Potentiation of Myocardial Salvage by Tissue Type Plasminogen Activator in Combination With a Thromboxane Synthetase Inhibitor in Ischemic Cat Myocardium

Allan M. Lefer, Richard Mentley, and Jian-zhong Sun

We studied the effects of a thrombolytic agent (t-PA) and a thromboxane synthetase inhibitor (CGS-13080) in a model of myocardial ischemia and reperfusion. Occlusion of the left anterior descending coronary artery for 2 hours followed by 4 hours of reperfusion in anesthetized cats results in a large washout of creatine kinase into the blood (32 ± 7 IU/mg protein) and an area of necrotic tissue comprising 52 ± 5% of the area at risk and 9 ± 0.6% of the left ventricle. Intravenous administration of t-PA (500 IU/kg • min) for 30 minutes alone at reperfusion or infusion of CGS-13080 (500 μg/kg • hr) had no effect on washout of creatine kinase or extent of necrotic tissue development. Administration of the same doses of both t-PA and CGS-13080 together markedly attenuated creatine kinase release to 10 ± 2 IU/mg protein (p<0.01) and reduced the area of necrotic tissue to 9 ± 2% of the area at risk and only 1.3 ± 0.3% of the left ventricle (p<0.001). No significant sustained effects of these agents were observed on mean arterial blood pressure, heart rate, or the pressure rate index in these experiments. Thus, t-PA and CGS-13080 exert synergistic effects in preserving myocardial integrity in cats subjected to acute myocardial ischemia followed by reperfusion. The mechanism of this beneficial effect does not appear to be via reduced myocardial oxygen demand, increased myocardial oxygen supply, or enhanced inhibition of thromboxane A₂ formation. The mechanism of this anti-ischemic effect is not clear but may involve a metabolic or a cytoprotective effect. (Circulation Research 1988;63:621–627)
Polyethylene catheters were inserted into the inferior vena cava via the left femoral vein for infusion of drugs or their vehicles and into the right femoral artery for measurement of mean arterial blood pressure (MABP) via a Statham P23AC pressure transducer (Gould, Cleveland, Ohio). Positive-pressure respiration was instituted with a Harvard Apparatus respirator (South Natick, Massachusetts) connected to a tracheal cannula, and changes in respiratory pressure were monitored via a Statham differential pressure transducer. A midsternal thoracotomy was performed, the pericardium was opened, and a 4-0 silk ligature was placed around the left anterior descending coronary artery (LAD) 8–10 mm from its origin. Standard lead III of the scalar electrocardiogram was used to determine heart rate (HR) and ST segment elevation. All variables were continuously monitored on a Beckman R411 oscillographic recorder (Fullerton, California). The pressure-rate index (PRI), an approximation of myocardial oxygen demand, was calculated as the product of MABP and HR divided by 1,000.

**Experimental Protocol**

After completing all surgical procedures, the cats were allowed to stabilize for 30 minutes. Baseline readings were taken, and the initial blood sample was drawn (designated time 0). Immediately thereafter, MI was produced by tightening the previously placed reversible ligature around the LAD. Infusion of CGS-13080 (500 μg/kg • hr) or its vehicle (i.e., 0.9% NaCl) was started 30 minutes following coronary artery occlusion at a rate of 0.3 ml/hr and continued for the duration of the experiment. Two hours after coronary artery occlusion, 400 μl of 2% lidocaine was injected intravenously to minimize the occurrence of postreinfusion life-threatening arrhythmias. At this time, a 30-minute infusion of 500 IU/kg • min of tissue plasminogen activator (t-PA) or its vehicle was started. The vehicle for the t-PA consisted of 0.16 M NaCl containing 4 mM sodium phosphate dibasic, 0.75 mM potassium biphosphate, 0.05% Tween 80, and 0.01% EDTA adjusted to pH 7.3. Tissue-type plasminogen activator derived from melanoma cells produced by recombinant DNA technology having an activity of 415,100 IU/mg protein and 20% in the two-chain form was employed in this study (CIBA-Geigy, Basle, Switzerland). The myocardium was then reperfused by opening the ligated LAD. All animals were then monitored for 4 hours. All variables were recorded every 20 minutes and blood samples were drawn hourly. This degree of blood sampling did not significantly change the final hematocrit (34 ± 3% to 32 ± 4%) in six cats in which this was studied. Animals were then divided into four groups: 1) MI + vehicle; 2) MI + t-PA (500 IU/kg • min) for 30 minutes starting at reperfusion; 3) MI + CGS-13080 (500 μg/kg • hr) starting 30 minutes following coronary artery ligation and continuing for the duration of the experiment; and 4) MI + t-PA + CGS-13080.

**Biochemical Analysis**

Arterial blood samples (2 ml) were drawn hourly from the right femoral artery catheter for measurement of plasma creatine kinase (CK) activity, TXB₂, and protein concentration. Blood was collected in polyethylene tubes containing 20 μl of 450 mM EDTA and 6 μl of 60 IU heparin sodium (Upjohn, Kalamazoo, Michigan; bovine lung, 10,000 U/ml). Samples were centrifuged at 2,000g and 4°C for 20 minutes, and the supernatant was removed for biochemical analysis.

### Table 1. Alterations in Mean Arterial Blood Pressure, Heart Rate, and Pressure-Rate Index During Myocardial Ischemia (0 to 2 Hours) and Reperfusion (2 to 6 Hours)

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MABP (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>120 ± 11</td>
<td>101 ± 10</td>
<td>120 ± 8</td>
<td>132 ± 8</td>
<td>137 ± 7</td>
<td>138 ± 6</td>
<td>136 ± 6</td>
</tr>
<tr>
<td>t-PA</td>
<td>122 ± 4</td>
<td>117 ± 6</td>
<td>126 ± 6</td>
<td>139 ± 8</td>
<td>137 ± 8</td>
<td>140 ± 6</td>
<td>144 ± 7</td>
</tr>
<tr>
<td>CGS-13080</td>
<td>118 ± 5</td>
<td>100 ± 11</td>
<td>105 ± 10</td>
<td>120 ± 6</td>
<td>130 ± 6</td>
<td>126 ± 6</td>
<td>128 ± 6</td>
</tr>
<tr>
<td>t-PA + CGS-13080</td>
<td>124 ± 3</td>
<td>114 ± 8</td>
<td>116 ± 6</td>
<td>127 ± 5</td>
<td>125 ± 6</td>
<td>124 ± 5</td>
<td>132 ± 5</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>198 ± 11</td>
<td>170 ± 12</td>
<td>178 ± 9</td>
<td>172 ± 10</td>
<td>174 ± 9</td>
<td>178 ± 11</td>
<td>172 ± 11</td>
</tr>
<tr>
<td>t-PA</td>
<td>192 ± 5</td>
<td>165 ± 11</td>
<td>152 ± 14</td>
<td>148 ± 10</td>
<td>150 ± 14</td>
<td>154 ± 12</td>
<td>160 ± 11</td>
</tr>
<tr>
<td>CGS-13080</td>
<td>180 ± 10</td>
<td>145 ± 20</td>
<td>142 ± 16</td>
<td>145 ± 11</td>
<td>154 ± 13</td>
<td>147 ± 12</td>
<td>142 ± 15</td>
</tr>
<tr>
<td>t-PA + CGS-13080</td>
<td>176 ± 8</td>
<td>150 ± 12</td>
<td>145 ± 12</td>
<td>148 ± 11</td>
<td>158 ± 12</td>
<td>158 ±14</td>
<td>163 ± 14</td>
</tr>
<tr>
<td><strong>PRI (mm Hg • beats/min x 10^-3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>23.9 ± 3.0</td>
<td>17.5 ± 2.7</td>
<td>21.4 ± 1.8</td>
<td>22.7 ± 2.0</td>
<td>23.8 ± 1.7</td>
<td>24.6 ± 1.8</td>
<td>23.2 ± 1.6</td>
</tr>
<tr>
<td>t-PA</td>
<td>23.5 ± 0.76</td>
<td>19.3 ± 1.3</td>
<td>19.0 ± 1.7</td>
<td>20.4 ± 1.6</td>
<td>20.4 ± 1.9</td>
<td>21.2 ± 1.4</td>
<td>22.8 ± 1.1</td>
</tr>
<tr>
<td>CGS-13080</td>
<td>21.4 ± 1.7</td>
<td>15.2 ± 3.5</td>
<td>15.3 ± 2.6</td>
<td>17.6 ± 2.0</td>
<td>20.1 ± 2.2</td>
<td>18.4 ± 1.5*</td>
<td>18.2 ± 1.9</td>
</tr>
<tr>
<td>t-PA + CGS-13080</td>
<td>21.9 ± 1.1</td>
<td>17.3 ± 2.2</td>
<td>17.0 ± 2.0</td>
<td>18.7 ± 1.3</td>
<td>19.2 ± 1.2*</td>
<td>19.4 ± 1.6</td>
<td>21.3 ± 1.6</td>
</tr>
</tbody>
</table>

Infusion of drugs or vehicle was started at 30 minutes. MABP, mean arterial blood pressure; HR, heart rate; PRI, pressure-rate index. *p<0.05 compared with Vehicle. Six cats were studied in each group.
biochemical assay. Plasma CK activity was measured using the method of Rosalki\textsuperscript{18} and plasma protein concentration was assayed using the biuret method.\textsuperscript{19} Concentrations of TXB\textsubscript{2}, the stable breakdown product of TXA\textsubscript{2}, was measured by specific radioimmunoassay by the method of Lewy et al.\textsuperscript{20}

Myocardial Tissue Analysis

At the end of the 6-hour experimental period, the ligature around the LAD was retied. The heart was rapidly excised and transferred to a perfusion apparatus that allows determination of the area at risk. The coronary vasculature was perfused with 0.5% Evans blue dye in 0.9% NaCl at room temperature through a cannula in the aorta. Perfusion pressure, determined by the height of the dye reservoir, was adjusted to match the mean arterial blood pressure of the animal according to the method of Darius et al.\textsuperscript{21} Each heart beat spontaneously and was perfused for five beats. Following perfusion, the right ventricular and atrial tissue and the great cardiac vessels were removed. The remaining left ventricular and interventricular septal tissue was sectioned transversely, parallel to the atrioventricular groove, into slices approximately 4-5 mm thick. The area at risk was negatively stained while the remaining myocardium was stained dark blue by the Evans blue dye. The area at risk was separated, sectioned into slices 1 mm thick, and placed in nitroblue tetrazolium dye (0.1% in Sorensen’s buffer) at 37°C for 10 minutes. The dye forms a blue formazan complex in the presence of coenzymes and dehydrogenases, thus delineating the necrotic tissue by negative staining.\textsuperscript{22} The necrotic and nonnecrotic regions of the area at risk were separated and, together with the nonischemic region, were dried for 48 hours at 80°C. The dry weight of each region was then determined. The results are expressed as the area at risk determined as percent of the total left ventricular mass, and the area of necrotic tissue computed as percent of the area at risk and as percent of the total left ventricular mass.

Statistical Analysis

All values in the text, figures, and tables are mean ± SEM. Comparison among groups was made using analysis of variance (ANOVA).

Results

Twenty-seven cats were used in this study. Of these, three died during reperfusion (i.e., two had been given the vehicle and one had received t-PA) alone. The remaining 24 cats were randomly distributed into the four experimental groups of six cats each.

Table 1 summarizes the hemodynamic findings in the four MI groups. All groups of cats initially exhibited a MABP of 118–124 mm Hg and HRs of 176–198 beats/min. There were no significant differences in MABP or HR initially among any of the four groups of cats. Moreover, although there were early changes (i.e., 20–40 minutes) in MABP, there were no significant differences at any of the hourly readings in MABP or BP in any of the groups. Thus, it is not surprising that there were essentially no significant changes in the PRI in the four groups of cats over the 6-hour observation period. Only the MI + CGS-13080 at 5 hours and the MI + t-PA + CGS-13080 at 4 hours exhibited a significant decrease from MI + vehicle (p<0.05). This lack of sustained changes in PRI suggests that neither t-PA nor CGS-13080 exert any physiologically meaningful effect on myocardial oxygen demand during myocardial ischemia or reperfusion.

Plasma CK activities were measured hourly during the course of the 6-hour experimental period. Figure 1 summarizes these results, and shows that a significant washout of CK into the circulating blood occurs following reperfusion in MI cats receiving only the vehicle. These cats experienced a marked CK washout as evidenced by an increase in CK of 16-fold at 3 hours (p<0.001). MI cats receiving either the thrombolytic agent t-PA or the thromboxane synthetase inhibitor CGS-13080 developed peak increases of 12-fold and eightfold, respectively, at 3 hours. However, these values were not significantly

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Time course of plasma creatine kinase (CK) activity in International Units per milligram protein. Coronary artery occlusion occurred at 0 minutes. All values are means, brackets indicate SEM for six cats in each group. *p<0.01 compared with myocardial ischemia (MI)+vehicle.}
\end{figure}
different from the MI + vehicle group. In contrast, MI cats receiving both t-PA and CGS-13080 developed only a fourfold increase in circulating CK activity at 3 hours. This value was significantly lower than that of the MI + vehicle group (p<0.01). Thus, the combination therapy significantly reduced the washout of CK following 2 hours of ischemia, presumably reflecting a reduced degree of cellular injury during myocardial ischemia.

The primary objective of this study was to ascertain the effects of t-PA and CGS-13080 on the degree of myocardial salvage of ischemic or necrotic tissue upon reperfusion following 2 hours of LAD occlusion. Toward this end, we measured the area at risk of the ischemic heart and the area of necrotic tissue expressed as a percentage of either the area at risk or of the total left ventricle. Figure 2 presents the area at risk data for the four groups of cats subjected to acute myocardial ischemia and reperfusion. All groups exhibited an area at risk representing 16–18% of the total volume of the left ventricle. None of these values for the four groups were significantly different from any of the other three groups of cats. Therefore, we can conclude that the extent of ischemic damage is relatively uniform in all four groups of cats subjected to ischemia and reperfusion.

The area of necrotic tissue expressed as percent of area at risk in the four groups of cats subjected to acute MI are presented in Figure 3. The untreated MI cats developed a severe degree of ischemic damage comprising 52% of the area at risk. Cats given either t-PA or CGS-13080 exhibited slightly reduced degrees of ischemic damage; however they were not significantly lower than that of MI cats receiving only the vehicle. In contrast, cats receiving both t-PA and CGS-13080 experienced a dramatic reduction in the area of necrotic tissue to only 9% of the area at risk (p<0.001), a salvage of approximately 82% of the degree of necrotic tissue occurring in the untreated cats (i.e., MI + vehicle group).

This remarkable degree of synergism between the thrombolytic agent t-PA and the anti-thromboxane agent, CGS-13080, in salvaging ischemic myocardial tissue, was also observed when the data were calculated using the area of necrotic tissue taken as a percentage of total left ventricular mass. Figure 4 summarizes these results. Untreated MI cats developed ischemic damage constituting 8.8% of the total left ventricle. Use of t-PA alone failed to significantly reduce this value. However, CGS-13080 treatment resulted in a small but significantly reduced degree of ischemic tissue (p<0.05) to 6.3% of the total left ventricle. This represents a salvage of 28%. In contrast to this moderate effect of CGS-13080 alone, administration of both t-PA and CGS-13080 markedly reduced the area of necrotic tissue to only 1.3%, a value highly significantly lower than...
Area of Necrotic Tissue (% of Total LV)

FIGURE 4. Degree of ischemic cardiac damage expressed as area of necrotic tissue referenced to percent of total left ventricular weight. Bar heights are means of six cats in each group ± SEM. The myocardial ischemia (MI)+ t-PA group was not significantly different from the MI+vehicle group. The MI+t-PA group exhibited a moderate reduction in ischemic damage (p<0.05), whereas the MI+t-PA and CGS-13080 group exhibited a marked cardioprotective effect (p<0.001).

Table 2. Plasma Thromboxane B2 Concentrations in Cats During Myocardial Ischemia Followed by Reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Arterial plasma thromboxane B2 concentrations (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI + vehicle</td>
<td>5</td>
<td>2.7±0.6*</td>
</tr>
<tr>
<td>MI + t-PA</td>
<td>4</td>
<td>2.6±0.8*</td>
</tr>
<tr>
<td>MI + CGS-13080</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>MI + t-PA + CGS-13080</td>
<td>4</td>
<td>ND</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. n, number of cats evaluated in each group. MI, myocardial ischemia. ND, <0.5 pmol/ml. *p<0.02 from initial values, which were <0.5 pmol/ml.

t-PA and TxA2 Inhibition in Myocardial Ischemia

Discussion

Our findings indicate that administration of a thromboxane synthetase inhibitor adjunctively with a thrombolytic agent (e.g., t-PA) can reduce the degree of injury to ischemic myocardial tissue in an ischemic and reperfused coronary vascular bed. Neither the thrombolytic agent (i.e., t-PA) nor the thromboxane synthetase inhibitor (i.e., CGS-13080) by themselves significantly attenuated the degree of necrotic tissue in the ischemic myocardium. Thus, there appears to be a synergistic effect between these two agents. Prior experiments in a cat model of MI without reperfusion indicated that CGS-13080 at 1 mg/kg/hr is necessary to achieve a cardioprotective effect. Also, prior studies in a model comparable to the present one indicate that 1,000 IU/kg of t-PA exerted a prominent cardioprotective effect, and that 500 IU/kg of t-PA exerted a moderate but significant anti-ischemic effect. Very little other data are available on cardioprotective actions of these agents in MI. The only other study involving the cardioprotective effects of t-PA focused on synergism of t-PA with superoxide dismutase in dogs. Higher doses of t-PA were required (i.e., 750,000 U/kg), and they were infused for 1 hour starting 15 minutes prior to reperfusion. Under these conditions, t-PA appeared to exert a direct cardioprotective effect; however, it also synergized with superoxide dismutase in salvaging ischemic myocardial tissue. Very recently, Mickelson et al. reported that CGS-13080, given adjunctively with streptokinase, more effectively maintained the patency of a thrombosed coronary artery, but there was no difference in infarct size between the two groups of dogs.

The use of cardioprotective adjunctive therapy with thrombolysis is an interesting and important concept since there is such a high restenosis rate following thrombolysis. Coronary venous TXB2 concentrations are known to increase following acute myocardial ischemia in cats within 30 minutes of the onset of ischemia, in dogs 5 minutes following the onset of ischemia, and in patients with documented myocardial infarction within 5 minutes after the onset of chest pain. Thus, activation of TXA2 formation, largely from platelets, is a consistent finding soon after the occurrence of myocardial ischemia.

Since TXA2 is known to be a potent inducer of platelet aggregation, a potent enhancer of membrane leakiness, and a potent constrictor of coronary vessels, TXA2 may contribute to the propagation of cellular injury in the ischemic myocardium. This is consistent with findings that either thromboxane synthetase inhibitors or thromboxane receptor antagonists given early after the onset of myocardial ischemia, can retard ischemic restitution.
processes and result in less ischemic damage in experimental myocardial ischemia. In clinical studies, Walinsky et al. have shown that there is a marked elevation of coronary venous TXB₂ concentrations 5 minutes following successful thrombolysis with streptokinase. Thus, thrombolysis effectively washes out thromboxane metabolites that are trapped in the circulation supplying the ischemic region. Upon successful reperfusion, this thromboxane is washed downstream into the coronary microvasculature where it may exacerbate tissue injury. In fact, the recovered TXB₂ in coronary venous blood following thrombolysis may actually underestimate the true amount of TXB₂ formed during ischemia since stenotic or thrombosed vessels may trap formed thromboxanes. This large washout of thromboxane metabolites upon thrombolysis has been confirmed in dogs using intracoronary administration of streptokinase. If such an effect were to occur, the mechanism for the synergism between t-PA and CGS-13080 is not via a more effective TXA₂ blockade.

The mechanism of the synergistic effect of CGS-13080 and t-PA on the ischemic myocardium is not totally clear at present. Neither agent exerts significant effects on either myocardial oxygen supply or demand. In this connection, neither t-PA nor CGS-13080 was found to alter cat coronary vascular tone. Moreover, neither agent acts as a systemic vasodilator, a negative inotropic or chronotropic agent, and neither agent altered mean arterial blood pressure, heart rate or the PRI in anesthetized cats in the present study. It is not known whether either CGS-13080 or t-PA exerts a myocardial cytoprotective effect as streptokinase is alleged to have. If such an effect were to occur, however, it could contribute to the beneficial effects on myocardial salvage observed in this study. The degree of myocardial protection achieved with t-PA and CGS-13080 is quite striking, and is greater than that obtained using the calcium channel blocker nisoldipine in this same model.

In summary, occlusion of the LAD coronary artery in cats for two hours followed by reperfusion for four hours results in a significant degree of necrotic myocardial tissue. Administration of the thrombolytic agent t-PA or the thromboxane synthetase inhibitor CGS-13080 alone did not exert a clear-cut degree of myocardial salvage. However, addition of both t-PA and CGS-13080 together markedly improved myocardial salvage to 82–85% of the area at risk. This anti-ischemic effect does not appear to be due to improvements in either myocardial oxygen supply or demand, or in more effective inhibition of thromboxane formation but apparently to metabolic or cytoprotective effects of t-PA and CGS-13080. Finally, this protective effect occurred at a dose of t-PA approximately 20–25% of the full thrombolytic dose of t-PA.

Acknowledgments

We gratefully acknowledge the technical assistance of Margaret Groh and Judith Komlosh during the course of these studies. We also thank Dr. Thomas M. Glenn of CIBA-Geigy Pharmaceutical Research, Summit, New Jersey, for the generous supply of CGS-13080, and Dr. M. Kuenzi of CIBA-Geigy, Basle, Switzerland, for the generous supply of t-PA.

References


KEY WORDS • plasma creatine kinase activity • pressure-rate index • cardioprotective effect • thromboxane B2 • area at risk
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