Thrombolytic Activity of a Novel Modified Tissue-Type Plasminogen Activator, YM866, in a Canine Model of Coronary Artery Thrombosis

Tomihisa Kawasaki¹, Masao Katoh¹, Seiji Kaku¹, Hiroshi Gushima¹, Toichi Takenaka¹, Yoshiki Yui² and Chuichi Kawai²

¹Cardiovascular and Atherosclerosis Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan
²Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto 606–01, Japan

Received January 27, 1993 Accepted May 27, 1993

ABSTRACT—The thrombolytic activity of a novel modified t-PA, YM866, was compared with that of a recombinant t-PA in a canine model of copper coil-induced coronary thrombosis. The coronary thrombus was allowed to age for 1, 3 or 6 hr before either drug was administered. YM866 was administered by i.v. bolus injection, while t-PA was given by the same method, as well as by 60-min i.v. infusion. YM866 showed thrombolytic activity 2 to 4 times as potent as that of t-PA when administered by bolus injection, the difference in thrombolytic effect being obvious in the 3- and 6-hr-old thrombi. Coronary reperfusion was achieved more rapidly with YM866 than with i.v. infusion of t-PA. In animals injected with doses of more than 0.1 mg/kg of YM866, no acute reocclusion occurred. Depletion of plasma fibrinogen to 70% of baseline levels was observed in animals given 0.2 mg/kg YM866, 0.4 mg/kg t-PA by bolus, and 0.6 mg/kg t-PA via infusion. The residual plasma YM866 and t-PA antigen 30 min after bolus injection was 25% and 3% of the peak levels, respectively. YM866, administered by i.v. bolus injection, was thus confirmed to exert a thrombolytic effect superior to that of bolus injection and infusion of t-PA, without systemic fibrinolytic activation. These results suggest the potential clinical applicability of YM866 as a thrombolytic agent that can be administered by i.v. bolus injection for acute myocardial infarction.

Keywords: Tissue-type plasminogen activator (t-PA) (modified), Thrombosis (copper coil-induced), Thrombi (aged), Coronary thrombolysis

Tissue-type plasminogen activator (t-PA) is a potent thrombolytic agent which, owing to its strict fibrin specificity, is superior to urokinase and streptokinase. However, due to its extremely short half-life, administration must be performed by high-dosage infusion; this increases the possibility of systemic bleeding, and the incidence of acute reocclusion is also increased (1, 2). To eliminate these shortcomings, attempts have been made to modify the t-PA molecule by genetic engineering techniques, and several mutants of t-PA that have prolonged plasma half-life and that can be administered by i.v. bolus injection have been reported (3–6).

YM866 is a novel modified t-PA with deletion of the K1 domain of the molecule and with a point mutation (⁴⁷²Arg → Glu) at the site of the K2 domain linkage to the L-chain (7). It has been shown to possess a pronounced affinity for fibrin and retain essentially the same specific activity as t-PA in vitro (8). A remarkably sustained plasma concentration of this novel modified t-PA has been demonstrated in rabbits (3). Antigenicity studies of the preparation in chimpanzees have shown no antigenic potential that is of any toxicological concern (T. Oohata et al., unpublished study). The present study was performed to assess the thrombolytic activity of YM866 and its effect on the parameters of the fibrinolytic system in a canine model of coronary arterial thrombosis.

It is now accepted that the early use of thrombolytic treatment in patients with acute myocardial infarction reduces mortality and salvages the ischemic myocardium. The time after onset until coronary recanalization is thought to constitute a critical point that makes the difference between success and failure of thrombolytic therapy (9). While the usefulness of t-PA in the thrombolysis of aged thrombi, owing to its affinity for fibrin, has been reported (10), little has been done to evaluate the effect of mutant t-PA on aged thrombi (11). In view of this, we
compared the thrombolytic effects of YM866 and t-PA on thrombi of three different ages (1, 3 and 6 hr) after formation.

MATERIALS AND METHODS

Thrombolytic agents

YM866 is a recombinant tissue-plasminogen activator analogue which contains a finger domain, EGF domain, kringle-2 domain, and serine protease domain, and a site mutation at the kringle-2-serine protease linkage site (del 92-173, 275Arg → Glu) (7). The schematic representation of the amino acid sequence of YM866 is shown in Fig. 1. A lyophilized pharmaceutical preparation of YM866 was dissolved in a specified volume of distilled water for injection and was then diluted with physiological saline. For control groups, a lyophilized preparation (placebo) with the same constituents as those above, minus YM866, was dissolved and diluted in the same manner. t-PA (ACTIVASE®, 50 mg/vial; Genentech, Inc., South San Francisco, CA, USA) was dissolved in distilled water for injection and was diluted with physiological saline for use in the experiments. The specific activities of YM866 and t-PA determined by the fibrin clot lysis assay calibrated with the international t-PA standard (83/157) were 570,000 and 600,000 IU/mg, respectively (8).

Preparation of coronary artery thrombi and determination of thrombolytic activity

One hundred and forty adult mongrel dogs of both sexes, weighing approximately 15 kg each, were used. Animals were anesthetized with 20 mg/kg sodium pentobarbital, intubated, and artificially ventilated with room air. Catheters were placed in the cephalic vein, femoral vein, and femoral artery for injection of test drugs, collection of blood samples, and blood pressure/heart rate monitoring, respectively. Continuous monitoring of the ECG was performed in precordial leads to detect arrhythmias. Coronary thrombosis was induced by placing a copper coil (8-mm-long and 2 mm in diameter) over an intracoronary wire into the left anterior descending coronary artery distal to the first diagonal branch, under fluoroscopy, as described previously (12). An occlusive thrombus was confirmed angiographically. The coronary thrombus was allowed to age for 1, 3 or 6 hr before administration of test drugs. Heparin (Novo Heparin®; Novo BioLabs, Soeborg, Denmark) was given to all animals as an intravenous bolus of 300 IU/kg prior to the start of the protocol. YM866 was injected by i.v. bolus injection in 30 sec, and t-PA was administered by the same method, as well as by 60-min i.v. infusion, using a constant-rate infusion pump (STC-523; Terumo Co., Ltd., Tokyo). Reperfusion, assessed by angiography, was performed at 5-min intervals for up to 60 min after bolus injection or after the initiation of the infusion. 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 confirmation of reperfusion in the bolus injection group or after the end of the administration in the infusion group. Reperfusion was defined as TIMI grade 2 or 3 and recoclusion as 0 or 1 (13). The reperfusion rate, time to reperfu-

Fig. 1. Schematic representation of the amino acid sequence of YM866.
sion, and reocclusion rate calculated from coronary angiography served as parameters for the thrombolytic activity of the test drugs. Lidocaine (Xylocaine®; Fujisawa Astra Co., Ltd., Osaka) was used to prevent arrhythmias emerging during the experimental processes of thrombotic occlusion and reperfusion.

Measurement of fibrinolytic system parameters

Citrated blood samples were collected periodically in 1 μM PPACK (d-Phe-Pro-Arg-chloromethylketone; Calbiochem Co., Ltd., San Diego, CA, USA) for the determination of fibrinogen, plasminogen, and α2-plasmin inhibitor (14). Plasma samples were preserved frozen at −70°C until assayed. Fibrinogen was determined by the thrombin time method (15) (Fibrinogen B Test®; Wako Pure Chemical Co., Ltd., Osaka), and plasminogen and α2-plasmin inhibitor were measured by the use of synthetic substrates (16) (Testzym PLG Kit® and Testzym APL Kit®; Daichi Pure Chemicals Co., Ltd., Tokyo).

Determination of YM866 and t-PA concentration in plasma

Plasma antigen levels of YM866 and t-PA were determined in PPACK-added plasma samples by enzyme-linked immunosorbent assay (17), which was 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 a copper coil, and occlusion of the artery was signaled by an elevation of the ST segment in the left precordial ECG. Figures 2, 3 and 4 illustrate the reperfusion rate, time to reperfusion, and reocclusion rate following administration of YM866 and t-PA in the models of 1-, 3- and 6-hr-old thrombi, respectively.

In the 1-hr-old thrombi (Fig. 2), the reperfusion rate with YM866 was twice as high as that with bolus injection of t-PA and was comparable with that following infusion of the latter. When compared at doses producing equal reperfusion rates, the time to reperfusion was 10 ± 4, 9 ± 2, and 36 ± 5 min at 0.05 mg/kg of YM866, 0.1 mg/kg of t-PA bolus, and 0.15 mg/kg of t-PA infusion, respectively.
of t-PA (bolus), and 0.075 mg/kg t-PA (infusion), respectively; and the time was 9 ± 3, 8 ± 1, and 30 ± 4 min at 0.1 mg/kg of YM866, 0.2 mg/kg of t-PA (bolus), and 0.15 mg/kg of t-PA (infusion), respectively. Thus, reperfusion occurred earlier with YM866 or t-PA (bolus) than with t-PA (infusion). Among the animals given YM866, some attained reperfusion even later than 20 min after injection, whereas all animals of the t-PA (bolus) group attained reperfusion within 15 min. The reocclusion rate tended to be higher at low doses for both drugs. This was particularly high with t-PA (bolus); there was a reduction in reocclusion rate with 0.1 mg/kg of YM866 as compared with 0.1

Fig. 5. Changes in plasma fibrinogen, plasminogen, and α2-plasmin inhibitor levels after i.v. bolus injection of YM866 [A] and t-PA [B] and during i.v. infusion of t-PA [C]. Values are the mean ± S.E.M. of 5 to 15 animals, used in Figs. 2, 3 and 4. Data are plotted as percentages of the baseline level.
mg/kg of t-PA (bolus).

In the 3-hr-old thrombi (Fig. 3), YM866 produced reperfusion rates 4 times as high as those produced with t-PA (bolus) and more than 3 times as high as those produced with t-PA (infusion). When compared at doses producing equal reperfusion rates, the time to reperfusion was 13±3, 10±2 and 21±4 min at 0.1 mg/kg of YM866, 0.4 mg/kg of t-PA (bolus), and 0.6 mg/kg t-PA (infusion), respectively; hence, reperfusion was obtained earlier with YM866 and with t-PA (bolus) than with t-PA (infusion). As in the 1-hr-old thrombi, among animals treated with YM866, there were those that achieved reperfusion even later than 20 min after injection, whereas reperfusion occurred within 15 min in all animals given t-PA (bolus). Subsequent reocclusion did not occur at highest doses of either drug.

In the 6-hr-old thrombi (Fig. 4), the bolus injection of YM866 afforded reperfusion with rates 4 times those reached after t-PA (bolus) and higher than those following t-PA (infusion). When compared at doses producing equal reperfusion rates, time to reperfusion was 23±5, 10±2 and 42±6 min at 0.2 mg/kg of YM866, 0.8 mg/kg of t-PA (bolus), and 0.3 mg/kg of t-PA (infusion), respectively. Thus, reperfusion occurred earlier with YM866 and with t-PA (bolus) than with t-PA (infusion). As in the 1- and 3-hr-old thrombi models, some of the animals injected with YM866 attained reperfusion later than 20 min after injection, whereas reperfusion occurred within 15 min in all animals given t-PA (bolus). No animals treated with YM866 developed reocclusion, and the number of reocclusion with t-PA was few.

Changes in fibrinolytic system parameters

Changes in plasma levels of fibrinogen, plasminogen, and α2-plasmin inhibitor observed following the administration of test drugs are shown in Fig. 5. Data are plotted as percentages of the baseline level. These factors showed little or no change following injection of placebo (data not shown). The plasma fibrinogen level decreased to 69±11, 72±5 and 64±13% of the baseline values in 60 min after i.v. bolus injection of YM866 at 0.2 mg/kg and t-PA at 0.4 mg/kg, and after the beginning of i.v. infusion of t-PA at 0.6 mg/kg, respectively. Plasma plasminogen levels exhibited essentially the same patterns of changes as those of fibrinogen. The α2-plasmin inhibitor concentration in plasma fell below 50% of the initial level after the same doses as those above. With the single bolus doses of YM866 and t-PA, plasma α2-plasmin inhibitor dropped rapidly and tended to reach an inverse plateau at about 15 min after i.v. bolus injection, whereas with i.v. infusion of t-PA, it tended to decline progressively with time.

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 a 60-min period after the bolus injection or until 60 min after the end of the infusion. The plasma YM866 antigen level compared with that of plasma t-PA was markedly sustained following a bolus dose. With i.v. infusion of t-PA, plasma t-PA antigen reached a plateau within about 15 min of infusion and fell rapidly after the end of the infusion. To delineate the difference in sustained plasma levels of YM866 and t-PA, residual plasma antigen levels following a 0.2 mg/kg, i.v. bolus dose of YM866 and t-PA were plotted as percent-

![Fig. 6. Plasma antigen concentrations of YM866 [A], t-PA (bolus) [B], and t-PA (infusion) [C]. Bolus group [A, B]: during the 60-min period after the bolus injection. Infusion group [C]: during the 60-min infusion period and after the end of the infusion. Values are the mean±S.E.M. of 5 to 15 animals, used in Figs. 2, 3 and 4.](image-url)
Fig. 7. Residual plasma antigen levels after i.v. bolus injection of YM866 and t-PA at a dose of 0.2 mg/kg. Values are the mean ± S.E.M. of 5 to 15 animals. Data are plotted as percentages of the value at 30 sec after i.v. bolus injection, used in Figs. 2, 3 and 4.

ages of the value at 30 sec after the injection (Fig. 7). At 30 min after the injection, the plasma antigen level of YM866 was maintained at about 25%, while that of t-PA was as low as about 3%.

DISCUSSION

The thrombus induced by the insertion of a copper coil in this study was fibrin-rich; such thrombi have generally been recognized as a useful model for the evaluation of the efficacy of thrombolytic agents (18). In acute myocardial infarction, the success rate of thrombolytic therapy diminishes with time after onset (19), probably due to progressive cross-linking of the fibrin network (20). Therefore, coronary artery recanalization should be accomplished as rapidly as possible, thus leading to improvement of left ventricular function and reduction of mortality (21). t-PA is more effective than urokinase and streptokinase for the lysis of aged thrombi because of its greater affinity for fibrin, the difference in efficacy increasing with the increasing age of the thrombus (10). YM866 is a mutant t-PA that essentially retains the specific activity and fibrin affinity of t-PA, but has a prolonged plasma biological half-life. Therefore, i.v. bolus injections of the drug would be expected to have greater efficacy than t-PA against aged thrombi. In view of this, we compared the thrombolytic activity of YM866 and t-PA against thrombi of various ages (1-, 3- and 6-hr-old).

The reperfusion rate with YM866 was 2 to 4 times as high as that with t-PA in these models in lysing thrombi following a bolus injection. The dose of YM866 that produced a reperfusion rate of 100% in the 1-, 3- and 6-hr thrombus models were 0.1, 0.1 and 0.2 mg/kg, respectively, compared to 0.2, 0.4 and 0.8 mg/kg of t-PA. The thrombolytic activity of t-PA diminished markedly with increasing thrombus age, while the activity of YM866 was less liable to be affected by thrombus age. These results agree with the report by Suzuki et al. that a bolus injection of a modified tissue-type plasminogen activator (E6010) had equivalent potency in lysing fresh and aged thrombi (11).

The time to reperfusion for both YM866 and t-PA, when administered in bolus doses, showed no obvious dose-dependence, occurring in all animals within 15 min following bolus doses of t-PA but occurring even later than 20 min after the bolus injection of YM866. This finding seems to reflect the plasma half-lives of these drugs. It has been reported that, despite the rapid disappearance of t-PA from the circulation, its thrombolytic effect was sustained due to its adhesion to the thrombus (22). In the present study, however, the thrombolytic effect of t-PA was evident for only 15 min after bolus injection. On the other hand, reperfusion occurred later than 15 min after the beginning of t-PA i.v. infusion in all animals. This indicates that the thrombolytic effect of t-PA was exerted only after its plasma concentration had reached a certain level. The usefulness of the clinical method applied in the United States and Europe, in which part of the total t-PA is given first in a bolus dose, followed thereafter by infusion of the rest of the dose (13), thus seems unquestioned. Several clinical trials of i.v. bolus injections of t-PA aimed at the early recanalization of the coronary vessels have recently been reported (23, 24). These studies obtained reperfusion rates comparable to those attained by i.v. infusion with bolus doses of t-PA. In this present study, bolus injections of t-PA produced reperfusion rates virtually equal to those produced by infusion; these injections reduced the time to reperfusion in 1- and 3-hr-old thrombi. In the 6-hr-old thrombi, on the other hand, bolus injections of t-PA were apparently less effective than infusion in lysing the thrombus. These findings imply that bolus injections of t-PA may afford essentially the same thrombolytic effect as that obtained by infusion when administered early after formation of thrombi, but that no such effect can be expected when this agent is given late.

The present findings show that the reocclusion rate tended to decrease with increasing dose, irrespective of thrombus age and irrespective of the drug the animals received. This suggests that the development of acute reocclusion has a close connection with thrombolytic activity in vivo. Clinically, a relationship between coronary reocclusion and residual thrombus after thrombolytic therapy has been described (25). Additional doses of a thrombolytic agent (2) or adjunctive therapy with an antiplatelet agent...
such as anti-GPIIb/IIIa antibody (26) or RGD peptide (27) or with an anticoagulant such as synthetic thrombin inhibitor (28) are considered useful to minimize the residual thrombus, as well as to prevent growth of the thrombus.

A reduction in plasma fibrinogen by approximately 30% occurred following the bolus injection of YM866 at 0.2 mg/kg and t-PA at 0.4 mg/kg and after i.v. infusion of t-PA at 0.6 mg/kg. The changes appeared to generally parallel the relative in vivo thrombolytic activity of YM866 and t-PA; such changes may not lead to enhanced systemic fibrinolysis, since the decrease was minimal as compared with that seen following the administration of urokinase (29) or streptokinase (30). The changes that occurred early after the bolus injection of YM866 or t-PA could be attributed to a sharp rise in plasma concentration after i.v. bolus injection.

Plasma antigen demonstrated a remarkably long-sustained concentration of YM866 as compared to that of t-PA. These findings are essentially consistent with the mean residence time of YM866 calculated from pharmacokinetics in rats (8) and can probably be extrapolated to man. It has been reported that deletion or substitution mutants within the heavy chain had longer half-lives and showed a superior thrombolytic effect than native t-PA in vivo (11, 31). We suggest that the superior thrombolytic effect of YM866 can be attributed to its sustained plasma concentration and its greater specific activity and affinity for fibrin.

Bolus injections of YM866, provided they achieve superior efficiency to t-PA without enhancing systemic fibrinolysis, might not only simplify treatment, but might also induce more rapid recanalization.

REFERENCES

1 Sherry, S.: Tissue plasminogen activator (t-PA). Will it fulfill its promise? N. Engl. J. Med. 313, 1014–1017 (1985)
2 Gold, H.K., Leinbach, R.C., Garabedian, H.D., Yasuda, T., Johns, J.A., Grossbard, E.B., Palacios, I. and Collen, D.: Acute coronary reocclusion after thrombolysis with recombinant human tissue-type plasminogen activator: prevention by a maintenance infusion. Circulation 73, 347–352 (1986)
3 Katoh, M., Shimizu, Y., Kawauchi, Y., Ishida, J., Takayama, M., Yokota, M., Yano, E., Kawakami, K., Yano, S., Morinaga, T., Tsuji, T., Kinoshita, A., Gomi, Y., Takemoto, T., Itoh, K., Ezoe, H. and Gushima, H.: Comparison of clearance rate of various tissue plasminogen activator (tPA) analogues. Thromb. Haemostas. 62, 542 (1989)
4 Yoshitake, S., Kato, H., Hashimoto, A., Ikeda, Y., Kuwada, M. and Mulvihill, E.: Characterization of various forms of modified human tissue plasminogen activators in vitro. Thromb. Haemostas. 62, 542 (1989)
5 Johannessen, M., Dines, V., Pingel, K., Petersen, L.C., Rao, D., Lioubin, P., O’Hara, P. and Mulvihill, E.: Fibrin affinity and clearance of t-PA deletion and substitution analogues. Thromb. Haemostas. 63, 54–59 (1990)
6 Cambier, P., Van de Werf, F., Larsen, G.R. and Collen, D.: Pharmacokinetics and thrombolytic properties of a non-glycosylated mutant of tissue plasminogen activator, lacking the finger and growth factor domains, in dogs with copper coil-induced coronary artery thrombosis. J. Cardiovasc. Pharmacol. 11, 468–472 (1988)
7 Kawauchi, Y., Morinaga, T., Yokota, M., Kinoshita, A., Kawamura, K., Suzuki, Y., Takayama, M., Furuki, K. and Gushima, H.: Gene construction and large scale production of a novel fibrinolytic agent (YM866) in CHO cells. Thromb. Haemostas. 65, 1193 (1991)
8 Katoh, M., Suzuki, Y., Miyamoto, I., Watanabe, T., Mori, K., Arakawa, H. and Gushima, H.: Biochemical and pharmacokinetic properties of YM866, a novel fibrinolytic agent. Thromb. Haemostas. 65, 1193 (1991)
9 Simoons, M.L., Serruys, P.W., Brand, M., Res, J., Verheugt, F.W.A., Krauss, H., Remme, W.J., Bar, F., Zwaan, C., Laarse, A., Vermeer, F. and Lubesn, J.: Early thrombolysis in acute myocardial infarction: Limitation of infarct size and improved survival. J. Am. Coll. Cardiol. 7, 717–728 (1986)
10 Marder, V.J. and Sherry, S.: Thrombolytic therapy: current status (first part). N. Engl. J. Med. 318, 1512–1520 (1988)
11 Suzuki, S., Saito, M., Suzuki, N., Kato, H., Nagaoka, N., Yoshitake, S., Mizuo, H., Yuzuriha, T., Yui, Y. and Kawai, C.: Thrombolytic properties of a novel modified human tissue-type plasminogen activator (E6010): a bolus injection of E6010 has equivalent potency of lysing young and aged canine coronary thrombi. J. Cardiovasc. Pharmacol. 17, 738–746 (1991)
12 Susawa, T., Yui, Y., Hattori, R., Takahashi, M., Aoyama, T., Takatsu, Y., Sakaguchi, K., Yui, N. and Kawai, C.: Heparin requirement in tissue-type plasminogen activator-induced experimental coronary thrombolysis: comparison with urokinase-induced coronary thrombolysis. Japan. Circ. J. 51, 431–435 (1987)
13 Chesebro, J.H., Knatterud, G., Roberts, R., Borer, J., Cohen, L.S., Daren, J., Dodge, H.T., Francis, C.K., Hillis, D., Ludbrook, P., Markis, J.E., Mueller, H., Passamani, E.R., Powers, E.R., Rao, A.K., Scherle, D., A.K., Robertson, T., Ross, A., Ryan, T.J., Sobel, B.E., Willerson, J., Williams, D.O., Zarret, B.L. and Braunwald, E.: Thrombolysis in myocardial infarction (TIMI) trial, phase I: a comparison between intravenous tissue plasminogen activator and intravenous streptokinase. Circulation 76, 142–154 (1987)
14 Tiefenbrunn, A.J., Robison, A.K., Kurnik, P.B., Ludbrook, P.A. and Sobel, B.E.: Clinical pharmacology in patients with evolving myocardial infarction of tissue-type plasminogen activator produced by recombinant DNA technology. Circulation 71, 110–116 (1985)
15 Ratnoff, O.D. and Menzie, C.: A new method for the determination of fibrinogen in small samples of plasma. J. Lab. Clin. Med. 37, 316–320 (1951)
16 Wohl, R.C., Sinio, L., Summaria, L. and Robbins, K.C.: Comparative activation kinetics of mammalian plasminogens. Biochim. Biophys. Acta 745, 20–31 (1983)
17 Guesdon, J.L., Ternynck, T. and Avrameas, S.: The use of avidin-biotin interaction in immunoenzymatic techniques. J. Histochem. Cytochem. 27, 1131–1139 (1979)
18 Bush, L.R. and Shebuski, R.J.: In vivo models of arterial
thrombosis and thrombolysis. FASEB J. 4, 3087–3098 (1990)

19 Topol, E.J., Bates, E.R., Walton, J.A., Baumann, G., Walfie, S., Maino, J., Baver, L., Gorman, L., Kline, E.M., O’Neill, W. and Pitt, B.: Community hospital administration of intravenous tissue plasminogen activator in acute myocardial infarction: improved timing, thrombolytic efficacy and ventricular function. J. Am. Coll. Cardiol. 10, 1173–1177 (1987)

20 Loven, M., Frade, L.J., Torrado, M.C. and Navarro, J.L.: Thrombus age and tissue plasminogen activator-mediated thrombolysis in rats. Thromb. Res. 56, 67–76 (1989)

21 Flameng, W., Van de Werf, F., Vanhaecke, J. and Collen, D.: Coronary thrombolysis and infarct size reduction after intravenous infusion of tissue-type plasminogen activator in nonhuman primates. J. Clin. Invest. 75, 84–90 (1985)

22 Eisenberg, P.R., Sherman, L.A., Tiefenbrunn, A.J., Ludbrook, P.A., Sobel, B.E. and Jaffe, A.S.: Sustained fibrinolysis after administration of t-PA despite its short half-life in the circulation. Thromb. Haemostas. 57, 35–40 (1987)

23 Tebbe, U., Transwell, P., Seifried, E., Feuerer, W., Scholz, K.H. and Herrmann, K.S.: Single bolus injection of recombinant tissue-type plasminogen activator in acute myocardial infarction. Am. J. Cardiol. 64, 448–453 (1989)

24 Khan, M.I., Hackett, D.R., Andreotti, F., Davies, G.J., Regan, T., Haider, A.W., McFadden, E., Haison, P. and Maseri, A.: Effectiveness of multiple bolus administration of tissue-type plasminogen activator in acute myocardial Infarction. Am. J. Cardiol. 65, 1051–1056 (1990)

25 Badimon, L., Assila, R., Badimon, J., Vallabhajosula, S., Chesebro, J.H. and Fuster, V.: Residual thrombus is more thrombogenic than severely damaged vessel wall. Circulation 78, Supp. II, II-119 (1988)

26 Mickelson, J.K., Simpson, P.J., Cronie, M., Homeister, J.W., Laywell, E., Kitzen, J. and Lucchesi, B.R.: Antiplatelet antibody [7E3F (ab)] prevents rethrombosis after recombinant tissue-type plasminogen activator-induced coronary artery thrombolysis in a canine model. Circulation 81, 617–627 (1990)

27 Yasuda, T., Gold, H.K., Leinbach, R.C., Yaoita, H., Fallon, J.T., Guerrero, L., Napier, M.A., Bunting, S. and Collen, D.: Kistrin, a polypeptide platelet GPIIb/IIIa receptor antagonist, enhances and sustains coronary arterial thrombolysis with recombinant tissue-type plasminogen activator in a canine preparation. Circulation 33, 1038–1047 (1991)

28 Yasuda, T., Gold, H.K., Yaoita, H., Leinbach, R.C., Guerrero, J.L., Jang, I.K., Holt, R., Fallon, J.T. and Collen, D.: Comparative effects of aspirin, a synthetic thrombin inhibitor and a monoclonal antiplatelet glycoprotein IIb/IIIa antibody on coronary artery reperfusion, reocclusion and bleeding with recombinant tissue-type plasminogen activator in a canine preparation. J. Am. Coll. Cardiol. 16, 714–722 (1990)

29 Korninger, C., Matsuo, O., Suy, R., Stassen, J.M. and Collen, D.: Thrombolysis with human extrinsic (tissue-type) plasminogen activator in dogs with femoral vein thrombosis. J. Clin. Invest. 69, 573–580 (1982)

30 Agnelli, G., Buchanan, M.R., Fernandez, F., Boneu, B., Ryn, J.V., Hirsh, J. and Collen, D.: A comparison of the thrombolytic and hemorrhagic effects of tissue-type plasminogen activator and streptokinase in rabbits. Circulation 72, 178–182 (1985)

31 Martin, U., Spener, G. and Strein, K.: Evaluation of thrombolytic and systemic effects of the novel recombinant plasminogen activator BM06.022 compared with alteplase, anistreplase, streptokinase and urokinase in a canine model of coronary artery thrombosis. J. Am. Coll. Cardiol. 19, 433–440 (1992)