Myocardial Blood Flow following Experimental Coronary Occlusion*

Effects of Diltiazem

Motomi Nakamura, M.D.; Yutaka Kikuchi, M.D.; Yutaka Senda, M.D.; Akira Yamada, M.D.; and Yasushi Koizaya, M.D.

To determine the effects of diltiazem, a new calcium antagonist, on myocardial ischemia, two experiments were performed. The results showed 1) the intravenous bolus injection of large doses of diltiazem reduced ST segment elevation in open-chest dogs with acute coronary occlusion. The regional myocardial blood flow in the control area, as well as moderately and mildly ischemic areas increased significantly after diltiazem.

The size of an acute myocardial infarction caused by acute coronary occlusion is an extremely important factor for determining the patient's prognosis. Reduction in myocardial infarct size will result if the severity of acute myocardial ischemia is improved by drug therapy.1 The calcium antagonist verapamil has been verified to improve the degree of ischemia following experimental acute myocardial infarction.2 Diltiazem, a calcium antagonist developed recently, is also effective clinically in preventing attacks of angina at rest and of effort angina,3,4 by dilating the collateral vessels.5 Weisshaar et al6 have demonstrated recently that diltiazem can minimize the consequences of acute myocardial ischemia, but its beneficial effects do not extend to all aspects of disturbed myocardial metabolism. We have confirmed that intravenous injection of diltiazem can increase the collateral flow after three weeks of coronary occlusion.7 Using dogs with acute coronary occlusion, we have studied the acute effect of a bolus injection of a relatively large dose of diltiazem under anesthetized open-chest conditions and the effect of 24-hour intravenous infusion of a small dose of the drug in the conscious state, particularly on myocardial blood flow. The results are presented in this article.

Material and Methods

Bolus Injection of Diltiazem (100 µg/kg) in Open-Chest Dogs

A part distal to the first diagonal branch of the left anterior descending coronary artery (LAD) of anesthetized open-chest mongrel dogs, weighing an average of 18 kg was completely occluded. Diltiazem, 100 µg/kg, was injected intravenously for 20 seconds 30 minutes after LAD occlusion. Similarly, saline solution was injected in control dogs. The group receiving diltiazem (11 dogs) and the group receiving saline solution (8 dogs) were compared.

Hemodynamic Changes: The aortic pressure became lowest one minute after injection of a bolus of diltiazem at a statistically significant level compared to the saline group; the mean aortic pressure was lowered to 97 mm Hg from the initial value of 118 mm Hg (p < 0.001). The lowered aortic pressure returned gradually to the preinjection level 15-20 minutes later. The mean heart rate before injection of the drug was 154 beats/min which decreased to 132 beats/min after injection of the drug. Lowering the heart rate in the diltiazem group was statistically significant compared to that of group receiving saline solution (p < 0.005). The lowered heart rate recovered gradually to 138 beats/min and 144 beats/min five and 20 minutes later, respectively, which was not significantly different from the heart rate of the group receiving saline solution.

PR intervals on the ECG in the group receiving saline solution were 0.09 sec before injection and remained unchanged after saline solution injection. On the other hand, the interval was prolonged to 0.11 sec (p < 0.001) one minute after injection of diltiazem. PR intervals 3, 5 and 10 minutes after injection of the drug were 0.11 (p < 0.001), 0.11 (p < 0.005) and 0.10 sec (p > 0.05), respectively. None of the experimental animals showed second degree or advanced atrioventricular block. Tension time indexes 1, 3 and 5 minutes after injection of the drug decreased significantly in all (p < 0.001 to 0.05) compared with the group receiving saline solution.

Effect of Diltiazem on Epicardial ST-segment Elevation: 2ST was obtained by the ECG tracing taken from the epicardium and the value, i.e., the degree of mean ST-segment elevation (2ST/2), was calculated by the division of 2ST with the derived number (2) indicating ST-segment elevation more than 5 mV. 2ST/2 began to decrease one minute after the drug. The decrease continued until the sacrifice of the dog 30 minutes after the drug, which was statistically significant compared to that in the group receiving saline solution during this period (p < 0.01). This may suggest that...
diltiazem can protect from ischemic injury for more than 30 minutes.

Effect of Diltiazem on Myocardial Blood Flow: The regional myocardial blood flow (MBF) was measured three times; it was measured before and after LAD occlusion and 1.5 minutes after a bolus injection of saline solution or diltiazem. MBF was measured using tracer microspheres 15 μm in diameter (described previously by us). Due to LAD occlusion, MBF of the LAD area decreased markedly. The decrease in MBF to the ischemic center was less than 10 percent of that in the nonischemic area (control area). The decrease in MBF was remarkable in the subendocardial layer, resulting in a significant decrease in subendocardial/subepicardial flow ratio (p < 0.005). MBF (1.2 ml/g/min) in the control area in the group receiving diltiazem increased about two times (2.14 ml/g/min) as high as that in the group receiving saline solution (Fig 1). In the subepicardial layer of moderate ischemic area where MBF decreased to 25-50 percent in the control area, MBF in the group receiving diltiazem increased significantly (p < 0.05) while that in dogs receiving saline solution remained unchanged. In mild ischemic area showing 51-75 percent myocardial blood flow in the control area, MBF in both subepicardial and subendocardial layers increased significantly after injection of diltiazem. While myocardial blood flow of severe ischemic area with less than 25 percent of MBF to the control area was increased to 0.28 ml/g/min (initial: 0.18 ml/g/min) after injection of diltiazem, (not statistically significant). In a region with less than 10 percent MBF to the control area, MBF remained unchanged even after injection of diltiazem.

The above results prove that a bolus injection of diltiazem at 100 μg/kg for 20 seconds in dogs with acute LAD occlusion (under anesthetized open chest condition) can induce an apparent decrease in aortic pressure as well as heart rate and tension time index and lowering of epicardial ST-segment elevation in ischemic area, and that although MBF is not increased in the central part of the ischemic area, MBF in the mild and moderate ischemic areas is increased by injection of diltiazem. Therefore, the decrease in myocardial oxygen consumption (MVO₂) or another mechanism rather than the increase in MBF may underlie the inhibition of ST-segment elevation.

Continuous Intravenous Infusion of a Small Dose of Diltiazem in Conscious (Closed Chest) Dogs

A snare type occluder was attached to the LAD in open-chest beagles weighing 7 to 13 kg while indwelling catheters remained in the left atrium, aorta and femoral vein. After closing the chests, the dogs were allowed to return to their preoperative state. The left anterior descending artery was acutely occluded under closed chest condition 5-7 days later. Seven dogs were started on intravenous infusion of a saline solution 15 minutes after LAD occlusion (saline group, group 1) while nine dogs were received intravenous diltiazem 20 μg/kg/min for 15 minutes and thereafter 10 μg/kg/min for 23 hours and 45 minutes until the dogs were sacrificed (diltiazem group, group 2). The following comparisons were made using Student's t-test. Changes within a group were analyzed by paired t-test.

Hemodynamics: Aortic pressures of both groups decreased gradually after occlusion of the LAD. Transient but significant elevation of left atrial pressure was noticed just after LAD occlusion, which stabilized thereafter. There was no significant hemodynamic difference between the two groups. The heart rate increased gradually after LAD occlusion. The precordial ECG tracings of group 2 revealed significant prolongation of PR intervals (0.11 sec to 0.13 sec) as in

Figure 1. Changes in regional myocardial blood flow (MBF) of subepicardial (left) and subendocardial (right) layers 30 min after LAD occlusion and 1.5 min after diltiazem (filled circle) or saline (open circle). Values are expressed as mean. The left ventricle was divided into five areas according to percentage of MBF in the nonischemic control area measured 15 min after LAD occlusion. 1) nonischemic area (≥100 percent); 2) nonischemic area (76-100 percent); 3) mild ischemia (51-75 percent); 4) moderate ischemia (25-50 percent); 5) severe ischemia (<25 percent). Statistical significance was obtained by paired test when compared with values in the previous stage. (* p < 0.05; ** p < 0.01; ***p < 0.005; ****p < 0.001)
experiment 1, a decrease in heart rate (3, 6 [p < 0.05] and
24 [p < 0.01] hours after occlusion), and significant de-
crease in frequency of premature ventricular contractions 22
and 24 hours after LAD occlusion (p < 0.01 at both
times).

Effect of Diltiazem on MBF: The left ventricle (free wall
and ventricular septum) was cut transversely into four layers
by the method of Falsetti et al8 and the layers were sectioned
radially into eight pieces each totaling 32 specimens. The
specimens were further sliced into subendocardial, mid-
and subepicardial layers. Thus, the left ventricle was divided
into a total of 96 segments. MBF of each segment was determined
by measuring the radioactivity of tracer microspheres of 9 μ

diameter as reported previously.8 Three determinations were
done before, 15 minutes and 24 hours (immediately before
sacrifice) after LAD occlusion by using three differently
labelled microspheres. All segments were fixed in formalin
and examined histologically after measurement of the radio-
activity to measure the size of myocardial necrosis.

Each segment was classified according to MBF values 15
minutes after LAD occlusion by size of ischemic center (<
0.10), severe ischemia (0.11-0.25), moderate ischemia (0.26-
0.50), mild ischemia (0.51-0.75) and normal area (> 0.76
ml/g/min).

RESULTS

Myocardial blood flow to the LAD area decreased
significantly following LAD occlusion, particularly
in the subendocardial layer, causing a significant
reduction of subendocardial/subepicardial flow
ratio.

The blood flow 24 hours after LAD occlusion was
compared between the two groups of dogs. As
shown in Figure 2, MBF to each segment in group 2
(diltiazem) tended to be high, but MBF of segments
from severe and moderate ischemic areas increased
significantly (p < 0.05 in both ischemic areas). MBF in all segments of the subendocardial layer,
however, was not increased significantly.

Because of tracer microsphere loss, MBF to seg-
ments of the ischemic center, severe and moderate
ischemia before LAD occlusion was about 10-15
percent as low as that in normal areas. However, no
significant difference in MBF existed in these areas
before and 15 minutes after LAD occlusion between
the two groups.

Results described above indicate that following
intravenous infusion of a small dose of diltiazem (10
μg/kg/min), ventricular extrasystole is reduced 24
hours later and MBF, especially the subepicardial
flow in severe and moderate ischemia, is increased
significantly without an accompanying obvious low-
ering of aortic pressure and elevation of the left
atrial pressure. Though PR intervals were prolonged
slightly, second degree atrioventricular block was
not found during infusion of diltiazem.

Tissue specimens were examined histologically to
clarify whether the increased MBF in the ischemic
area which was induced by infusion of diltiazem can
salvage myocardial cells from ischemic injury.

Necrosis

Subepicardial and subendocardial layers were
examined histologically from each tissue segment
used for measurement of MBF. The ratio (%) of
necrotic area of each segment, a plane parallel to the

Figure 2. MBF to total layer 24 hours after LAD occlusion in groups receiving saline (open
circle) and diltiazem (filled circle). Note significantly higher MBF in diltiazem group of
segments from severe and moderate ischemic areas.
cardiac surface at almost the central part of each segment, was examined against the total area calculated as percentage of necrosis. The percentage of necrosis in the control area was usually zero, whereas at the ischemic center (region with MBF of less than 0.1 ml/g/min) it was close to 100 percent. MBF (ml/g/min or percent of control MBF) was inversely proportional to percentage of necrosis. Mean values of coefficient of correlation in group 1 (saline) were \(-0.76 \pm 0.06\) (mean \(\pm SE\)) and \(-0.88 \pm 0.05\) in subepicardial and subendocardial layers respectively; in group 2 the ratio was \(-0.73 \pm 0.07\) and \(-0.90 \pm 0.02\) in subepicardial and subendocardial layers respectively. Figure 3 charts the correlation of a typical case. The percentage of necrosis at zero MBF was compared between both groups. The values in the subendocardial layer were \(88 \pm 8.8\) percent (mean \(\pm SE\)) and \(77.8 \pm 8.6\) percent in group 1 (saline) and group 2 respectively. The values in the subepicardial layer were \(64.5 \pm 10.5\) percent and \(52.9 \pm 11.7\) percent in the two groups, respectively. However, the values of group 2 were not statistically different from those of group 1 \((p > 0.05)\).

Assuming weights of subendocardial layer and subepicardial layer of the entire left ventricle to be 100 g each, the weight (g) of myocardial necrosis occupying the layers was determined as percentage of weight (\%) of necrotic myocardium and compared between the two groups. The values in the subepicardial layer were \(19.6 \pm 10.4\) percent (mean \(\pm SD\)) and \(13.2 \pm 9.2\) percent in groups 1 and 2, respectively, which were lower in group 2 than in the group receiving saline solution. However, the values were not statistically different between both groups. The values in the subendocardial layer were \(25.1 \pm 6.2\) percent and \(19.6 \pm 8.5\) percent in group 1 vs group 2, indicating a lower value in the latter without significant difference from the value in the former.

Each segment was classified, according to the ratio (\%) of MBF of each segment 15 minutes after LAD occlusion in MBF to the control area, by 0-10 percent, 11-25 percent, 26-50 percent, 51-75 percent, 76-100 percent and more than 100 percent of the control flow. Mean of \% necrosis was compared between the groups. Percentage of necrosis of group 2 in general tended to be small in all classified percentages of control flow level, which was statistically small in such segments showing especially 26-50 percent, 51-75 percent and 76-100 percent of the control flow in the subendocardial layer and 51-75 percent of the control flow in the subepicardial layer (Fig 4).

Figure 3. Typical example of correlation chart between level of MBF (ml/min/g) and extent of myocardial necrosis as \% necrosis area. Equations calculated are \(Y = -116X + 101\) \((r = -0.95)\) from the subendocardial layer (open circle), and \(Y = -114X + 88\) \((r = -0.90)\) from the subepicardial layer (filled circle). Note a highly significant negative correlation between them both in the subendocardial and subepicardial layers.

Figure 4. Percentage of necrosis of myocardial segment divided into six levels of MBF according to percentage of control MBF 15 min after LAD occlusion in groups receiving diltiazem (filled circle) and saline (open circle).
Conclusions

Intravenous infusion of diltiazem at a small dose level of 10 μg/kg/min could thus increase MBF of a circumscribed region at the ischemic center of acute myocardial infarct, particularly MBF of the subepicardial layer, and induce a tendency to a slight reduction of myocardial infarct size.

In general, it seems likely that augmentation of collateral flow before the establishment of necrosis of preservation of ATP by lowering MVo2 of ischemic myocardium is necessary to reduce myocardial infarct size after coronary occlusion. In contrast to a bolus injection of diltiazem at a high dose level, continuous intravenous infusion of the drug at a low dose level could not induce lowering of the tension time index. However, there is a possibility of decreasing myocardial oxygen demand because peripheral vascular resistance is decreased by diltiazem. If ischemic myocardial contractility can be depressed selectively by diltiazem, as proved by the study of verapamil,10 MVo2 in the ischemic myocardium may also be decreased, resulting in reduction of necrotic size. The consequent studies will be designed to evaluate the effect of diltiazem on ischemic myocardial contractility, to examine distribution of the drug given after the onset of LAD occlusion into the ischemic area, and to assess whether the collateral flow can actually be increased to salvage the tissue necrosis.

Probably due to the presence of many preformed collaterals in the subepicardial layer in dogs, MBF in the subepicardial layer could be increased significantly following intravenous infusion of diltiazem. Even though MBF in areas of severe and moderate ischemia was significantly increased after intravenous infusion of the drug at a low dose level, the increase in MBF in normal areas was not significant. The mechanism of this action is unknown. The small number of dogs used might be one of the causes.

Schaper et al11 reported that the collateral flow began to increase significantly in dogs after four to six hours of acute coronary occlusion and the collateral vessels showed a remarkably high resistance within 24 hours after occlusion. It remains unknown whether the increase in collateral flow within 24 hours after occlusion can be expected in drug therapy. It is unknown whether the increased subepicardial flow in areas of severe and moderate ischemia seen in the present study should be attributed to the increase in collateral flow or whether such should be attributed to the increase in blood flow to the normal myocardium. On the other hand, it is reported that subepicardial blood flow in the ischemic myocardium three weeks after coronary occlusion of dogs is increased significantly by therapy with diltiazem7 and both retrograde pressure and flow are also increased by action of the drug,8 which suggest an action of the drug to increase collateral flows. Further study is required to clarify whether the collateral flow can be increased by diltiazem administered early before myocardial infarction occurs due to coronary occlusion.

If the size of the myocardial infarction resulting from coronary occlusion in dogs could be assumed to be reduced by diltiazem, a similar effect on dogs might be observable in pigs having lesser preformed collaterals. Further study is needed.

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