LABORATORY INVESTIGATION

MYOCARDIAL INFARCTION

Comparative effects of nicardipine, a new calcium antagonist, on size of myocardial infarction after coronary artery occlusion in dogs

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ABSTRACT  To examine whether nicardipine, a dihydropyridine derivative, limits size of myocardial infarction, and to compare the protective effects of nicardipine administered before and early and late after coronary artery occlusion, 99mTc-labeled albumin microspheres were injected into the left atrium during 5 min temporary coronary artery occlusion to determine the extent of the hypoperfused zone (the area at risk). The coronary arteries were then reperfused for 45 min before 6 hr permanent coronary artery occlusion. Fifteen minutes before permanent occlusion, dogs were randomly assigned to (1) a control group (n = 11), (2) a pretreatment group (n = 9), which received at this point 10 µg/kg of nicardipine as a loading dose followed by a continuous infusion of 8 µg/kg/hr for 6 hr, (3) an early treatment group (n = 9), in which nicardipine treatment was initiated 15 min after occlusion, or (4) a late treatment group (n = 8), in which nicardipine administration was delayed for 3 hr. Six hours after coronary artery occlusion, the hearts were excised and the left ventricle of each was cut into 3 mm thick slices and stained with triphenyltetrazolium chloride. The extent of myocardial necrosis was measured by planimetry of the unstained areas. Thereafter, the same slices were autoradiographed and the extent of the hypoperfused zone was measured by planimetry of the "cold spot." The extent of the hypoperfused zone was identical among the four groups. In the control group, the ratio of the extent of myocardial necrosis to the extent of the hypoperfused zone was 95.8 ± 3.8% (mean ± SEM). However, it was significantly smaller in the pretreatment group (59.9 ± 13.3%, p < .05) and the early treatment group (49.0 ± 10.6%, p < .01) than in the control group. In the late treatment group, this value was not different from that in the control group (86.5 ± 7.1%). There was a close inverse correlation between reduction of infarct size and the extent of the hypoperfused zone in the pretreatment and early treatment groups. Thus, nicardipine administered before or early after coronary artery occlusion limited infarct size by 37% to 49%, whereas when administration was delayed for 3 hr infarct size was not reduced. Furthermore, nicardipine had more striking effects on the ischemic myocardium of dogs with small hypoperfused zones than on that of dogs with large hypoperfused zones.


IN 1969, Maroko et al. 1,2 demonstrated that myocardial infarction is a dynamic process and that the ultimate size of myocardial infarction can be altered by many interventions applied early after coronary artery occlusion when myocardial injury is still in the reversible phase. A variety of interventions have been shown in animal experiments to be effective in reducing the extent of myocardial necrosis after coronary artery occlusion. 3–5 Calcium antagonists that inhibit transmembrane calcium influx in cardiac and vascular smooth muscle dilate systemic and coronary vascular beds and depress cardiac contractility. 6 These effects would be expected to be beneficial to ischemic myocardium since they may increase coronary blood flow and decrease myocardial oxygen demand by reducing afterload, preload, and cardiac contractility. Furthermore, by inhibiting calcium uptake by ischemic myocardial cells calcium antagonists may exert a direct myocardial protective effect since calcium overload in the myocardium seems to play an important role in the development of irreversible injury. 7,8 Several studies have shown that calcium antagonists have beneficial effects on the ischemic myocardium, 9–21 although this protective effect is not consistent. 22–24 Nicardipine, a new
calcium antagonist synthesized by Takenaka and his colleagues, is a dihydropyridine derivative and a potent coronary and cerebral vasodilator (figure 1).25-33 Nicardipine has been shown to relax the isolated canine coronary arterial strip twice as much as nifedipine, six times as much as nitroglycerin, 10 times as much as verapamil, and 80 times as much as diltiazem.27 In anesthetized dogs nicardipine increases coronary blood flow to the same extent as nifedipine and three times as much as nitroglycerin, while it has much less effect on atrioventricular conduction and cardiac contractility when compared with verapamil and diltiazem.

Therefore, this study was performed to examine whether nicardipine has beneficial effects on the ischemic myocardium and to compare the protective effects of nicardipine administered before and early and late after coronary artery occlusion. In addition, the effects of nicardipine on hemodynamics and ventricular arrhythmias were studied.

Methods

Experimental preparation. Thirty-seven mongrel dogs of both sexes weighing 12 to 34 kg (average weight, 20.5 ± 0.8 kg) were initially anesthetized with intravenous pentobarbital sodium (25 mg/kg body weight) and supplemental anesthetic was administered as needed. Respiration with room air was maintained through an endotracheal tube with a volume-limited respirator (Shinano, Model SM-480-4, Tokyo, Japan). The heart was exposed by a left thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. Polyethylene catheters filled with normal saline were placed in the left carotid artery to record systemic arterial pressure, in the left jugular vein for drug administration, and in the left atrium for injection of radiolabeled albumin microspheres and for measurement of left atrial pressure. A Swan-Ganz catheter (No. 7F) was positioned via the right femoral vein in the pulmonary artery for measurement of cardiac output and central venous pressure. The coefficient of variation for cardiac output measurement with the thermodilution technique was less than 5%. Systemic arterial pressure and electrocardiographic lead II were recorded throughout the experiment on a multimonitor (Hewlett-Packard, Model 78342A, USA). The left anterior descending coronary artery proximal to its first diagonal branch was dissected free from the adjacent tissue and a ligature was passed underneath.

Experimental protocol. After recording baseline hemodynamic and electrocardiographic measurements the left anterior descending coronary artery was occluded with a Schwartz arterial clamp for 5 min. One minute after occlusion, 1.0 to 1.5 × 10⁶ human albumin microspheres (5M Co., St. Paul, MN) labeled with 5 mCi of ⁹⁹mTc were injected into the left atrium for the subsequent assessment of the hypoperfused zone of the myocardium.11, 34 The microspheres had been shaken vigorously by hand before injection to prevent aggregation. The injection of the microspheres lasted 10 sec and was followed by an injection of 10 ml of normal saline solution over a period of 10 sec. Five minutes later coronary reperfusion was performed by removing the Schwartz clamp and 45 min was allowed for the left ventricle to recover before the second coronary artery occlusion.

Thirty minutes after the start of coronary artery reperfusion, i.e., 15 min before the second, permanent, coronary artery occlusion, each dog was randomly assigned to one of four groups: (1) a control group of 11 dogs that received no drug, (2) a pretreatment group of nine dogs that received intravenous nicardipine beginning immediately after randomization until 6 hr after coronary occlusion, (3) an early treatment group of nine dogs that received nicardipine beginning 15 min after permanent coronary artery occlusion and continuing until 6 hr after occlusion, and (4) a late treatment group of eight dogs in which nicardipine treatment was initiated 3 hr after coronary artery occlusion until 6 hr after occlusion. In nicardipine-treated dogs a loading dose of 10 μg/kg was administered over 5 min followed by a continuous infusion of 8 μg/kg/hr by infusion pump (Natsune, Model KN-204, Tokyo, Japan). Fifteen minutes after randomization of dogs to groups, the left anterior descending coronary artery of each was again occluded, this time permanently with a silk suture at precisely the same site.

The reason that coronary artery occlusion was carried out twice at exactly the same site in each dog and that radiolabeled albumin microspheres were injected during the first temporary occlusion and before the start of nicardipine infusion was to exclude the possible effects of nicardipine on the preexisting collateral vessels and collateral blood flow. Nicardipine could have affected the extent of the hypoperfused zone in the pretreatment group had the albumin microspheres been injected into the left atrium after permanent coronary artery occlusion. We have measured the changes in radioactivity of arterial blood after injection of radiolabeled albumin microspheres into the left atrium, and ascertained that the radioactivity of arterial blood almost completely disappears 5 min after the first coronary artery occlusion, indicating that almost all radiolabeled albumin microspheres will be trapped in the peripheral vascular beds before coronary artery reperfusion is carried out.
Six hours after the second permanent coronary artery occlusion, all dogs were killed by intravenous administration of 20 meq of potassium chloride. No antiarrhythmic agents such as lidocaine were administered throughout the experiment because we were interested in the effect of nicardipine on ventricular arrhythmias after coronary artery occlusion, and also because lidocaine has been shown to reduce infarct size during experimental myocardial infarction.35

Postmortem tissue preparation. After the dogs were killed the heart of each was excised and the distance from the ostium of the coronary artery to the site of occlusion was measured. The free wall of the right ventricle, atria, valves, great vessels, and epicardial fat were trimmed off from the left ventricle. The left ventricle was frozen at −70°C and cut into 15 to 20, 3 mm thick slices parallel to the atrioventricular groove with an electric meat slicer.

Assessment of infarct size. To assess the extent of myocardial necrosis (infarct size), all slices were placed in a 1.5% solution of triphenyltetrazolium chloride (TTC) for 10 min at 37°C, which stained the normal myocardium bright red and rendered the infarcted myocardium pale yellow.36,37 The slices were then immersed in a 10% solution of formaldehyde to enhance the difference in color between the normal and infarcted myocardium. The areas of necrosis were traced onto transparent plastic sheets and measured by planimetry. Infarct size was expressed as a percent of the total volume of the left ventricle since all slices were of uniform thickness.

Assessment of the hypoperfused zone of the myocardium. All slices were autoradiographed by placing them on x-ray films (Cronex 4, Dupont, Tokyo, Japan) for 12 hr. The zone of hypoperfusion was a clearly demarcated area of decreased radiographic density (“cold spot”), whereas the areas with normal perfusion were “hot.”38,39 Soft x-ray exposures (32 kV, 10 mA, 135 cm, Mo filter) were made to delineate the outline of each slice. These were superimposed on the autoradiographs to allow visualization of the inner and outer arcs and borders of the hypoperfused zone (figure 2). The outline of each slice and the hypoperfused zone were traced onto plastic sheets and measured with planimetry. The extent of the hypoperfused zone was expressed as a percent of the total volume of the left ventricle.

In each dog the ratio of the extent of myocardial necrosis (infarct size or IS) to the extent of the hypoperfused zone (HZ) was determined by IS/HZ × 100.

The dogs were then classified according to the extent of the hypoperfused zone: those with hypoperfused zones of greater than 30% of the left ventricle and those with hypoperfused zones of 30% or less of the left ventricle. The effectiveness of treatment was assessed by calculating the infarct size/hypoperfused zone ratio for the treated dogs, dividing it by the same ratio for the corresponding class of control dogs, and subtracting the resulting number from 1.

Analysis of ventricular arrhythmias. Dogs were considered free from ventricular arrhythmias if they had less than two ventricular premature contractions per minute. More than three successive ventricular contractions constituted an episode of ventricular tachycardia. Since it has been shown that ventricular arrhythmias after coronary artery occlusion are not due to a single mechanism,39 we evaluated the effects of nicardipine on ventricular arrhythmias at different times after coronary occlusion. Furthermore, we compared the incidence of ventricular arrhythmias in nicardipine-treated and untreated groups at different time intervals. In these comparisons, from 0 to 15 min after coronary occlusion the dogs considered untreated included control and early and late treatment groups, while from 16 to 179 min after occlusion, control and late treatment groups were regarded as untreated.

Statistical analysis. All data are expressed as mean ± SEM. A Student’s unpaired t test was used for comparisons between two groups. The one-way analysis of variance was used to compare the effects of nicardipine on infarct size and the baseline characteristics among the four groups, followed by the Bonferroni method when analysis of variance indicated a significant difference among groups.39 The multigroup repeated measurements of hemodynamic parameters after coronary artery occlusion were analyzed by comparing the means of all measurements and the slopes among groups by one-way analysis of variance. The relationships between the extent of the hypoperfused zone and infarct size in each group and between the extent of the hypoperfused zone and the effectiveness of treatment were examined by regression analysis.40 Comparison of regression lines was performed by determining the significance of differences in residual variances, slopes, and elevations by F test.40 The χ2 test was used to evaluate the incidence of ventricular arrhythmias and mortality between groups. A probability value less than .05 was considered indicative of a significant difference.

Materials. All biochemicals and reagents used were commercially available. Nicardipine hydrochloride was kindly supplied by Dr. T. Takenaka of Yamanouchi Pharmaceutical Co., Tokyo, Japan.

Results

Data from three dogs (all in the pretreatment group) with hypoperfused zones smaller than 10% of the left ventricle were excluded from the analyses of the effects of nicardipine on the incidence of ventricular arrhythmias and myoccardial infarct size after coronary artery occlusion, because it has been demonstrated that both the occurrence of ventricular arrhythmias and the effectiveness of an intervention to reduce infarct size depend on the extent of the hypoperfused zone, and that the smaller the hypoperfused zone, the
less frequent the ventricular arrhythmia and the more effective the treatment.35, 41

Ventricular arrhythmias and mortality. Ventricular fibrillation did not occur during reperfusion after temporary (5 min) coronary artery occlusion. The incidence of ventricular arrhythmias and mortality during permanent coronary artery occlusion are listed in table 1. The incidence of ventricular premature contractions and ventricular tachycardia was not different among the four groups or between nicardipine-treated and untreated groups at different times after coronary artery occlusion. Mortality was similar in all groups.

Dogs that died from ventricular fibrillation were excluded from further study. Therefore, all analyses were based on the results in seven dogs each in control and early treatment groups and in six dogs each in pretreatment and late treatment groups.

Hemodynamics. Before group randomization, i.e., 15 min before coronary artery occlusion, hemodynamic variables such as heart rate, systolic, diastolic, and mean arterial pressures, mean left atrial pressure, cardiac output, total peripheral resistance, and rate-pressure product in the four groups did not differ significantly (table 2). The hemodynamic changes during coronary artery occlusion are shown in figure 3. In the control group, the repeated-measures analysis of variance indicated a significant variation in heart rate, mean arterial pressure, cardiac output, and total peripheral resistance after coronary artery occlusion, while rate-pressure product did not change. Analysis of regression coefficients showed a significant time trend of cardiac output toward decreasing values and of total peripheral resistance toward increasing values after coronary artery occlusion. In the pretreatment group, analysis for heart rate, rate-pressure product, and cardiac output showed a significant variation over time, with a significant trend toward decreasing values after coronary artery occlusion, while mean arterial pressure and total peripheral resistance only varied significantly over time. In the early treatment group, a significant variation of cardiac output and total peripheral resistance across time and a significant time trend of cardiac output toward decreasing values after coronary artery occlusion were noted, while heart rate, mean arterial pressure, and rate-pressure product were not altered. In the late treatment group there were significant overall changes in heart rate, rate-pressure product, cardiac output, and total peripheral resistance.

TABLE 1
Incidence of ventricular arrhythmias and mortality after coronary artery occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>VPCs</th>
<th>VT</th>
<th>n</th>
<th>VPCs</th>
<th>VT</th>
<th>n</th>
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<th>VT</th>
<th>n</th>
<th>Mortality</th>
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<tr>
<td>Late treatment</td>
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<td></td>
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</tbody>
</table>

VPCs = ventricular premature contractions; VT = ventricular tachycardia; n = number of dogs alive at the beginning of that period.

TABLE 2
Baseline characteristics of 26 dogs immediately before random group assignments

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Pretreatment (n = 6)</th>
<th>Early treatment (n = 7)</th>
<th>Late treatment (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>133 ± 8</td>
<td>137 ± 9</td>
<td>136 ± 8</td>
<td>144 ± 14</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>95 ± 4</td>
<td>104 ± 7</td>
<td>99 ± 4</td>
<td>97 ± 9</td>
</tr>
<tr>
<td>Rate-pressure product (×10³)</td>
<td>15.1 ± 1.1</td>
<td>17.2 ± 1.6</td>
<td>16.5 ± 1.0</td>
<td>17.4 ± 2.8</td>
</tr>
<tr>
<td>Mean left atrial pressure (mm Hg)</td>
<td>0.9 ± 0.5</td>
<td>0.8 ± 0.7</td>
<td>1.1 ± 2.3</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>2.77 ± 0.29</td>
<td>2.98 ± 0.38</td>
<td>3.26 ± 0.35</td>
<td>2.91 ± 0.23</td>
</tr>
<tr>
<td>Total peripheral resistance (dyne·sec·cm⁻²)</td>
<td>2852 ± 235</td>
<td>2933 ± 267</td>
<td>2614 ± 289</td>
<td>2751 ± 421</td>
</tr>
<tr>
<td>Occlusion distance (cm)</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Hypoperfused zone (%LV)</td>
<td>29.0 ± 2.2</td>
<td>25.5 ± 4.8</td>
<td>23.5 ± 2.9</td>
<td>31.0 ± 5.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
LV = left ventricle.
°Distance from the ostium to the site of occlusion.
Heart Rate
(beats/min)

Mean Arterial Pressure
(mmHg)

Heart Rate x Systolic Arterial Pressure
($\times10^3$)

Cardiac Output
(l/min)

Total Peripheral Resistance
($\times10^3$ dynes/sec-cm$^2$)

Time after Coronary Artery Occlusion (hrs)

FIGURE 3. The hemodynamic changes during coronary artery occlusion in the four groups. Open circles indicate control group (n = 7), closed circles pretreatment group (n = 6), closed squares early treatment group (n = 7), and closed triangles late treatment group (n = 6). Bars indicate the SEM. *p < .01, significant variation across time, †p < .05; ‡p < .01, significant time trend after coronary artery occlusion.

over time and a significant trend of cardiac output toward decreasing values after coronary artery occlusion, while mean arterial pressure did not change.

In spite of these hemodynamic changes within the four groups, the comparison of the means of all measurements and the slopes of hemodynamic parameters showed no significant overall differences or differences in trends among the four groups during coronary artery occlusion.

Effects on infarct size. The distance from the ostium of the left coronary artery to the site of occlusion was similar in dogs in the four groups (table 2). The extent of the hypoperfused zone of the myocardium after coronary artery occlusion varied greatly (from 12.5% to 48.3% of the left ventricle); however, the average in each group was identical statistically (table 2), indicating that all groups were comparable. The infarct size expressed as a percent of the left ventricle was 27.8 ± 2.6% (n = 7) in the control group, 17.9 ± 6.7% (n = 6) in the pretreatment group, 13.2 ± 3.7% (n = 7) in the early treatment group, and 27.1 ± 5.1% (n = 6) in the late treatment group. The ratio of the extent of myocardial necrosis to the extent of the hypoperfused zone was 95.8 ± 3.8% (n = 7) in the control group, 59.9 ± 13.3% (n = 6, p < 0.05 vs control) in the pretreatment group, 49.0 ± 10.6% (n = 7, p < 0.01 vs control) in the early treatment group, and 86.5 ± 7.1% (n = 6, NS vs control) in the late treatment group (figure 4). Furthermore, there was a close correlation between the extent of the hypoperfused zone (HZ) and the infarct size (IS) in each group (figure 5). In the control group, the regression line was expressed as IS = 1.07 × HZ − 3.19 (r = .92, p < .01). The linear regression lines for pretreatment and early treatment groups were shifted downward, indicating a smaller infarct size for the same extent of the hypoperfused zone. Comparison of regression lines showed that there was a significant difference in elevations of the regression lines for the pretreatment (p < .05) and early treatment (p < .01) groups and those of the
regression line for the control group. The regression line for the late treatment group did not differ from that for the control group. Thus, this analysis again demonstrated beneficial effects of nicardipine in pretreatment and early treatment groups.

With the use of a linear regression equation for the control group, we calculated the predicted infarct size for each dog in the pretreatment and early treatment groups in which nicardipine reduced the mean infarct size and compared these values with the actual infarct size (table 3). In 10 dogs the actual infarct size was at least 30% smaller than predicted, while three dogs (two in the pretreatment group and one in the early treatment group) had an actual infarct size greater than 80% of that predicted. There were no differences in hemodynamic changes during coronary artery occlusion between these three dogs in which nicardipine did not provide protection and the other 10 protected dogs. However, the unprotected dogs had significantly larger hypoperfused zones (i.e., 33.5%, 45.1%, and 34.4% of the left ventricle, respectively) than did the protected dogs (20.5 ± 1.8% of the left ventricle, p < .01).

We examined further the influence of the extent of the hypoperfused zone on the effectiveness of nicardipine in reducing infarct size in the pretreatment and early treatment groups. The ratio of the extent of myocardial necrosis to the extent of the hypoperfused zone was 83.3 ± 7.8% when the extent of the hypoperfused zone was more than 30% of the left ventricle (n = 4, p = NS vs respective controls) and 41.4 ± 8.1% when the hypoperfused zone was less than 30% of the left ventricle (n = 9, p < .01 vs respective controls).

**FIGURE 4.** Comparison of the ratio of the extent of myocardial necrosis (IS) to the extent of the hypoperfused zone (HZ) in control, pretreatment, early treatment, and late treatment groups. Bars indicate the SEM.

**FIGURE 5.** Relationship between the extent of the hypoperfused zone (HZ, on the abcissa) and infarct size (IS, on the ordinate) in the control group (open circles), pretreatment group (closed circles), early treatment group (closed squares), and late treatment group (closed triangles). LV = left ventricle.
TABLE 3  
Predicted and actual infarct size in pretreatment and early treatment groups

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Pretreatment group</th>
<th>Early treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted infarct size (%)</td>
<td>Actual infarct size (%)</td>
</tr>
<tr>
<td>1</td>
<td>12.3</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>13.7</td>
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<td>45.1</td>
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<td>6</td>
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<td>7</td>
<td>11.5</td>
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</tbody>
</table>

*Predicted infarct size was calculated from a linear regression equation for control group.

*Dog not protected by nicardipine.

When the effectiveness of treatment was assessed by examination of the reduction in the infarct size/hypoperfused zone ratio, reduction of infarct size was 13.3% ± 8.2% in dogs with hypoperfused zones of more than 30% of the left ventricle and 57.0% ± 8.5% in dogs with hypoperfused zones of less than 30% of the left ventricle (p < .05). Furthermore, there was a close inverse correlation between reduction in infarct size and the extent of the hypoperfused zone (figure 6): infarct size reduction (percent) = -2.69 hypoperfused zone (% of the left ventricle) + 109.24 (n = 13, r = -.83, p < .01). Thus, nicardipine had more striking effects on the ischemic myocardium in dogs with small hypoperfused zones than in dogs with large hypoperfused zones.

Discussion

Nicardipine, which was synthesized by Takenaka et al.,25 is a dihydropyridine derivative, 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-[2-(N-benzyl-N-methylamino)] ethyl ester 5-methyl ester hydrochloride (YC-93). It has a potent cerebral and coronary vasodilating action due to a calcium antagonistic mechanism.27, 30-33 Experimentally, nicardipine has been shown to relax the isolated coronary arterial strip and increase coronary blood flow.27 In the clinical setting, it has been used as an antihypertensive agent42 and is undergoing intense investigation as an antianginal agent.43 Nicardipine is not light-sensitive and does not decompose in solvent when exposed to ordinary light sources, in contrast with nifedipine, which is highly light-sensitive.

Effect of nicardipine after coronary artery occlusion.

Our results demonstrated that intravenous nicardipine administered before or early after coronary artery occlusion reduced the extent of myocardial necrosis by 37% to 49% in anesthetized, open-chest dogs subjected to a 6 hr coronary artery occlusion, while treatment with nicardipine initiated 3 hr after coronary occlusion had no detectable effect. The incidence of ventricular arrhythmias after coronary artery occlusion was not different in nicardipine-treated and control groups and therefore nicardipine does not appear to have antiarrhythmic properties. The dosage of nicardipine used in this study, i.e., a loading dose of 10 μg/kg over 5 min followed by a continuous infusion of 8 μg/kg/hr, did not cause significant differences in the time trends of hemodynamic parameters among the four groups during coronary artery occlusion.

Methodologic consideration.

In this investigation we used the TTC staining technique to determine the extent of myocardial necrosis.36, 37 Recently, several investigators have reported an excellent correlation between myocardial infarct size determined by the TTC technique and histologically42 or electron microscopically determined areas of myocardial necrosis49 and have concluded that the TTC technique is a reliable and practical means for localizing and quantifying

![FIGURE 6. Relationship between the extent of the hypoperfused zone (HZ, on the abscissa) and reduction in infarct size, i.e., the amount of myocardium salvaged by nicardipine (on the ordinate). Closed circles indicate pretreatment group (n = 6) and closed squares the early treatment group (n = 7). LV = left ventricle.](http://circ.ahajournals.org/content/120/20/S21/-/DC1/fig6.png)
myocardial necrosis, even during the early phase of myocardial infarction.

To assess the hypoperfused zone of the myocardium after coronary artery occlusion, we employed the autoradiographic technique.11, 34, 35 The advantage of our technique compared with the postmortem intracoronary injection of dyes46-48 or barium gels49, 50 is that radiolabeled albumin microspheres can be injected in vivo under physiologic hemodynamic conditions. Thus, we could assess the hypoperfused zone, taking into account both the reduction in coronary blood flow due to coronary occlusion itself and the coexisting collateral blood flow from the normal area to the one rendered hypoperfused. Furthermore, since the ratio of the extent of myocardial necrosis to the extent of the hypoperfused zone was determined, variability among individual dogs due to differences in the anatomy of the coronary arterial tree and in the degree of development of coexisting collateral blood vessels was minimized. The coefficient of variation for infarct size alone was 24.8% in the control group, while after correction for the extent of the hypoperfused zone it was 10.5%.

To determine the extent of the hypoperfused zone before administration of nicardipine in the pretreatment group, we performed coronary artery occlusion at exactly the same site twice in each dog and injected radiolabeled albumin microspheres during the first 5 min (temporary) coronary occlusion. Then 30 min of coronary artery reperfusion was carried out before randomization to allow the left ventricle to recover. The 5 min temporary coronary occlusion and 30 min of reperfusion were chosen because (1) almost all radiolabeled albumin microspheres can be trapped in the peripheral vascular beds during 5 min of coronary occlusion, which was confirmed by our observation that the radioactivity of the arterial blood was cleared by the end of temporary occlusion, and (2) it has been shown that abnormal contractile function of the myocardium induced by a 5 min coronary occlusion can be normalized by 30 min of reperfusion,51 which was also confirmed by our finding that any hemodynamic parameters obtained after 30 min of reperfusion were not significantly different from baseline values. The same procedures were also performed in control and early and late treatment groups, enabling us to quantify the extent of the hypoperfused zone before the start of drug therapy in all groups.

Influence of the extent of the hypoperfused zone on myocardial protection. In this investigation we excluded three dogs with hypoperfused zones of less than 10% of the left ventricle to prevent the overestimation of the beneficial effects of nicardipine. These dogs, which were pretreated with nicardipine and had hypoperfused zones of 9.0%, 8.3%, and 8.1% of the left ventricle, respectively, had marked reductions in infarct size, i.e., the infarct size/hypoperfused zone ratios were 24.8%, 0%, and 0%, respectively. Three unprotected dogs in the nicardipine-treated group had significantly larger hypoperfused zones than those in protected dogs. Furthermore, a close inverse correlation between the reduction in infarct size and the extent of the hypoperfused zone was found. Thus, the initial extent of the hypoperfused zone after coronary artery occlusion is an important factor in determining the effectiveness of nicardipine in reducing infarct size, which is in accordance with previous report.41 This can be explained by the better penetration of the drug and/or more collateral blood flow into smaller ischemic zones due to a higher surface/volume ratio.41, 52

Calcium antagonists for protection of ischemic myocardium. A large number of investigators have studied the protective effects of various calcium antagonists on the ischemic myocardium using a variety of experimental preparations and different end points.8-24 Most of the experimental studies with verapamil10-12 and diltiazem15-18 have reported beneficial effects on the ischemic myocardium, while some controversy remains regarding a dihydropyridine compound such as nifedipine.13, 14, 19-21, 23 Henry et al.13 showed less myocardial creatine kinase depletion from 24-hr-old infarcts in conscious dogs treated with nifedipine, while Melin et al.14 reported smaller infarct size in nifedipine-treated conscious dogs. In contrast, Geary et al.22 and Alp et al.20 failed to show beneficial effects of nifedipine in baboons, which are known to have a small native collateral network. Using the new dihydropyridine calcium antagonist nicardipine, however, Alp et al.20 reported a marked protective effect in anesthetized baboons subjected to 6 hr coronary artery occlusion. In their study, infarct size was determined with a nitroblue tetrazolium staining technique, and the vascular area at risk was determined with postmortem coronary arteriography. Although there are differences in the animals and in the techniques used to assess the area at risk for infarction between the present investigation and that of Alp et al., two studies have demonstrated the beneficial effects of nicardipine on the ischemic myocardium.

Influence of delay of administration of treatment on myocardial protection. Several studies have examined the time course over which myocardial cell death occurs after coronary artery occlusion.53-55 In anesthetized dogs, myocardial necrosis is almost complete by 3 to 6
hr after coronary occlusion and therefore little or no salvage could be achieved after 3 to 6 hr of ischemia.\textsuperscript{53}

Several investigators have studied the influence of delay in the application of interventions on their effectiveness in reducing infarct size.\textsuperscript{14, 56–58} Miura et al.\textsuperscript{56} and Rasmussen et al.\textsuperscript{57} have reported that propranolol administered before coronary occlusion reduced infarct size by 39% to 53%, whereas it was less effective when given 3 hr after occlusion, reducing infarct size by 16% to 28%. Melin et al.\textsuperscript{14} have shown that nifedipine exerted a protective effect even when treatment was delayed for 3 hr. Hillis et al.\textsuperscript{58} have reported that hyaluronidase given 20 min or 3 or 6 hr after coronary occlusion was effective in salvaging the ischemic myocardium, while when administered 9 hr after coronary occlusion the drug did not have any effect. Although in our study nicardipine administered 3 hr after coronary occlusion did not result in myocardial salvage, direct comparisons with these investigations are difficult for several reasons. First, Miura et al. did not assess the area at risk, which is a major determinant of infarct size.\textsuperscript{46, 55} Second, Rasmussen et al. quantified myocardial necrosis from histologic sections of transmural slices through the posterior papillary muscle, which does not necessarily reflect total left ventricular necrosis. Third, Melin et al.\textsuperscript{59} used conscious dogs, in which evolution of myocardial infarction is expected to be slower than in anesthetized dogs because of lower myocardial oxygen demand for a given reduction in myocardial blood flow. Finally, Hillis et al.\textsuperscript{58} used epicardial ST segment elevation and depletion of cardiac creatine kinase as indexes of ischemic area and infarct size.

**Mechanisms of action.** Mechanisms of myocardial salvage by nicardipine are unknown. One of the possible explanations is reduced myocardial oxygen demand due to decreases in afterload, preload, and contractility. However, there were no significant differences in the time trend of hemodynamics among the four groups. Therefore, it is unlikely that reduction in myocardial oxygen demand is the major mechanism of the marked beneficial effects of nicardipine.

Intravenous nicardipine in the dosage used in this study has been shown to increase collateral blood flow to the ischemic endocardium by 90% and to the ischemic epicardium by 32% when administered after coronary artery occlusion in anesthetized dogs. Furthermore, pretreatment with the same dosage of nicardipine reduced the extent of decrease in collateral flow to the ischemic region after coronary occlusion.\textsuperscript{*}

Thus, an increase in collateral flow appears to play at least a partial role in salvaging the ischemic myocardium with nicardipine.

Myocardial ischemia is characterized by a reduction in myocardial ATP stores, which may raise intracellular calcium ions by several mechanisms. The resultant increase in intracellular free calcium may be deleterious to the ischemic myocardium. It may activate calcium-activated ATPase, which augments ATP usage. Mitochondrial calcium overload may depress ATP production further. An increase in calcium may activate sarcoplasmic phospholipases and proteases such as calcium-activated neutral protease, which could impair the integrity of the cell membrane. Thus, nicardipine may exert a direct myocardial protective effect by interfering with calcium overload in the ischemic myocardium, retarding the ischemic process. Further studies will be required to clarify the role of calcium in ischemia and the exact mechanisms of the beneficial effects of nicardipine on ischemic myocardium.

**Limitations of this study.** Because of the short-term time frame of ischemia, i.e., 6 hr of coronary artery occlusion, there are several limitations to this study. The question of whether this drug actually limits the ultimate extent of infarction or simply delays the evolution of myocardial necrosis was not addressed in this investigation. It has been reported that calcium antagonists have beneficial effects on the ischemic myocardium even when infarct size is measured 2 to 7 days after coronary occlusion,\textsuperscript{14–16} although this finding has not been consistent.\textsuperscript{52} Furthermore, it is uncertain whether this drug has a detrimental effect on scar formation, which has been demonstrated with methylprednisolone.\textsuperscript{60} Accordingly, experiments assessing the effects of nicardipine on infarct size and scar formation much later after coronary occlusion are in progress in our laboratory to resolve these issues.

**Clinical implication.** The extrapolation of the results of our study to patients should be performed with extreme caution, since the animal preparation is obviously different from acute myocardial infarction in humans. However, if nicardipine has effects in patients with acute myocardial infarction similar to those obtained in this study, it would be expected that those patients reaching the hospital early after the onset of acute myocardial infarction, and especially those admitted before the onset of infarction, e.g., with a diagnosis of unstable angina, may benefit from treatment with the drug to salvage the ischemic myocardium.

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