PROTECTION OF CORONARY REPERFUSION INJURY BY A CALCIUM ANTAGONIST

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The anterior descending branch of the left coronary artery (LAD) of the dog was ligated for 2 hours and thereafter reperfusion was continued for 1 hour. The regional myocardial blood flow (RMBF) during reperfusion was measured by a radioactive microsphere method. Whether a decrease in RMBF in the reperfused region could be prevented by injection of a calcium antagonist “diltiazem hydrochloride (diltiazem)” to LAD during reperfusion was simultaneously studied. RMBFs in ischemic epicardial (Epi) and endocardial (End) layers and End/Epi ratio 5 minutes after reperfusion were 1.87 ml/min/g, 1.75 ml/min/g and 1.06 respectively, showing an increase in all values compared to the values in normal region. RMBFs in the Epi and End and End/Epi ratio 60 minutes after reperfusion were 0.99 ml/min/g, 0.91 ml/min/g and 1.05 respectively, showing a decrease in all values without occurrence of transmural flow gradient. The decrease in RMBFs in the Epi and End 60 minutes after reperfusion could be prevented by diltiazem to show 1.68 ml/min/g and 1.42 ml/min/g respectively which were within normal limits. RMBFs in normal Epi which could be perfused by diltiazem were 1.31 and 1.70 ml/min/g before and after injection of diltiazem while those in normal End before and after injection of the drug were 1.27 and 1.46 ml/min/g respectively; RMBFs in both layers could be increased by diltiazem, with the significant increase in RMBF of normal Epi to show a decrease in the End/Epi ratio after injection of the drug. The conclusions are as follows: (1) Diltiazem increases RMBF in normal myocardium. (2) The gradual decrease in RMBF after reperfusion can be inhibited by the drug so that myocardial injury may be reduced. (3) A possibility exists that clinically the drug is a promising agent for early revascularization.

Key Words:
Regional myocardial blood flow
Calcium antagonist
Diltiazem hydrochloride
Radioactive microsphere
Reperfusion

(Received on December 12, 1979; Accepted on February 14, 1980)

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Japanese Circulation Journal Vol. 44, June 1980 461
mic region and its marginal cells and consequently RMBF decreases gradually, especially in the internal tunic. In addition to this, hemorrhage and necrosis occur in the myocardium, resulting in reperfusion injury. Furthermore, calcium ions are reported to participate in these changes. If the influx of calcium ions into the cells is inhibited by a calcium antagonist, the decrease in myocardial blood flow after reperfusion and reperfusion injury may be prevented. A calcium antagonist of a benzothiazepine derivative, diltiazem hydrochloride (diltiazem) having a strong coronary vasodilator action, has been proved to exert an excellent effect on angina pectoris, particularly on rest angina. The present study was designed to assess whether diltiazem can inhibit a decrease in RMBF of the ischemic region resulting from reperfusion.

MATERIALS AND METHODS

I. Animal preparation
Thirteen mongrel dogs weighing 10–18 kg with an average of 14.5 were anesthetized intravenously with pentobarbital sodium 30 mg/kg, and maintained under an Aika R-60 Respirator through which oxygen gas was administered at a rate of 1–3 l/min so as to keep the arterial Po2 over 80 mmHg. The chest was opened through the left fifth intercostal space to prepare the pericardial cradle, and the anterior descending branch of the left coronary artery (LAD) was detached 1–2 cm from the origin. A 25-gauge hypodermic needle with a silicone tube was inserted into LAD and was secured with Aron Alpha®. A physiological saline solution (saline) was infused continuously at a rate of 0.15 ml/min by a Holter pump. For the reperfusion study a snare ligature was attached to a part distal to the first diagonal branch of LAD and ischemia was produced in a considerable area of the left ventricular anterior wall. Reperfusion was accomplished by loosening the snare ligature. An 8F NIH catheter was inserted into the aortic origin through the right carotid artery to monitor the mean aortic pressure, whereas the other catheter for injecting microspheres was introduced into the left atrium. A catheter was also introduced into the right femoral artery to collect reference samples.

II. Measurement of RMBF
Radioactive microspheres of 15 μ in diameter (3MCo), previously well agitated by a Vortex mixer, were injected into the left atrium. The microspheres trapped in the tissue were measured as RMBF. About 2 × 10^6 microspheres were injected for one measurement of RMBF, and were flushed with 10 ml of saline immediately after injection. RMBF was measured 3 times per dog by using 3 kinds of radionuclides (141Ce, 51Cr, 85Sr). Along with injection of microspheres, blood samples of one-minute free flow from the femoral artery were collected in calibrated cylinders to measure RMBF per minute. RMBF measured by this procedure was compared with that by blood flow obtained at a constant rate in the other 5 dogs. As a result it was verified that there was agreement between RMBF obtained by both procedures. The heart was excised after injection of the last radioactive microspheres and cut at a right angle against LAD in round slices. The tissues from the reperfused ischemic region of the left ventricular free wall, border region adjacent to the ischemic region and normal region of the LAD perfusing area distal to the border region were used as myocardial tissue samples which were subsequently divided.
Coronary Reperfusion and Ca Antagonist

### TABLE II

<table>
<thead>
<tr>
<th>Region</th>
<th>Normal region</th>
<th>Border region</th>
<th>Ischemic region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>60 min</td>
<td>70 min</td>
</tr>
<tr>
<td><strong>Epi</strong></td>
<td>1.46 ± 0.32</td>
<td>1.15 ± 0.20</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td><strong>End</strong></td>
<td>1.44 ± 0.22</td>
<td>1.20 ± 0.24</td>
<td>1.29 ± 0.24</td>
</tr>
<tr>
<td><strong>End/Epi</strong></td>
<td>1.05 ± 0.07</td>
<td>1.01 ± 0.07</td>
<td>1.13 ± 0.09</td>
</tr>
</tbody>
</table>

Mean ± SEM; *: P < 0.05; The asterisks refer to statistical comparison between each observation and the one immediately preceding it. 
Epi = Epicardial layer; End = Endocardial layer

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into endocardial (End) and epicardial (Epi) layers. Each tissue sample was weighed and the radioactivity was measured by an Aloka 3-channel scintillation counter. Separation of the isotope energy was calculated by a YHP programmable desk calculator. RMBF was expressed as ml/min/g by admitting the measured blood to relate to the reference sample of the arterial blood.

\[
RMBF = \frac{\text{Blood flow of reference blood sample}}{\text{Count of reference blood sample}} \times \text{Count of tissue}
\]

During snare ligature of LAD and reperfusion, ventricular arrhythmia occurred frequently but the symptom could be depressed by 1 or 2 bolus injections of lidocaine in a dose of 2 mg/kg which has been reported to have no influence on the coronary blood flow.

### III. Experimental procedure

After stabilization of hemodynamics (more than 30 minutes after completion of surgical procedure in dogs) the control hemodynamics was measured. Thereafter the snare ligature was applied tightly on LAD to obstruct the coronary artery for 2 hours. The coronary reperfusion was done by loosing the snare ligature. RMBF of Group I (N = 7) was measured 5 minutes after reperfusion and saline was given directly into LAD at a rate of 0.15 ml/min by a Holter pump. The second RMBF was measured 60 minutes after reperfusion. Diltiazem was then given at a rate of 2 γ/kg/min for 3 minutes into LAD, and the third RMBF was measured 70 minutes after reperfusion. In Group II (N = 6) the first RMBF was measured 5 minutes after reperfusion, then diltiazem was injected via LAD at a rate of 0.5 γ/kg/min for 55 minutes, and the second RMBF was measured 60 minutes after reperfusion. Only the saline was given subsequently and the third RMBF was measured 10 minutes later. All values obtained were analyzed statistically by Student’s t-test for paired observation.

TABLE III  REGIONAL MYOCARDIAL BLOOD FLOW (ml/min/g) DURING CORONARY REPERFUSION IN GROUP II (N=6)

<table>
<thead>
<tr>
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<th>Normal region</th>
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<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>60 min</td>
<td>70 min</td>
</tr>
<tr>
<td>Epi</td>
<td>1.31 ± 0.12</td>
<td>1.70 ± 0.19</td>
<td>1.39 ± 0.15*</td>
</tr>
<tr>
<td>End</td>
<td>1.27 ± 0.11</td>
<td>1.46 ± 0.14</td>
<td>1.19 ± 0.09*</td>
</tr>
<tr>
<td>End/Epi</td>
<td>0.98 ± 0.07</td>
<td>0.87 ± 0.05</td>
<td>0.90 ± 0.10</td>
</tr>
</tbody>
</table>

Mean ± SEM; *: P < 0.05; **: P < 0.01
The asterisks refer to statistical comparison between each observation and the one immediately preceding it.
Epi = Epicardial layer; End = Endocardial layer

RESULTS

As shown in Table I, the changes in heart rate and mean aortic blood pressure after reperfusion were not significantly different between Group I and Group II, suggesting that diltiazem given via LAD had no influence on the systemic hemodynamics.

I. RMBF in Group I

Fig. 1 and Table II show RMBF in the ischemic region after the saline injection to the coronary artery for 60 minutes starting immediately after reperfusion and RMBF in the same region after subsequent diltiazem injection into the coronary artery. RMBF in the ischemic region increased 5 minutes after reperfusion compared to that in the normal region but it decreased significantly by 53% and 52% in Epi and End layers respectively 1 hour after reperfusion compared with RMBF 5 minutes after reperfusion. However, the End/Epi ratio showed no significant change. RMBFs in Epi and End increased by 13% and 24% respectively 70 minutes after reperfusion, i.e., after diltiazem injection into LAD, but the changes were not significant indicating no response of the blood flow to the drug. RMBF in Epi of the border region 5 minutes after reperfusion was similar to that of the normal region whereas RMBF in End increased by 38% compared with that of normal region. Therefore the End/Epi ratio increased significantly by 27% in comparison with that of the normal region (p < 0.05).

RMBF in Epi and End 70 minutes after reperfusion, i.e., after diltiazem injection, showed no significant change. The normal region of Group I was an area proximal to the site of a catheter which was inserted into LAD, so that the area had not been perfused by diltiazem. RMBF in Epi and End of the normal region and its End/Epi ratio showed no significant change even 5, 60 and 70 minutes after reperfusion.

II. RMBF in Group II

The results of Group II are indicated in Fig. 2 and Table III. RMBFs in Epi and End of the ischemic region were increased by 23% and 30%...
respectively 5 minutes after reperfusion, which were significantly higher than those of the normal region. The increased RMBFs in Epi and End of the ischemic region 60 minutes after reperfusion did not decrease significantly compared to those 5 minutes after reperfusion. RMBFs in Epi and End of the ischemic region 10 minutes after interruption of diltiazem, ie, 70 minutes after reperfusion, were decreased by 32% and 20% respectively. The End/Epi ratios 5 minutes after reperfusion and during diltiazem injection were 0.96 ± 0.22 and 0.83 ± 0.09 respectively, showing a slight decrease during injection of the drug. The ratios returned to the values of pre-reperfusion 10 minutes after cessation of diltiazem injection. RMBF in Epi of the border region increased after reperfusion but that in End was the same as that of the normal region. RMBF in Epi of the border region 60 minutes after reperfusion, ie, just after injection of the drug, remained unchanged compared to that 5 minutes after reperfusion while that in End increased. The End/Epi ratio increased accordingly. RMBFs in Epi and End of the border region 10 minutes after cessation of diltiazem injection were decreased by 19% and 23% respectively, suggesting the effect of diltiazem.

The normal region in Group II was the myocardial area adjacent to the border region, which could be perfused by diltiazem. There was no appreciable change in RMBF of the normal region 5 minutes after reperfusion, but the values in Epi and End 60 minutes after reperfusion, that is, during infusion of diltiazem were increased 30% and 15% respectively. Therefore, the End/Epi ratio decreased. RMBFs in Epi and End 10 minutes after the end of dilatiazem were decreased by 18% and 8% respectively, revealing that RMBF in Epi was mainly increased by diltiazem.

DISCUSSION

Reperfusion in the ischemic myocardium causes intracellular and intercellular edema which leads to gradual increase in coronary vascular resistance and in extreme case 'no reflow' phenomenon appears. Tissue injury and hemorrhage develop additionally in the reperfused region. In this sense, performance of bypass operation after the onset of acute myocardial infarction is reported to elicit the development of arrhythmia or spread of myocardial necrosis. The present study proved the possibility that the decrease in RMBF after reperfusion can be prevented by a calcium antagonist 'diltiazem'. The influx of calcium ions into cells is needed for the development of myocardial tissue injury due to reperfusion and the positive myocardial scintigram by Tc-pyrophosphate indicates the necessity of accumulation of calcium ions in cells for the formation of myocardial tissue injury. It is reasonable to assume that the influx of calcium ions into cells can be prevented by a calcium-blocking agent resulting in protection against myocardial injury. Parker et al. described that RMBF was decreased by 50% and 29% in Epi and End respectively 4 hours after reperfusion, which suggested the appearance of an epicardium-favoring transmural flow gradient. In our study, the transmural flow gradient was not found after reperfusion and the decrease in RMBF was nearly equal in both Epi and End to show an increase in coronary vascular resistance. Although diltiazem was injected to the coronary artery 1 hour after reperfusion, the coronary vasodilation was not significant. This was suggestive of the organic change in the coronary vessel. Therefore the calcium antagonist should be administered immediately after reperfusion in order to prevent the injury. According to the study by Parker et al., a transmural flow gradient increased between 2 and 4 hours after reperfusion. On the basis of the result, a short time (60 minutes in our study) of reperfusion seems to underlie one of the reasons for no appearance of a transmural flow gradient in our study. Recently Christlieb et al. reported in abstract form almost the same results as ours with the use of nifedipine instead of diltiazem hydrochloride. They showed that nifedipine completely preserved stroke work index and left ventricular dP/dt and an injured left ventricular volume measured morphometrically.

In the present study it was shown that the increase in coronary vascular resistance could be inhibited by injection of a calcium antagonist immediately after reperfusion. RMBF in the normal region being perfused by LAD was increased by injection of diltiazem, particularly the blood flow in Epi was increased to show a decrease in End/Epi ratio. On the other hand, it is known that a calcium antagonist has a dilating action on large coronary arteries and therefore the steal phenomenon hardly occurs. The inhibitory action of diltiazem on the increased coronary vascular resistance resulting

*Japanese Circulation Journal Vol. 44, June 1980*
from reperfusion may be attributed to the coronary vasodilation on the one hand and to the disturbance of calcium influx in cells which leads to protection against the onset of tissue injury on the other. Two possibilities were verified as the clinical significance of diltiazem in this study; when the drug is used immediately after the onset of myocardial infarct, the size of infarct is reduced in addition to expansion of indications to surgery of early revascularization, and in the field of internal medicine reperfusion injury due to thrombolytic agents may be prevented.

REFERENCES