (16) standardized against YM866 and t-PA, respectively.

**RESULTS**

**Thrombolytic activity of YM866 and t-PA**

An occlusive thrombus usually developed within 10 to 20 min after the insertion of the copper coil; occlusion of the artery was signaled by an elevation of the ST segment in the left precordial ECG and confirmed as complete occlusion by coronary angiography. Figures 2 and 3 illustrate the reperfusion rate, time to reperfusion, and reocclusion rate following i.c. administration of YM866 and t-PA in the 6-hr-old thrombi.

By single injection (Fig. 2), the reperfusion rate of YM866 was 4 times higher than that of t-PA. Time to reperfusion of t-PA was shorter than that of YM866: all animals of the t-PA group attained reperfusion within 10 min after the cessation of injection, whereas many animals given YM866 did not attain reperfusion until more than 10 min after injection. The incidence of reocclusion was zero with YM866 and low with t-PA.

By multiple injection (Fig. 3), the reperfusion rate and time to reperfusion of two agents were comparable. All animals in both groups attained reperfusion, after the 2nd injection at the earliest. There was no clear difference in reocclusion rate between the agents.

**Changes in fibrinolytic parameters**

Changes in plasma levels of fibrinogen, plasminogen, and α2-plasmin inhibitor following the administration of test drugs are shown in Figs. 4 and 5. Data are plotted as percentages of the baseline. No change in plasma fibrino-
Fig. 4. Changes in plasma fibrinogen, plasminogen, and α2-plasmin inhibitor levels after a single injection of YM866 (A) and t-PA (B). Values are the mean ± S.E.M. of 5 animals. Data are plotted as percentages of the baseline. (A), ○, 0.025; △, 0.05; ■, 0.1 mg/kg; (B), ○, 0.1; △, 0.2; ■ 0.4 mg/kg.

Fig. 5. Changes in plasma fibrinogen, plasminogen, and α2-plasmin inhibitor levels after multiple injection of YM866 (A) and t-PA (B). Values are the mean ± S.E.M. of 5 animals. Data are plotted as percentages of the baseline. ○, 0.05; △, 0.1; ■, 0.2 mg/kg.
Fig. 6. Plasma antigen concentrations after a single injection (A) at a dose of 0.1 mg/kg or multiple injection (B) at a dose of 0.2 mg/kg of YM866 and t-PA. Values are the mean±S.E.M. of 5 animals. ●, YM866; ○, t-PA.

Changes in plasma antigen levels of YM866 and t-PA

Figure 6 illustrates the time-course of plasma YM866 and t-PA antigen levels over the 60 (single injection) and 120 (multiple injection)-min periods after the start of injection of both agents. By single injection (Fig. 6A), plasma YM866 antigen levels were markedly sustained compared with that of t-PA. By multiple injection with t-PA at 0.2 mg/kg or t-PA at 0.4 mg/kg or multiple injections of YM866 at 0.2 mg/kg decreased to 67%, 69% and 60% of the baseline, respectively. With the single injection of t-PA (Fig. 4), plasma α2-plasmin inhibitor dropped rapidly and tended to reach an inverse plateau, whereas with both regimens of YM866, it declined progressively over time.

DISCUSSION

The benefit of thrombolysis is enhanced by early treatment and the reestablishment of coronary patency. The ideal thrombolytic agent should therefore allow simple and rapid administration by the intravenous route, and provide effective and rapid restoration of coronary patency without adverse side effects (17). Theoretically, t-PA is only activated at the site of a fibrin-bound thrombus, and should thus be as effective when given intravenously as when given intraarterially. In practice, however, a number of clinical trials (9, 10, 18) have shown intraarterial use to be superior, in that lysis could be attained substantially faster and with smaller doses of thrombolytic agent. The aim of the present study was to explore the potential of i.c. thrombolysis with YM866 as a supplement to conventional thrombolytic methods. In the previous study, the residual plasma YM866 and t-PA antigen at 10 min after i.v. bolus injection was 61% and 20% of the peak levels, respectively (7). Therefore in this study, we administered both agents by a single injection (10 min) or multiple injection (4 × 10 min).

By single i.c. injection, the reperfusion rate with YM866 was 4 times higher than that with t-PA. By multiple injection, reperfusion with YM866 was comparable to that with t-PA. We previously reported that the intravenous doses of the agents producing a 100% reperfusion rate in the same thrombus model were 0.2 mg/kg of YM866, 0.8 mg/kg of t-PA by bolus injection, and 0.3 mg/kg of t-PA by infusion (7). Both agents were twice as potent when given by single i.c. injection than by i.v. bolus injection. By multiple i.c. injection, however, t-PA was two to three times as potent as by i.v. infusion, whereas YM866 showed only comparable potency to administration by i.v. bolus injection.

For both agents, time to reperfusion was faster when administered by single injection than when given by multiple injection. By single injection, reperfusion with t-PA was seen in all animals within 10 min after the end of injection, whereas reperfusion with YM866 was as late as 20 min or more after injection. This finding seems to reflect the respective plasma half-lives of the agents, since both agents had the same affinity for fibrin (6) and the same resistance to plasminogen activator inhibitor-1 (PAI-1) (M. Katoh et al., unpublished data). By multiple injection, successful reperfusion with both agents occurred later than at the end of the 2nd injection. This indicates that the thrombolytic effect of both agents was exerted only after its plasma concentration had reached a certain level.

With both drugs, the rate of reocclusion was lower after a single injection than after multiple injections. The occurrence of acute reocclusion in this thrombosis model is probably closely related to the extent of residual thrombi at the site of thrombus; the higher concentrations of both agents by single injection may be credited with decreasing residual thrombi. A relationship between coronary reocclusion and residual thrombus after thrombolytic therapy has been described clinically (19). Additional doses of a thrombolytic agent (1), or adjunctive therapy with an antiplatelet agent such as anti-GPIIb/IIIa antibody (20) or
von Willebrand Factor inhibitor (21), or with an anticoagulant such as synthetic thrombin inhibitor (22) help to minimize residual thrombus, as well as to prevent the growth of the thrombus.

\[ \alpha_2 \text{-Plasmin inhibitor levels in the plasma decreased to within 60% to 70% of the baseline after the single injection of YM866 at 0.1 mg/kg or t-PA at 0.4 mg/kg or multiple injection of YM866 at 0.2 mg/kg. However, no change in plasma fibrinogen or plasminogen was detected in any group. At doses that produced a 100% reperfusion rate when YM866 was given intravenously, plasma fibrinogen and } \alpha_2 \text{-plasmin inhibitor levels were decreased to approximately 30% and 60%, respectively (7). The administration of smaller amounts of thrombolytic agents offers higher reperfusion rates and the likelihood of a reduced incidence of systemic side effects.} 

Concentrations of plasma antigen were sustained markedly longer with YM866 than with t-PA. These findings are essentially consistent with the mean residence time of YM866 as calculated from its pharmacokinetics in rats (6) and dogs (I. Miyamoto et al., unpublished data), which can probably be extrapolated to humans. By multiple injection, antigen levels of YM866 were maintained at 2 to 3 times that of t-PA, but the reperfusion rate with YM866 was comparable to that of t-PA. This may be a reflection of the finding that the clot lysis activity of YM866 in the plasma of dogs was about one-third that of t-PA (6). In the assay of fibrin clot lysis using purified human fibrinogen, in contrast, the specific activities of the agents were shown to be comparable. This discrepancy may be explained by a difference between the agents in their resistance to putative inhibitors other than PAI-1 of the thrombolytic system in plasma.

The results of this study suggest that YM866 by single intracoronary injection produces excellent thrombolysis and prevents acute reocclusion without systemic fibrinolytic activation. Furthermore, this regime may provide safe and effective thrombolysis in patients in whom early i.v. thrombolysis is unsuccessful and may allow angioplasty in patients who were not previously suitable for this procedure.

REFERENCES


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