YM866, a Novel Modified Tissue-Type Plasminogen Activator, Affects Left Ventricular Function and Myocardial Infarct Development in Dogs With Coronary Artery Thrombi

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ABSTRACT—YM866 is a novel modified tissue-type plasminogen activator (t-PA). Its effects on left ventricular function and myocardial infarct development in dogs with copper coil-induced coronary artery thrombosis were compared with those of a native t-PA, alteplase. YM866 (bolus injection) and alteplase (bolus plus infusion) were administered 15 min after coronary artery occlusion. YM866 and alteplase produced reperfusion in all animals, with a median time to reperfusion of 10 min. In contrast, no reperfusion occurred in the vehicle control group. Left ventricular ejection fraction (LVEF) significantly decreased 15 min after coronary occlusion. YM866 and alteplase improved LVEF 3 hr and 4 hr after administration, respectively, while LVEF did not improve in the vehicle control group. Only slight myocardial infarct areas were observed in both YM866- and alteplase-administered groups, while the area in the vehicle control group accounted for 18.2% of left ventricular myocardial area. In conclusion, although both YM866 and alteplase reperfused occluded coronary arteries, inhibited myocardial infarct development and improved LVEF in dogs with coronary artery thrombi, only a single bolus injection of YM866 was necessary to achieve these improvements. Therefore, YM866 shows promise as an improved clinical agent in treating acute myocardial infarction.

Keywords: Modified tissue-type plasminogen activator, Left ventricular ejection fraction (LVEF), Myocardial infarction, Coronary artery thrombosis, Thrombolysis

Ten years ago, tissue-type plasminogen activator (t-PA) held promise as a thrombolytic agent with a low possibility of systemic bleeding due to strict control pathways regulating its effects. Although part of that promise has held true, exhibiting a coronary reperfusion rate of 70% or higher in patients with acute myocardial infarction (1–4), t-PA administration must be performed by high-dose intravenous infusion because of its extremely short biological halflife. This extreme dosing regimen increases the possibility of systemic bleeding and consequently, acute coronary artery reocclusion (5). Furthermore, infusion is a more complicated and inconvenient method of drug delivery than bolus injection especially in an emergency clinical setting. Therefore, a thrombolytic agent that can exert effective thrombolytic activity after a single bolus injection is urgently sought after by emergency medical practitioners.

YM866 is a novel modified t-PA. The first kringle (K1) domain of the molecule is deleted and a point mutation \(^{(275}{\text{Arg}}\rightarrow{\text{Glu}})\) is present at the site of the second kringle (K2) domain linkage to the L-chain (6). It has been demonstrated that YM866 possesses a pronounced affinity for fibrin while retaining essentially the same specific activity as a native t-PA in vitro. It also has a remarkably persistent plasma concentration in vivo, compared with native t-PA (7, 8). It has been also shown that, due to its remarkably sustained plasma concentration, YM866 administered by intravenous bolus injection exerts a thrombolytic effect superior to that of native t-PA administered by intravenous infusion in canine coronary artery thrombosis models (9). Therefore, YM866 shows promise as a highly effective thrombolytic agent, which can be quickly and easily administered by intravenous bolus injection to patients with coronary artery thrombosis.

Ischemia in myocardial tissue due to coronary artery thrombosis promptly causes tissue necrosis and deterioration of left ventricular function in dogs (10–13). Conversely, rapid reperfusion of occluded coronary arteries has
been shown to inhibit myocardial infarct development and improve left ventricular function. It is also recently accepted that the early use of thrombolytic treatment in patients with acute myocardial infarction reduces mortality (2) and rescues ischemic myocardial tissue (14–18). Therefore, the present study was performed to assess the thrombolytic activity of YM866 and its effect on left ventricular ejection fraction (LVEF) when administered by intravenous bolus injection to dogs with induced coronary artery thrombosis. The action of YM866 in this model was compared with the action of alteplase that was administered by a combination dosing regimen of intravenous bolus injection followed by infusion, similar to the procedure currently used in clinical practice.

MATERIALS AND METHODS

Preparation of coronary artery thrombi and determination of thrombolytic activity

Adult beagle dogs weighing 10–13 kg were used. Animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v. bolus + 3–5 mg/kg/hr infusion) and artificially ventilated with room air. Catheters were placed in the femoral vein for administration of test drugs and the femoral artery for blood pressure/heart rate monitoring. Continuous monitoring of the ECG was performed in precordial leads to detect arrhythmias. Coronary thrombosis was induced by placating a copper coil (5-mm-long and 2 mm in diameter) over an intracoronary wire into the left anterior descending coronary artery distal to the first diagonal branch using fluoroscopic visualization, as described previously (19). No copper coil was inserted into the coronary artery in the sham-operated control group. Confirmation of coronary occlusions was performed by angiography every 5 min after copper coil-insertion as follows: A Sones catheter (8 Fr; Bird Japan, Tokyo) was inserted into left coronary artery, and contrast medium (Optry®; Yamanouchi Co., Ltd., Tokyo) was injected via the catheter under fluoroscopy. YM866 (0.05 mg/kg) was administered by intravenous bolus injection, and alteplase (total dose: 0.38 mg/kg) was administered by intravenous bolus injection (0.04 mg/kg) followed by infusion (0.34 mg/kg/hr for 1 hr). YM866 and alteplase treatments began 15 min after coronary occlusions were confirmed. Heparin (300 U/kg) (Novo Heparin; Novo BioLabs, Soeborg, Denmark) was administered intravenously 10 min before administration of vehicle and test drugs in the vehicle control, YM866-administered and alteplase-administered groups. Since no reperfusion was observed in the vehicle control group, it is supposed that pretreatment with heparin had no effect on the thrombolytic activities of YM866 and alteplase. Confirmation of reperfusion, assessed by angiography, was performed every 5 min for up to 60 min after starting administration of test drugs. Animals showing no evidence of coronary reperfusion by 60 min were considered not to have attained reperfusion. Reperfusion was defined as TIMI (thrombolysis in myocardial infarction) grade 2 or 3 and reocclusion defined as 0 or 1 (20). Lidocaine (1 mg/kg, i.v.) (Xylocaine; Fujisawa-Astra Co., Ltd., Osaka) was used to prevent arrhythmia as the experiment proceeded through thrombotic occlusion and reperfusion, since almost half of the animals died from arrhythmia such as ventricular fibrillation, especially when coronary arteries were occluded and reperfused in the preliminary study.

LVEF

Left ventriculography was performed before copper coil-insertion, 15 min after confirmation of coronary occlusions and 1, 2, 3 and 4 hr after beginning of test drug administration as follows: A pig tail catheter (8 Fr, Bird Japan) was inserted into left ventricle and contrast medium (Optry®) was injected via the catheter in the 30° right anterior oblique position (RAO 30) using fluoroscopic visualization. Left ventriculography was recorded with videotape (WV-H2; Sony, Tokyo). Videotaped systolic and diastolic left ventriculography were traced by an image analyzer (KD4300; Graphitec Co., Ltd., Tokyo), LVG; analysis software (Goodman Co., Nagoya) was used to determine LVEF.

Myocardial infarct area

The animals were sacrificed with a lethal dose of pentobarbital sodium following these experiments. Their hearts were excised and cut into cross-sections 1-cm-thick at 1 and 2 cm below the position of copper coil-insertion. The slices were stained with 1% TTC (2,3,5-triphenyl tetrazolium chloride; Sigma Chemical, St. Louis, MO, USA) for 5 min at 37°C (21). The area of the myocardial infarct was identified as the area that was not stained by TTC. Myocardial infarct area was calculated using an area measuring program (System Supply, Nagano).

All experiments were performed in compliance with the regulations of the Animal Ethics Committee of Yamanouchi Pharmaceutical Co., Ltd.

Drugs

YM866 (Yamanouchi Co., Ltd.) and alteplase (Grtpa®; Tanabe Co., Ltd., Osaka) were dissolved in physiologic saline for use in the experiments. For the vehicle control group, the YM866 vehicle was diluted in the same manner. All drugs were administered in a volume of 0.5 ml/kg body weight. The specific activity of alteplase determined by the fibrin clot lysis assay calibrated with an international t-PA standard (83/157) was 580,000 IU/mg.
YM866 was given at the dose of 0.05 mg/kg and alteplase was given at the dose of 0.38 mg/kg (217,500 IU/kg) beginning 15 min after coronary occlusion.

**Statistics**

Time to reperfusion was expressed as the value observed for each animal. The median and statistical analysis of data for intergroup comparison was performed by the Steel-Dwass test. Cardiovascular parameters, LVEF and myocardial infarct area were expressed as the mean±S.E. Statistical analyses of data for intergroup comparison was performed by Tukey's test.

**RESULTS**

**Cardiovascular parameters**

No significant differences in heart rate or mean blood pressure were observed between sham-operated control and vehicle control groups in pre-operation values, values 15 min after coronary occlusion and values 4 hr after beginning of test drug administration (Table 1). YM866 (0.05 mg/kg, i.v. bolus) and alteplase (total volume: 0.38 mg/kg, i.v., 0.04 mg/kg, i.v. bolus + 0.34 mg/kg/hr infusion for 1 hr) did not affect heart rate or mean blood pressure.

**Thrombolytic activity**

An occlusive thrombus usually developed within about 20 min after copper coil-insertion. Thrombotic coronary occlusion was signaled by an elevated ST segment in the left precordial ECG. YM866 (0.05 mg/kg, i.v.) reperfused occluded coronary arteries in all animals treated with drug (5/5, Fig. 1). YM866 reperfusion occurred 10 min (median time) after administration. Alteplase (0.38 mg/kg, i.v.) also reperfused occluded coronary arteries in all animals (5/5) with a time to reperfusion of 10 min (median time) after beginning drug administration. The vehicle could not reperfuse occluded coronary arteries throughout the 4-hr observation period following vehicle administration in the vehicle control group (data not shown). No reocclusion after reperfusion was observed for up to 4 hr after beginning drug administration in the YM866- and alteplase-administered groups (data not shown).

**LVEF**

LVEF before copper coil-insertion was 61.2–63.6%, and it decreased to 35.0–36.0% within 15 min after coronary artery occlusion (Fig. 2). LVEF recovered gradually in both YM866 (0.05 mg/kg, i.v.)- and alteplase (0.38 mg/kg, i.v.)-administered groups, showing significant recovery 3 hr (YM866) and 4 hr (alteplase) after beginning drug administration compared with the vehicle control group. LVEF in both YM866- and alteplase-administered groups recovered to the same degree as that in sham-operated control group 2 hr and 4 hr after beginning drug administration. No significant difference in LVEF at each time-point after beginning of drug administration was observed between YM866- and alteplase-administered groups. In contrast, no recovery of LVEF was observed within the 4-hr observation period after vehicle administration in the vehicle control group.

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**Table 1. Changes in heart rate and mean blood pressure due to administration of YM866 or alteplase in dogs with induced coronary artery thrombi**

<table>
<thead>
<tr>
<th>Cardiovascular parameters/Groups</th>
<th>Pre-operation values</th>
<th>Values 15 min after coronary occlusions</th>
<th>Values 4 hr after beginning of test drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated</td>
<td>136±10</td>
<td>158±14</td>
<td>156±16</td>
</tr>
<tr>
<td>Vehicle</td>
<td>130±9</td>
<td>142±7</td>
<td>133±10</td>
</tr>
<tr>
<td>YM866</td>
<td>140±11</td>
<td>163±11</td>
<td>130±6</td>
</tr>
<tr>
<td>Alteplase</td>
<td>153±13</td>
<td>159±15</td>
<td>134±19</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated</td>
<td>120±9</td>
<td>123±4</td>
<td>140±7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>96±5</td>
<td>123±7</td>
<td>113±13</td>
</tr>
<tr>
<td>YM866</td>
<td>101±7</td>
<td>129±10</td>
<td>125±8</td>
</tr>
<tr>
<td>Alteplase</td>
<td>118±8</td>
<td>121±8</td>
<td>118±11</td>
</tr>
</tbody>
</table>

1) Just before test drug administration. Administration of test drugs began 15 min after coronary occlusion was confirmed. YM866 (0.05 mg/kg) was administered by a single intravenous bolus injection, and alteplase (total dose: 0.38 mg/kg) was administered by intravenous bolus injection (0.04 mg/kg) followed by infusion (0.34 mg/kg/hr for 1 hr). Each value represents the mean±S.E. from 5 animals. No significant differences in heart rate or mean blood pressure were observed between the groups in pre-operation values, values 15 min after coronary occlusion and values 4 hr after beginning of test drug administration (Tukey's test).
Fig. 1. Thrombolytic activity of YM866 and alteplase in dogs with induced coronary artery thrombi. Administration of test drugs began 15 min after coronary occlusion was confirmed. YM866 (0.05 mg/kg) was administered by a single intravenous bolus injection, and alteplase (total dose: 0.38 mg/kg) was administered by intravenous bolus injection (0.04 mg/kg) followed by infusion (0.34 mg/kg/hr for 1 hr). Reperfusion was confirmed by observations for up to 60 min after the start of test drug administration. Each point indicates the value of each animal and — indicates the median. Black symbols indicate reperfused animals and white symbols indicate non-reperfused animals. *P < 0.05: a significant difference from the vehicle control group (Steel-Dwass test). N.S. indicates no significant difference between the groups (Steel-Dwass test).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Vehicle</th>
<th>YM866</th>
<th>Alteplase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reperfusion rate</td>
<td>0 / 5</td>
<td>5 / 5</td>
<td>5 / 5</td>
</tr>
</tbody>
</table>

Fig. 2. Effects of YM866 and alteplase on left ventricular ejection fraction in dogs with induced coronary artery thrombi. Administration of test drugs began 15 min after coronary occlusion was confirmed. YM866 (0.05 mg/kg) was administered by a single intravenous bolus injection and alteplase (total dose: 0.38 mg/kg) was administered by intravenous bolus injection (0.04 mg/kg) followed by infusion (0.34 mg/kg/hr for 1 hr). △: sham-operated, ○: vehicle, ●: YM866, ◆: alteplase. Each value represents the mean ± S.E. from 5 animals. *P < 0.05, **P < 0.01: a significant difference from the sham-operated control group (Tukey's test).
Myocardial infarct area

Anterior transmural myocardial infarction was observed in the vehicle control group. The ratio of myocardial infarct area in left ventricular myocardium area was 18.2±4.2% in the vehicle control group (Fig. 3: a and d). No infarct was observed in the sham-operated control group. Only slight myocardial infarct areas were observed in the YM866 (0.05 mg/kg, i.v.)- and alteplase (0.38 mg/kg, i.v.)-administered groups. The ratio of these infarct areas in left ventricular myocardium were 0.6±0.5% for YM866 and 0.8±0.5% for alteplase (Fig. 3: b, c and d). No difference in the ratio of infarct area was observed between YM866- and alteplase-administered groups.

DISCUSSION

Thrombi induced by copper coil-insertion into canine coronary arteries have generally been recognized as useful models of acute myocardial infarction to evaluate the efficacy of thrombolytic agents (9, 22–24).

This is because these induced thrombi, much like many that cause acute myocardial infarction in humans, was fibrin-rich (25). The canine model is also useful for evaluating drug effects on left ventricular function in the acute stage after thrombolysis (26). In the present study, the thrombolytic effect of YM866 and its effect on left ventricular function were examined after intravenous bolus injection in dogs with induced coronary artery

**Fig. 3.** Effects of YM866 and alteplase on myocardial infarct area in dogs with induced coronary artery thrombi. Administration of test drugs began 15 min after coronary occlusion was confirmed. YM866 (0.05 mg/kg) was administered by intravenous bolus injection, and alteplase (total dose: 0.38 mg/kg) was administered by intravenous bolus injection (0.04 mg/kg) followed by infusion (0.34 mg/kg/hr for 1 hr). The hearts were excised from sacrificed animals 4 hr after start of test drug administration. The heart slices were stained with TTC (2,3,5-triphenyl tetrazolium chloride). Pictured are representative slices from vehicle control (a), YM866 (b)- and alteplase (c)-administered groups. Myocardial infarct area was identified as the area that was not stained by TTC. The scale bar indicates 2 cm. d: Each value represents the mean ± S.E. from 5 animals. **P < 0.01:** a significant difference from the vehicle control group (Tukey's test). N.S. indicates no significant difference between the groups (Tukey's test).
thrombi.
YM866 administered by bolus injection rapidly reperfused occluded coronary arteries in all animals treated with drug. These results were in good agreement with results previously described by Kawasaki et al. (9). In human acute myocardial infarction, the efficacy of coronary artery recanalization with thrombolytic agents diminishes with time after the onset of the event (27), probably due to progressive cross-linking of the fibrin networks (28). Kawasaki et al. previously demonstrated that YM866 produced rapid recanalization of coronary artery occluded by fresh and aged thrombi when administered by bolus injection (9). This effect of YM866 is probably due to its pronounced affinity for fibrin and persistence in plasma (7–9).

It has been reported that the native t-PA alteplase reperfused occluded coronary arteries and preserved left ventricular function in patients with acute myocardial infarction (15–18). In those studies, alteplase was administered by infusion due to its extremely short half-life. In the present study, the thrombolytic effects of YM866 administered by bolus injection were nearly the same as those of alteplase administered by bolus injection followed by infusion. YM866 administered by bolus injection has shown thrombolytic activity 2–4 times as potent as that of alteplase administered by bolus injection (9). The greater efficacy of YM866 can partly be attributed to its persistence in plasma (7–9). A thrombolytic agent administered solely by bolus injection including YM866 may be more beneficial and convenient in emergency medicine. This is because management of infusion is more troublesome than that of bolus injection, introducing the possibility of mistakes in treatment. More importantly, the availability of a single injection treatment available to properly qualified personnel would greatly improve the survival rate in acute myocardial infarction.

YM866 as well as alteplase almost completely inhibited myocardial infarct development. The total time that the coronary artery was occluded was less than 25 min in the drug-administered group because these drugs were administered 15 min after coronary occlusion, which was confirmed, and they reperfused occluded coronary arteries within 10 min after administration. Thus, rapid reperfusion resulted in a significant reduction of infarct size. These present results are thought to correspond to a report of a study in dogs which stated that myocardial infarction does not develop for up to 15 min after coronary artery occlusion but begins developing about 30 min after occlusion (10). The inhibitory effect on myocardial infarction by YM866 may therefore be due to rapid coronary recanalization.

YM866 improved LVEF that had decreased by coronary artery occlusion. This result is similar to the effects of E6010 (26, 29), a modified t-PA like YM866. Left ventricular function in dogs deteriorates promptly after occlusion of the coronary artery, but recovers with rapid reperfusion of the occluded vessel (10–13). It has been previously demonstrated that the early use of thrombolytic treatment in patients with acute myocardial infarction salvages ischemic myocardial tissue and preserves left ventricular function (15, 30). Improvement of left ventricular function by YM866 is probably similarly due to rapid reperfusion of occluded coronary arteries which salvages ischemic myocardial tissue. YM866 may therefore be able to improve patient prognosis (2).

In conclusion, YM866 administered by a single intravenous bolus injection showed thrombolytic activity and improvement of left ventricular function in dogs with induced coronary artery thrombi. These effects were equal to the currently approved clinical treatment of alteplase administered by a combination regimen of intravenous bolus injection followed by infusion. These results suggest that a single bolus injection of YM866 may be an effective thrombolytic agent that can rapidly reperfuse occluded coronary arteries and preserve left ventricular function in patients with acute myocardial infarction.

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