Myocardial Salvage by Reperfusion 12 Hours After Coronary Ligation in Dogs

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It is not clear why late reperfusion therapy in patients with acute myocardial infarction is effective. An investigation was carried out to see whether or not reperfusion conducted 12 h after coronary occlusion causes myocardial salvage in dogs. Coronary arteries were occluded in 11 mongrel dogs and a portion of the occlusion (late reperfusion area; LR area) reperfused 12 h later; the other part was left occluded (permanent occlusion area; PO area). The dogs were maintained for 4 weeks after reperfusion. Regional myocardial blood flow (Qm) was measured by the non-radioactive colored microsphere method. In both areas, the transmurality of necrosis was measured by triphenyl tetrazolium chloride staining, and the amount of viable myocardium and the extent of fibrosis was determined by Azan-Mallory staining. Qm decreased markedly after coronary occlusion to similar levels in both areas until 12 h. Qm transiently increased in the LR area only following reperfusion after 12 h. The transmurality of necrosis in the PO area was 83.8±10.5%, but that in the LR area was 58.7±21.3%, a significant decrease (p<0.01). In the outer layer, the amount of viable myocardium was significantly greater, and the extent of myocardial fibrosis was significantly less in the LR area. Evaluation in the same heart of dogs confirmed the myocardial salvage effects of late reperfusion (12 h after coronary occlusion). (Jpn Circ J 1998; 62: 294–298)

Key Words: Acute myocardial infarction; Reperfusion therapy; Myocardial salvage

C oronary thrombolytic (CT) therapy is one of the most useful treatments for acute myocardial infarction, significantly reducing the number of deaths in the acute stage. CT therapy performed within 6 h after the onset, salvages the myocardium in the infarct-related artery and prevents a decrease in left ventricular function, thereby reducing mortality. Thus, reperfusion while myocardial injury is still reversible, reperfusion has myocardial salvage effects. However, many recent clinical studies have shown a decrease in mortality even when CT therapy is performed more than 6 h after onset. Although late reperfusion therapy is often effective, the mechanism is unclear. We occluded the coronary arteries in dogs, then reperfused part of the occlusion after 12 h but left the other part occluded. Using this model, which allows accurate comparison of the extent of necrosis between the two areas, we determined the myocardial salvage effects of late reperfusion.

Methods

Myocardial Infarction in Dogs (Fig 1)

Eleven adult mongrel dogs (body weight 13.8±5.2 kg, mean±SD) were fixed in the right decubitus position on an X-ray table under anesthesia with intravenous pentobarbital (20 mg/kg). The chest was opened using a sterile technique under positive-pressure breathing with a tidal volume of 200–250 ml, respiration rate 30/min, and end expiratory pressure 5 cmH2O. Arterial blood gas analysis revealed pH 7.425±0.010, pO2 125±10 mmHg, pCO2 33±5 mmHg and base excess −1.3±0.3. The heart was exposed on a pericardial cradle. Fluoroscopy was used to guide a thermoligation catheter into the ascending aorta and to perform coronary angiography to confirm complete occlusion or reperfusion after coronary artery ligation or release, respectively. The left anterior descending coronary artery (LAD) was dissected approximately 0.5 cm downstream from the bifurcation of the 2nd diagonal artery (2nd DG) and was ligated with silk. The 2nd DG was dissected approximately 0.5 cm downstream from the branching area and was ligated with a rubber thread to prevent arterial wall injury at the time of releasing the ligation. An aliquot of 10 mg of lidocaine was injected as a bolus intravenously 10 min before ligation of the arteries to prevent ventricular fibrillation. The left atrial appendage was exposed, and the tip of an 8F multihole polyethylene catheter was inserted about 2.5 cm into the left atrium for the purpose of infusing non-radioactive colored microspheres (F-Z Trac, USA) and of injecting ice-cold 5% glucose solution to measure cardiac output. Because of the rich collateral circulation in dogs and marked individual differences in the degree of development, all arteries peripheral to the LAD, the 2nd DG, and the left circumflex coronary artery (LCX) were ligated. This was necessary in order to examine the effect of late reperfusion by antegrade flow, and to minimize the effect of perfusion by the collaterals. The electrocardiogram as well as aortic blood pressure were monitored. A thermoligation catheter was inserted into the ascending aorta under fluoroscopic control and cardiac output was measured using a thermoligation technique. After 12 h, ligation of the 2nd DG was released (late reperfusion...
Measurement of Regional Myocardial Blood Flow (Q_m) and Preparation of Resected Myocardial Specimens

Q_m was measured using non-radioactive colored microspheres before as well as immediately (about 5 sec), 3 h, and 12 h after ligation of the LAD and 5 min, 1 h, and 4 weeks after release of the ligation of the 2nd DG alone (total, 7 times). The details of Q_m measurement using non-radioactive colored microspheres have been described elsewhere. In brief, we injected into the left atrium 4x10^6 microspheres diluted with 10 ml of 13.3% glucose solution followed by flushing of the catheter with 10 ml of 5% glucose solution within 2-3 sec. We used 7 differently colored microspheres to determine Q_m 7 times. Following measurement after 4 weeks, the heart was resected. The entire heart weighed 97.6±18.3 g, and the left ventricle weighed 55.2±9.2 g. In the resected heart, the middle area between the apex and the branching site of the 2nd DG from the LAD was cross-sectioned into a 5-mm slice (middle slice). In addition, 1-cm areas on both the base side and the apex side of this middle slice were cross-sectioned (upstream slice and downstream slice, respectively). Thus, 3 slices were prepared. The midline between the arrangement of the 2nd DG and that of the LAD was drawn, and the area from the 2nd DG to this line was defined as the LR area, and that from the midline to the LAD as the PO area. Each of the upstream and downstream slices in the LR and the PO areas was macroscopically divided into 3 parts (inner, middle, and outer layers). As a normal area, the myocardium at the lateral base in the LCX region of the atrioventricular groove was obtained for the measurement of Q_m using microspheres.

The total myocardium at the inner, middle, and outer layers of the myocardial slices in the LR area weighed 0.277±0.182, 0.181±0.124, and 0.227±0.086 g, respectively, and those in the PO area weighed 0.270±0.205, 0.182±0.107, and 0.240±0.104 g, respectively. These myocardial slices were all thermolysed with strong alkali, agitated, washed twice with a counting reagent (E-Z Trac) and centrifuged, and microspheres were recovered in the sediment fraction of the centrifugation tube. When non-radioactive colored microspheres are used, Q_m can be calculated using the same equation as that when radioactive microspheres are used. However, as loss of microspheres occurs with time in the infarcted portion, the flow cannot be measured as an absolute value. Therefore, the ratio to the preocclusion value was calculated, and this ratio in the infarcted portion to that in the non-infarcted area (% of non-infarcted) was obtained as Q_m:

\[ Q_m = \frac{Q_1}{Q_2} \]

where Q_1 is the number of microspheres infused before coronary occlusion and Q_2 is the number of microspheres infused at the time of measurement.

Q_m (% of non-infarcted) thus obtained was used as an index of changes in the regional myocardial blood flow.

Measurement of Transmurality of Myocardial Infarction

The middle slices were stained with triphenyltetrazolium chloride (TTC), and the area surrounded by the LAD and the 2nd DG was divided using its midline as the border into the PO and LR areas. In these areas, the percentage of the part that did not stain red with TTC was regarded as transmurality.

Measurement of Myocardium Score and Extent of Fibrosis

After the measurement of transmurality, the middle slices were stretched on a plate, avoiding distortion, and fixed in 10% formaldehyde solution for about 1 month, then Azan-Mallory staining was performed. The infarct portions of the specimens were photographed under a light microscope (×100). One photograph included a 0.685×0.456 mm tissue area. Approximately 70 photos were taken for the entire PO or LR area. On these photos, the extent of fibrosis was determined by 3 researchers who were not informed about whether the photo represented the LR or PO area. In 6 photos, the...
Table 1  Hemodynamics and Regional Myocardial Blood Flow (Qm: % of Non-Infarcted) in Permanent Occlusion (PO) and Late Reperfusion (LR) Areas

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion (control)</th>
<th>Coronary artery occlusion</th>
<th>After reperfusion only at LR area</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Immediately after (5 sec)</td>
<td>3 h</td>
<td>12 h</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td>153±27</td>
<td>158±25</td>
<td>171±28</td>
</tr>
<tr>
<td><strong>Ventricular extrasystole (%)</strong></td>
<td>0±0</td>
<td>1±1</td>
<td>10±10*</td>
</tr>
<tr>
<td><strong>Mean aortic pressure (mmHg)</strong></td>
<td>126±28</td>
<td>112±20</td>
<td>130±11</td>
</tr>
<tr>
<td><strong>Cardiac output (L/min)</strong></td>
<td>2.20±0.02</td>
<td>2.17±0.8</td>
<td>1.76±0.48</td>
</tr>
</tbody>
</table>

| Inner layer | PO | 100 | 9.4±9.3 | 11.1±9.7 | 9.4±7.2 | 13.7±8.8 | 17.8±12.3 |
| LR | 100 | 6.7±4.5 | 9.7±7.6 | 7.1±7.4 | 5.3±4.0** | 26.7±21.2 |

| Middle Layer | PO | 100 | 6.7±6.1 | 12.3±9.4 | 10.3±7.0 | 19.4±17.1 | 25.7±16.5 |
| LR | 100 | 6.1±4.0 | 14.3±13.8 | 7.9±6.2 | 49.7±30.7** | 29.2±24.5 |

| Outer Layer | PO | 100 | 11.7±6.9 | 20.3±18.9 | 16.3±12.9 | 25.5±20.8 | 52.1±41.7 |
| LR | 100 | 12.0±8.8 | 19.3±12.2 | 17.2±10.2 | 82.8±38.5** | 65.3±43.3 |

Mean±SD. Ventricle extrasystole (% of total heart beat). Difference from the control. *p<0.05, **p<0.01.

PO, permanent occlusion; LR, late reperfusion. Difference between the PO and LR. **p<0.01.

Table 2 The Amount of Viable Myocardium (Myocardium Score) and Extent of Fibrosis at the Permanent Occlusion (PO) and Late Reperfusion (LR) Areas

<table>
<thead>
<tr>
<th>Myocardium score (0–100%)</th>
<th>Extent of fibrosis (0–5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner layer</td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td>14.5±23.8</td>
</tr>
<tr>
<td>LR</td>
<td>33.8±26.0</td>
</tr>
<tr>
<td>Middle Layer</td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td>16.5±25.7</td>
</tr>
<tr>
<td>LR</td>
<td>40.3±31.9</td>
</tr>
<tr>
<td>Outer Layer</td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td>32.4±21.6</td>
</tr>
<tr>
<td>LR</td>
<td>71.4±13.7**</td>
</tr>
</tbody>
</table>

Mean±SD. PO, permanent occlusion; LR, late reperfusion; Difference between the PO and LR areas. *p<0.05, **p<0.01.

values among 3 researchers varied and the mean value of 3 was regarded as the value in each area. For determination of the myocardium score, a transparent film with a lattice (10×10 lines) covering the entire photo was superimposed on each photo. The proportion of viable myocardium was calculated from a total of 100 crossings on the lattice. Viable myocardium was defined as cardiac muscle with cross-striations. Fibrotic tissue was stained blue by Azan – Mallory staining. The extent of fibrosis was macroscopically graded from 0 (red color in the entire myocardium, normal) to 5 (fibrosis in the entire myocardium). This grade was considered to be the extent of fibrosis.

Statistical Analysis

Values are expressed as means±standard deviation. Differences in the mean value between the PO and the LR areas were analyzed by paired t-test. The time course changes in hemodynamics after coronary occlusion were evaluated by ANOVA and Scheffé’s multiple comparisons test. p values <0.05 were considered significant.

Results

Hemodynamic Changes

Heart rate was gradually increased after coronary occlusion accompanied by a fall in aortic pressure or cardiac output (Table 1). Ventricle extrasystoles appeared after coronary occlusion, increased gradually toward 12 h, and were almost absent at 4 weeks.

Serial Changes in Qm

Qm decreased markedly to 6.1–12.0% (% of non-infarcted) immediately after coronary occlusion, but gradually recovered during the subsequent 12-h period (Table 1). These changes were similar in the LR and the PO areas. Five min after reperfusion of the 2nd DG, Qm increased markedly, reaching 82.8±38.5% in the outer layer. After 4 weeks, Qm was slightly higher in the LR area than in the PO area.

Transmurality of Myocardial Infarction

TTC staining revealed 100% transmurality of myocardial infarction in both the LR and the PO areas in 1 animal, but a lower percentage in the LR area (58.7±21.3%) than in the PO area (83.8±10.5%) in the other animals. Transmurality of myocardial infarction was significantly less in the LR area (p<0.01).

The Myocardium Score and the Extent of Fibrosis

The myocardium score was consistently high in the LR area than in the PO area, and the difference was significant in the outer layer. The extent of fibrosis in each layer (outer, middle, inner) was less in the LR than in the PO area with significant differences in the outer layer (p<0.01) and inner layer (p<0.05).

Discussion

In this study, reperfusion even 12 h after coronary occlusion significantly decreased the transmurality of myocardial infarction. In the outer layer, the myocardium score increased significantly and the extent of fibrosis decreased significantly. These results show a certain degree of myocardial salvage by reperfusion even 12 h after occlusion and provide important data for clarifying the mechanism of the clinical effectiveness of late reperfusion therapy.

Late Reperfusion and Myocardial Salvage

In our previous study, reperfusion 7 h after coronary occlusion in dogs reduced the infarct size considerably.
In 1977, Reimer et al. evaluated in detail the process of completion of myocardial necrosis after ligation of the canine coronary artery. They reported that 62% of the myocardium in the risk area was salvaged by reperfusion after 40 min, but only 29% was salvaged by reperfusion after 6 h. Based on their results, significant salvage cannot be expected by reperfusion after 12 h.

Although experimental studies have suggested the failure of late reperfusion to salvage myocardium, many clinical studies have reported its effectiveness. The mechanism of this effectiveness has been suggested to be inhibition of left ventricular enlargement and remodeling, suppression of arrhythmias, promotion of the healing process of necrotic myocardium or increase in stiffness. Many of these studies cited the results in rats reported by Hochmann and Choe as a basis for using these mechanisms. Hochmann and Choe did not observe myocardial salvage but found inhibition of thinning and expansion of the infarct area in rats 2 h after reperfusion had been performed in that area. Similar findings were reported in rats by Hale and Klener and in pigs by Althaus et al. However, there are fewer collateral vessels and myocardial necrosis progresses more rapidly in rats and pigs than in dogs. The heart of the dog is more similar to the human heart than that of the pig in the development of collaterals. As late reperfusion was aimed originally at myocardial salvage based on the time lag until the wavefront of myocardial necrosis reaches the tissue from the inner to the outer layers, the use of rats or pigs that show rapid myocardial necrosis and a short lag may not have been appropriate.

Effects of Late Reperfusion

The results of this study are inconsistent with previous clinical or experimental studies that showed the absence of myocardial salvage effects of late reperfusion. In our previous study, the blood flow was only slightly decreased at the epicardial rim by coronary occlusion in dogs. Five sec after coronary occlusion, the blood flow was relatively large (62.3±7.1%) at a depth of 0–10% from the epicardial surface, but decreased markedly (32.9±9.4%) at a depth of 10–20%, and further decreased (18.2±2.7%) at a depth of 20–40%. In an experimental study by Hearse et al., tolerable ischemia was observed when blood flow decreased to 40–50% and fell into critical ischemia when Qm was 20–40%. These findings indicate that the viable myocardium remains at a depth of 0–10% even after complete occlusion, and critical ischemia at a depth of 10–20%. Therefore, it is speculated that myocardial salvage occurs in these areas even when reperfusion was considerably delayed. Assuming that a part of the myocardium, such as the epicardial rim, does not undergo necrosis, the myocardium on its inner side may not undergo necrosis, but may lose contractile force because of reduction in Qm and the development of hibernation. In these layers, late reperfusion may induce release of hibernation and the resulting recovery in myocardial contractility may prevent infarct expansion. The decrease in fibrosis in the infarct portion and the myocardial salvage observed after reperfusion in this study appear to play important roles in the inhibition of left ventricular enlargement and remodeling, and these roles may be associated with the effects of late perfusion.

In patients, infarct-related coronary arteries are either occluded or reperfused; in contrast, in the present experimental model the PO and the LR areas are present in a single heart. Coronary reperfusion in the LR area may elicit some changes to the PO area and vice versa. Accordingly, reperfusion of the LAD might not be exactly same as that of the 2nd DG. On the other hand, production of the PO and the LR areas in the same heart excluded individual differences among the dogs. Furthermore, it should be determined whether the LAD and the 2nd DG are similarly impaired by coronary occlusion. In our previous study, dogs were maintained after ligation of the LAD, and Qm, the infarct size, and an index of myocardial fibrosis were measured. The risk area on the left ventricular anterior wall was divided into 2 parts on the interventricular septum side and the free wall side (the 2nd DG side) to compare the two areas. We found no differences between the two areas. Moreover, in the present study, the decrease in Qm during coronary occlusion was similar in the PO and the LR areas. This suggests that the PO and the LR areas were similarly impaired by coronary occlusion in the present study.

Many of the previous studies did not demonstrate any measurable phenomenon of myocardial salvage by late reperfusion. In these studies, using animals such as dogs, the risk area was stained with TTC, and the infarct size was expressed as a percentage of the risk area. However, a considerable amount of viable myocardium was included in the area demonstrated to be infarcted by TTC staining. The percentage of viable myocardium among the infarcted areas varied, and thus the conventional method of measuring the infarct size by TTC staining may have problems. This study was characterized by evaluation of salvage by measurement of viable myocardium and the extent of fibrosis in the infarct portion. This may be why myocardial salvage was demonstrated in the present study.

Another important reason might have been the determination of Qm at the PO and the LR areas. In this study, the amount of viable myocardium as well as the extent of fibrosis were compared in the PO and the LR areas, where Qm was reduced equally during occlusion. In most previous studies, Qm was not determined in the reperfused or occluded areas. Thus, variations in the measured values may exist.

Calculation of risk area might be necessary in experimental animals such as dogs in which the collateral supply is plentiful and individual variation is great. Measurement of risk area by injecting dye into the coronary artery was not performed in this study in order to obtain unstained myocardial sections for histologic analysis of myocardial viability. Thus, we could not compare the infarct size by the conventional method. In the present study, however, Qm at occlusion was measured and the values at the PO and the LR during occlusion were the same, suggesting that the blood supply by collaterals was similar.

Clinical Implications

Marino et al. reported that reperfusion of infarct-related artery, even when too late to salvage a large amount of myocardium, prevents left ventricular enlargement and reduces the incidence of left ventricular aneurysms. The myocardial salvage effect demonstrated in this study may not always be detected by clinical
measurement of the infarct size using radioisotopes or of viability. In a previous study in our laboratory\(^{30}\) a group of dogs with permanent occlusion and another group with reperfusion 12 h after occlusion were investigated and demonstrated myocardial salvage by late reperfusion. In the present study, we confirmed the previous results using a different experimental design. The effects of late reperfusion appear to consist of myocardial salvage mainly at the outer layer and the release of hibernation. The myocardial salvage effect, although slight, increases myocardial tension in the left ventricular outer wall, inhibiting left ventricular expansion and eliminating electrical instability. On the other hand, the release of hibernation occurs early after reperfusion, which restores the myocardial contractile force. All of these effects may improve the prognosis in patients with late reperfusion.

References


