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# Ventricular Function and Cardiac Hypertrophy after Coronary Thrombolysis with Tissue-type Plasminogen Activator (t-PA) in Dogs with Coronary Artery Thrombi

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**Abstract**

Subacute prognosis of cardiac function after thrombolysis with a modified tissue-type plasminogen activator (t-PA) YM866 was determined in dogs with coronary artery thromboses induced by injection of a thrombin, fibrinogen and autogenous blood mixture.

The left ventricular ejection fraction (LVEF) decreased 30 min after occlusion and had not improved 1 week later. Examination after sacrifice revealed myocardial infarction as well as increases in both the left ventricular myocardial area and heart mass. Occluded coronary arteries reperfused by YM866 (0·1 mg $kg^{-1}$ i.v.) treatment 30 min after occlusion, by contrast, had improved LVEF and inhibited myocardial infarction development. In addition, the left ventricular myocardial area and heart mass were significantly reduced compared with the vehicle control group 1 week after administration. Although occluded coronary arteries reperfused by YM866 (0·1 mg $kg^{-1}$ i.v.) treatment 3 h after occlusion did not show an improvement in the LVEF or inhibition of myocardial infarction development, the left ventricular myocardial area and heart mass decreased significantly compared with the vehicle control group 1 week after administration.

In conclusion, early reperfusion by t-PA treatment 30 min after occlusion improved the ventricular function and cardiac hypertrophy, whereas late reperfusion by t-PA treatment 3 h after occlusion did not improve the ventricular function but did inhibit hypertrophy in dogs with coronary artery thrombi.

Coronary artery thrombosis induces myocardial ischaemia, which in turn quickly causes tissue necrosis and deterioration of the left ventricular function (Jennings et al 1960; Reimer et al 1977; Reimer & Jennings 1979; Lavallee et al 1983). Conversely, rapid reperfusion of occluded coronary arteries inhibits myocardial infarction development and improves the left ventricular function. Indeed, early use of thrombolytic treatment in patients with acute myocardial infarction reduces mortality (Sheehan et al 1988) and prevents extensive damage of ischemic myocardial tissue (Ishikawa et al 1988; National Heart Foundation of Australia Coronary Thrombolysis Group 1988; Van de Werf 1988; Wilcox et al 1988; Linderer & Schroder 1993). Early reperfusion after acute myocardial infarction also prevents left ventricular remodelling, leading to a good long-term prognosis (Hochman & Choo 1987; White et al 1987; Lavie et al 1990). Left ventricular remodelling characterized by an extension of the myocardial infarction region and efferent enlargement of the non-infarcted area begins to develop during the subacute stage (about 1 week after onset) after acute myocardial infarction (Pfeffer & Braunwald 1990). This process may lead to heart failure and serious ventricular arrhythmia as it develops, increasing the probability of an unfavourable long-term prognosis. Clinical results also indicate that reperfusion 6 h after the onset of acute myocardial infarction (late reperfusion) inhibits left ventricular remodelling, although the cardiac function does not improve (Topol et al 1992; Hirayama et al 1993). In animals, early reperfusion by thrombolysis improves the short-term outcome in an animal acute myocardial infarction model with coronary

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artery thrombi (Saito et al 1995; Suzuki et al 1995). There are few reports, however, on the long-term outcome after thrombolytic treatment in a coronary artery thrombi model.

A canine model employing coronary artery thrombi induced by closed-chest injection of a thrombin and autogenous blood with fibrinogen has been developed (Suzuki et al 1999). Coronary artery thrombi were easily prepared without open-chest surgery. This model can be used in long-term studies of myocardial infarction. In this study, subacute prognosis of cardiac function after thrombolysis with tissue-type plasminogen activator (t-PA) treatment 30 min or 3 h after occlusion was determined in dogs with coronary artery thromboses induced by closed-chest injection of a thrombin, fibrinogen and autogenous blood mixture.

## Methods

*Induction of coronary artery thrombi and determination of thrombolytic activity*

All experiments complied with the regulations of the Animal Ethics Committee of Yamanouchi Pharmaceutical Co., Ltd. Adult beagle dogs weighing 10–14 kg (aged about 6–12 months) were used. The dogs were anesthetized with sodium thiopental (20 mg $kg^{-1}$ i.v.). Anesthesia was maintained with a 0·5–1% halothane/room air mixture. Catheters were placed in the femoral vein to administer the drugs and in the femoral artery to monitor blood pressure and heart rate. A sheath (9 Fr; Tonokura, Tokyo, Japan) was placed in the common carotid artery for insertion of a cardiovascular catheter and a balloon catheter. Continuous monitoring of the ECG was conducted in the precordial leads to detect arrhythmias. A balloon catheter (5 Fr; Clinical Supply, Gifu, Japan) was inserted into the left anterior descending coronary artery distal to the first diagonal branch using an intracoronary wire and fluoroscopic visualization. The catheter had three ports and channels: the first for balloon inflation and the second and third for local delivery of various substances into the artery. Blood flow distal to the balloon was inhibited by balloon inflation. Coronary thrombus was induced by simultaneous injection of thrombin (300 IU; Mochida, Tokyo, Japan) using the second port and channel of the balloon catheter and autogenous blood mixed with fibrinogen (5 mg; Sigma Chemicals, St Louis, MO) using the third port and channel. This method has been described previously (Suzuki et al 1999). The balloon was deflated 5 min after injection of the agents. Confirmation of coronary occlusion was performed by angiography 5 min after deflation of the balloon as follows. A Sones catheter (7 Fr; Bird Japan, Tokyo, Japan) was inserted into the left coronary artery, and contrast medium (Optiray, Yamanouchi Co. Ltd, Tokyo, Japan) was injected via the cardiovascular catheter under fluoroscopy. t-PA YM866 was administered intravenously 30 min or 3 h after confirmation of coronary artery occlusion. Confirmation of reperfusion, also assessed by angiography, was performed every 10 min for up to 60 min after drug administration. Dogs showing no evidence of coronary reperfusion by 60 min were considered not to have attained reperfusion. Reperfusion was defined as thrombolysis in myocardial infarction grade 2 or 3 and occlusion was defined as 0 or 1 (Chesebro et al 1987). Confirmation of spontaneous reperfusion was performed 6 h, 1 day, 2 days, 3 days and 1 week after occlusion.

*Left ventricular ejection fraction*

Left ventriculography was performed before balloon catheter insertion, just prior to and 1 week after YM866 administration. A pigtail catheter (8 Fr; Bird, Japan) was inserted into the left ventricle, and Optiray contrast medium was injected via the catheter in the 30° right anterior oblique position using fluoroscopic visualization. The left ventriculography was recorded on video tape (WV-H2, Sony, Tokyo, Japan). The videotaped left systolic and diastolic ventriculographies were traced using a KD4300 image analyzer (Graphtec Co., Ltd, Tokyo, Japan). LVG analysis software (Goodman Co., Nagano, Japan) was used to determine left ventricular ejection fraction (LVEF).

*Myocardial infarction area, left ventricular myocardial area and heart mass*

The dogs were killed by means of a lethal dose of sodium pentobarbital 1 week after coronary artery occlusion. Their hearts were removed and the heart masses were measured. The hearts were cut into transverse sections 1 cm thick at points 1 and 2 cm cranial to the apex of the heart. The slices were stained with 1% 2,3,5-triphenyl tetrazolium chloride (TTC) (Sigma Chemicals) for 5 min at 37°C (Ishikawa et al 1992). The area of the myocardial infarction was identified as the area that was not stained by TTC. The myocardial infarction and left ventricular myocardial areas were calculated using an area measuring program (System Supply, Japan).

*Drugs*

YM866 (Yamanouchi Co., Ltd, Tokyo, Japan) is a novel modified t-PA. The gene has been engineered to delete the first kringle (K1) domain of the natural protein and to introduce a point mutation that changes the amino acid residue at position 275 from arginine to glutamic acid (Kawauchi et al 1991). YM866 possesses a pronounced affinity for fibrin while retaining essentially the same specific activity as native t-PA in-vitro. It also has a markedly persistent in-vivo plasma concentration compared with native t-PA (Katoh et al 1989, 1991). YM866 was dissolved in physiologic saline for use. For the vehicle control group, the YM866 vehicle was diluted in the same manner. YM866 and the vehicle control were administered at a volume of $0{\cdot}5\,mL\,kg^{-1}$ body mass. YM866 was given at a dose of $0{\cdot}1\,mg\,kg^{-1}$, since this dose reperfused coronary occlusion in all dogs in a preliminary study.

*Statistics*

The statistical analysis of parametric and non-parametric data for intergroup comparison was performed using Tukey's test and Steel-Dwass' test, respectively.

## Results

*Recanalization*

An occlusive thrombus was observed in all the dogs that were injected with the thrombin, fibrinogen and autogenous blood mixture. Thrombotic coronary occlusion was signalled by an elevated ST segment in the left precordial ECG. Spontaneous reperfusion of the occluded coronary arteries did not occur for up to 1 day after occlusion, but was observed 3 days after occlusion in all dogs in the vehicle control group.

Reperfusion due to YM866 ($0{\cdot}1\,mg\,kg^{-1}$ i.v.) treatment administered 30 min after occlusion occurred in all dogs (5/5) within 10 min (median time) of administration (Figure 1). Coronary perfusion was maintained, as open arteries were observed in all dogs (5/5) 1 week after administration. Similarly, reperfusion due to YM866 ($0{\cdot}1\,mg\,kg^{-1}$ i.v.) treatment administered 3 h after occlusion occurred in all dogs (6/6) within 15 min (median time) of administration. Coronary perfusion was maintained, as open arteries were observed in all dogs (5/5) 1 week after administration.

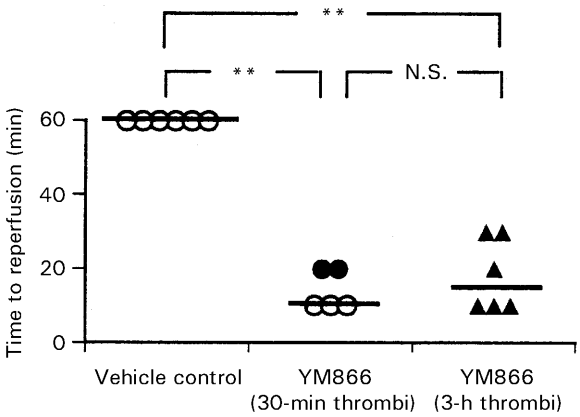


Figure 1. Reperfusion rate and time to reperfusion after t-PA treatment in dogs with occluded coronary arteries. YM866 ($0{\cdot}1\,mg\,kg^{-1}$) was administered intravenously 30 min or 3 h after coronary artery occlusion (30-min thrombi and 3-h thrombi, respectively). Confirmation of reperfusion was observed for up to 60 min after administration. Each point indicates the value for each dog and ⊖ indicates the median. Solid symbols indicate reperfused dogs and open symbols indicate non-reperfused dogs. $^{**}P < 0{\cdot}01$, significantly different from the vehicle control group (Steel-Dwass' test); N.S. = no significant difference between the groups (Steel-Dwass' test).

One of the six dogs in the vehicle control group died 1 day after coronary artery occlusion. While no dog in the group administered YM866 30 min after occlusion died within 1 week (0/5), one of the six dogs in the group administered YM866 3 h after occlusion, died 6 days after administration.

*Cardiovascular measurements*

No significant differences in heart rate were observed between the sham-operated control and the vehicle control groups in the values obtained just after occlusion, but the heart rate decreased significantly in the vehicle control groups compared with the sham-operated control group 1 week after occlusion (Table 1). LV dP/dt decreased just after occlusion and increased during the week following occlusion as compared with the sham-operated control group. One week after occlusion, the heart rate, mean blood pressure and LV dP/dt showed no effect of administration of YM866 ($0{\cdot}1\,mg\,kg^{-1}$ i.v.) 30 min or 3 h after occlusion (Table 1).

*Left ventricular ejection fraction*

The LVEF decreased to $26{\cdot}0 \pm 2{\cdot}1\%$ from $59{\cdot}7 \pm 2{\cdot}8\%$ within 30 min of occlusion and decreased to $26{\cdot}2 \pm 1{\cdot}9\%$ from $54{\cdot}8 \pm 4{\cdot}6\%$ within 3 h of occlusion (Figure 2). The decreased LVEF did not improve in the vehicle control group during the 1 week following occlusion ($38{\cdot}4 \pm 3{\cdot}0\%$).

Table 1. Changes in cardiovascular parameters after administration of t-PA in dogs with occluded coronary arteries.

| Cardiovascular parameters | Before coronary occlusion | Just after coronary occlusion | 1 week after drug administration |
|---|---|---|---|
| Heart rate (beats/min) | | | |
| Sham-operated control | 94·0 ± 9·3 | 96·0 ± 4·0 | 126·0 ± 13·0 |
| Vehicle control | 84·2 ± 7·4 | 93·3 ± 7·7 | 75·0 ± 6·7* |
| YM866 (30-min thrombi)[a] | 96·0 ± 3·7 | 111·0 ± 5·6 | 99·0 ± 6·0 |
| YM866 (3-h thrombi)[a] | 88·3 ± 6·0 | 98·3 ± 9·4 | 88·0 ± 11·9 |
| Mean blood pressure (mmHg) | | | |
| Sham-operated control | 93·9 ± 7·0 | 83·7 ± 5·9 | 113·6 ± 8·0 |
| Vehicle control | 100·3 ± 4·4 | 81·9 ± 7·0 | 87·8 ± 13·1 |
| YM866 (30-min thrombi) | 93·8 ± 8·3 | 82·3 ± 9·8 | 92·7 ± 6·0 |
| YM866 (3-h thrombi) | 85·2 ± 2·8 | 72·8 ± 9·2 | 88·7 ± 9·3 |
| LV dP/dt (mmHg/s) | | | |
| Sham-operated control | 3420 ± 159 | 3360 ± 196 | 3320 ± 263 |
| Vehicle control | 3467 ± 222 | 2883 ± 271 | 3700 ± 217 |
| YM866 (30-min thrombi) | 3420 ± 297 | 2880 ± 166 | 3520 ± 296 |
| YM866 (3-h thrombi) | 3467 ± 131 | 2817 ± 189 | 3460 ± 238 |

[a]YM866 (0·1 mg kg$^{-1}$ i.v.) was administered 30 min or 3 h after coronary artery occlusion (30-min thrombi and 3-h thrombi, respectively). Each value represents the mean ± s.e.m. (n = 5–6). *$P < 0.05$, significantly different from the sham-operated control group (Tukey's test).

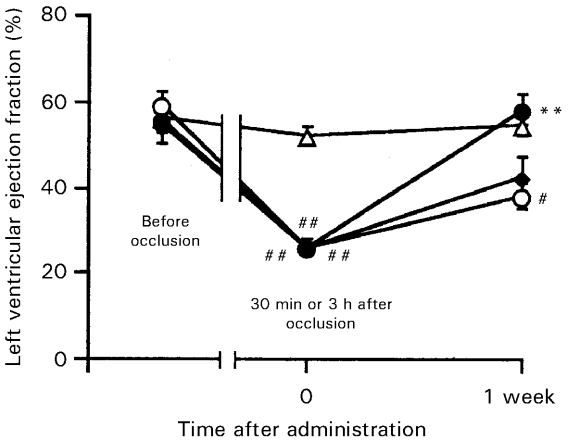


Figure 2. Left ventricular ejection fraction after t-PA treatment in dogs with occluded coronary arteries. YM866 (0·1 mg kg$^{-1}$) was administered intravenously 30 min or 3 h after coronary occlusion (30-min thrombi and 3-h thrombi, respectively). △, Sham (n = 5); ○, vehicle control (n = 6); ●, YM866 (30-min thrombi, n = 5); ◆, YM866 (3-h thrombi, n = 6). Each value represents the mean ± s.e.m. **$P < 0.01$, significantly different from the vehicle control group (Tukey's test); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, significantly different from the sham-operated control group (Tukey's test).

When YM866 was administered 30 min after occlusion, however, the LVEF improved to 58·4 ± 3·7%, a level similar to that in the sham-operated control group (55·0 ± 2·4%). When YM866 was administered 3 h after occlusion, however, the decreased LVEF showed no significant improvement during the week following administration (42·6 ± 4·9%).

*Myocardial infarction development*

Anteroinferior transmural myocardial infarctions were observed in the vehicle control group (Figure 3A). Similar infarctions were also observed in the group administered YM866 3 h after occlusion (Figure 3C). In contrast, myocardial infarctions were located in the subendocardial region in the group administered YM866 30 min after occlusion (Figure 3B). The ratio of myocardial infarction area to left ventricular myocardial area was 51·5 ± 7·8% in the vehicle control group (Figure 3D). When YM866 was administered 30 min after occlusion, the ratio was 26·4 ± 6·1%, significantly smaller than that in the vehicle control group. When YM866 was administered 3 h after occlusion the ratio was 46·6 ± 4·1%, similar to that in the vehicle control group.

*Left ventricular myocardial area*

The ratio of left ventricular myocardial area to body mass was 96·8 ± 4·6 mm$^2$ kg$^{-1}$ in the sham-operated control group (Figure 4). In the vehicle control group, the ratio was 121·8 ± 3·9 mm$^2$ kg$^{-1}$, significantly higher than that in the sham-operated control group. YM866 was administered either 30 min or 3 h after occlusion. The ratios were 100·0 ± 4·6 mm$^2$ kg$^{-1}$ for the 30-min group and 97·2 ± 7·2 mm$^2$ kg$^{-1}$ for the 3-h group. These ratios were significantly smaller than those of the vehicle control group.

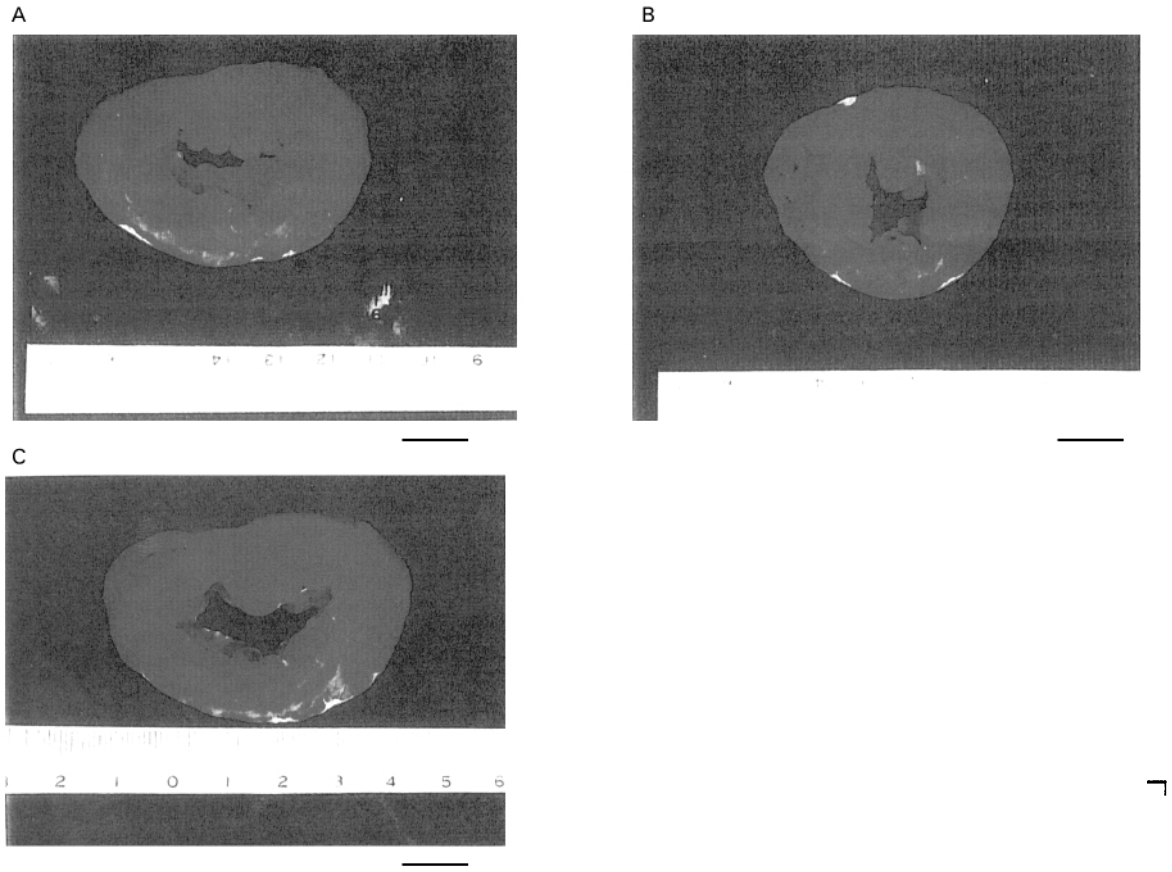


Figure 3. Myocardial infarction development after t-PA treatment in dogs with occluded coronary arteries. YM866 (0·1 mg $kg^{-1}$) was administered intravenously 30 min or 3 h after coronary artery occlusion (30-min thrombi and 3-h thrombi, respectively). The hearts were excised 1 week after administration. Heart slices were stained with TTC. The photos show representative slices from (A) the vehicle control, (B) the 30-min thrombi group and (C) the 3-h thrombi group. The myocardial infarction area was identified as the area that was not stained by TTC. A bar indicates 1 cm. (D) Each value represents the mean ± s.e.m. (n = 5). *$P < 0.05$, significantly different from the vehicle control group (Tukey's test); N.S. = no significant difference between the groups (Tukey's test).

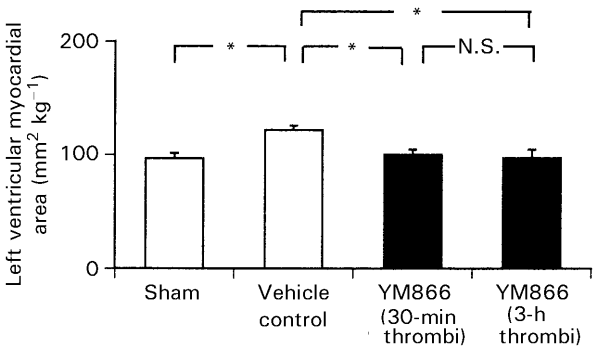


Figure 4. Left ventricular myocardial area after t-PA treatment in dogs with occluded coronary arteries. YM866 (0·1 mg $kg^{-1}$) was administered intravenously 30 min or 3 h after coronary occlusion (30-min thrombi and 3-h thrombi, respectively). The hearts were excised 1 week after administration. Each value represents the mean ± s.e.m. (n = 5). *$P < 0.05$, significantly different from the groups (Tukey's test); N.S. = no significant difference between the groups (Tukey's test).

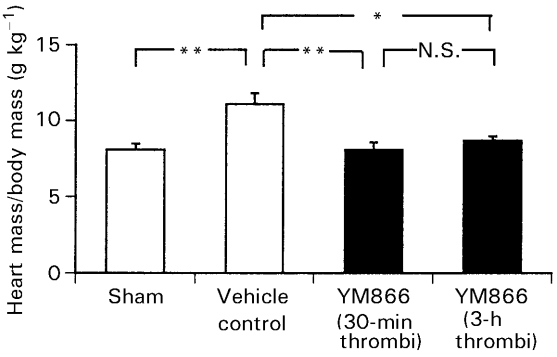


Figure 5. Heart mass after t-PA treatment in dogs with occluded coronary arteries. YM866 (0·1 mg $kg^{-1}$) was administered intravenously 30 min or 3 h after coronary occlusion (30-min thrombi and 3-h thrombi, respectively). The hearts were excised 1 week after administration. Each value represents the mean ± s.e.m. (n = 5). *$P < 0.05$, **$P < 0.01$, significantly different from the groups (Tukey's test); N.S. = no significant difference between the groups (Tukey's test).

*Heart mass*

The ratio of heart mass to body mass was $8{\cdot}1 \pm 0{\cdot}4\,g\,kg^{-1}$ in the sham-operated control group (Figure 5). In the vehicle control group, the ratio was $11{\cdot}1 \pm 0{\cdot}7\,g\,kg^{-1}$, significantly higher than that in the sham-operated group. YM866 was administered either 30 min or 3 h after occlusion. The ratios were $8{\cdot}1 \pm 0{\cdot}5\,g\,kg^{-1}$ for the 30-min group and $8{\cdot}7 \pm 0{\cdot}3\,g\,kg^{-1}$ for the 3-h group. These ratios were significantly smaller than those of the vehicle control group.

## Discussion

A technique for assessing the long-term prognosis of dogs with coronary artery thrombi has been developed (Suzuki et al 1999). Coronary artery thrombi induced by closed-chest injection of a thrombin and autogenous blood with fibrinogen were prepared easily without open-chest surgery. In the present study, subacute prognosis of cardiac function after thrombolytic treatment was determined using the canine model. Myocardial infarction development was inhibited by a modified t-PA YM866 administered 30 min after occlusion, but myocardial infarction development was not significantly inhibited by YM866 administered 3 h after occlusion. These results are in agreement with reports that myocardial infarction in dogs begins to develop within 40 min of the onset of ischaemia and that most myocardial tissue in the ischaemic area dies 3 to 6 h after occlusion (Reimer et al 1977; Reimer & Jennings 1979).

When YM866 was administered 30 min after occlusion, reperfusion occurred within 10 min and the LVEF had improved compared with the vehicle controls 1 week later. When YM866 was administered 3 h after occlusion, by contrast, the LVEF had not improved 1 week later, in spite of the occurrence of reperfusion within 15 min. Thrombi were created by stopping the coronary artery flow using an inflated balloon and inducing a clot in this model. The total time that the coronary arteries were occluded was about 50 min in the group administered YM866 30 min after occlusion. Because confirmation of thrombotic occlusion was performed 10 min after balloon inflation, the drug was administered 30 min after confirmation of occlusion and reperfusion occurred about 10 min after administration. The present results of effects on cardiac function are, therefore, in agreement with previous findings that cardiac function in dogs improves 1 week after coronary artery ligation for 1 h, but does not improve 1 week after ligation for 3 h (Lavallee et al 1983). It has previously been demonstrated that the early use of thrombolytic treatment in patients with acute myocardial infarction salvages ischaemic myocardial tissue and preserves the left ventricular function (National Heart Foundation of Australia Coronary Thrombolysis Group 1988; Linderer & Schroder 1993). The improvement of the left ventricular function is probably similarly due to rapid reperfusion.

Native t-PA administration must be performed by high-dose intravenous infusion because of its extremely short biological half-life. This high dosing regimen increases the possibility of systemic bleeding and, consequently, acute coronary artery reocclusion (Gold et al 1986). Furthermore, infusion is a more complicated and inconvenient method of drug delivery than bolus injection, especially in an emergency clinical setting. A thrombolytic agent that can exert effective thrombolytic activity after a single bolus injection is, therefore, desired by emergency medical practitioners. YM866 is a novel modified t-PA. It has been demonstrated in-vitro that YM866 possesses a pronounced affinity for fibrin, while essentially retaining the same specific activity as native t-PA. In-vivo, it persists in plasma for a markedly longer time than native t-PA (Kawasaki et al 1993, 1994). Due to this sustained plasma concentration, therefore, YM866 administered by intravenous bolus injection exerts a pronounced thrombolytic effect in dogs with induced coronary artery thrombi (Kawasaki et al 1993). Its effects, including recanalization rate and time to reperfusion, are similar to those of native t-PA treated by high-dose infusion. The outcome after coronary ischaemia depends on the time between coronary occlusion and recanalization. Indeed, the acute-stage improvement (several hours after occlusion) of cardiac function occurring with treatment with YM866 bolus injection is similar in degree to that occurring with treatment with native t-PA high-dose infusion in dogs with induced coronary artery thrombi (Suzuki et al 1998). Thus, both YM866 and a high-dose native t-PA may preserve the subacute-stage cardiac function to a similar degree.

Left ventricular myocardial area and heart mass, which indicate the ventricular hypertrophy, did not increase 1 week after YM866 treatment 30 min after occlusion (Hochman & Buckley 1982; Pfeffer & Braunwald 1990). The degree of remodelling is dependent on the size of the myocardial infarction (Hochman & Buckley 1982). These results may therefore be due to early reperfusion and inhibition of further myocardial infarction development. The observation that left ventricular hypertrophy was lower than that observed in the vehicle control groups 1 week after reperfusion, although myo-

cardial infarction development was not inhibited when YM866 was administered 3 h after occlusion, is of great interest. The clinical results also indicate that reperfusion 6 h after the onset of acute myocardial infarction (late reperfusion) inhibits left ventricular remodelling, although cardiac function does not improve (Topol et al 1992; Hirayama et al 1993). Several theories have been proposed to explain the apparently conflicting results that left ventricular hypertrophy was inhibited although LVEF did not improve. It has been suggested that the mechanism of inhibition of hypertrophy is due to the inhibition of remodelling and improvement of hibernating myocardium contraction (Kim & Braunwald 1993). Prevention of left ventricular remodelling by coronary reperfusion offers a good long-term prognosis in patients with acute myocardial infarction (Hochman & Choo 1987; White et al 1987; Lavie et al 1990). Since coronary thrombolysis with t-PA administered 30 min or 3 h after occlusion prevents left ventricular hypertrophy, it may be that coronary reperfusion prevents the progression of heart failure during left ventricular remodelling, independent of the length of time of ischemia.

In this study, YM866 treatment 30 min after occlusion improved cardiac function, whereas YM866 treatment 3 h after occlusion did not improve ventricular function but prevented hypertrophy in dogs with coronary artery thrombi. Lavallee et al (1983) have demonstrated that reperfusion 1 and 2 h after occlusion improves subacute-stage myocardial function but that reperfusion 3 h after occlusion does not improve it in dogs with coronary ligation. Reperfusion carried out 6 h after coronary occlusion causes less dilation of the abnormally contracting myocardial segment in the canine model (Kim & Braunwald 1993). Kimura et al (1998) showed a certain degree of myocardial salvage by reperfusion even 12 h after occlusion in dogs with coronary ligation. Reperfusion up to at least 2 h after the onset can improve cardiac function, therefore, and reperfusion up to 12 h after the onset may improve hypertrophy, but not cardiac function, in patients with acute myocardial infarction.

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