Experimental Studies

Beneficial Effects of Intracoronary Nicardipine on Hypoxic Myocardium

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SUMMARY

Regional effects of intracoronary administration of nicardipine, a dihydropyridine derivative with calcium blocking activity, were studied in 13 open-chest dogs. Hypoxic, constant-flow perfusion was done in the area supplied by the distal left anterior descending artery. The 5-min regional hypoxic perfusion did not result in significant alterations in either heart rate or aortic pressure in 6 control dogs perfused with original Krebs-Henseleit solution (KHS), which was deoxygenized by mixed gas (95%N₂ and 5%CO₂), and in 7 dogs perfused with nicardipine-containing (0.2mg/dl) KHS. A transmural biopsy after 5 min of perfusion revealed a less severe metabolic deterioration in nicardipine-treated dogs than in control dogs, as indicated by a higher ATP content (2.84±0.43 vs. 2.23±0.45 μmol/g, wet weight). The sequence of regional contractile deterioration was not different between the 2 groups. In conclusion, regional nicardipine administration showed a myocardial protective effect which was not mediated by afterload reduction in the whole heart.

Additional Indexing Words:
Myocardial protection Intracoronary nicardipine Regional hypoxic perfusion

NICARDIPINE, a dihydropyridine derivative with calcium blocking activity, has been recently developed¹ and is under clinical trials for hypertension² and angina pectoris.³⁻⁶ Nicardipine has been shown to be a potent vasodilator with minimal effects on atrioventricular conduction and cardiac contractility.⁷⁻¹⁰ Several studies have shown that calcium antagonists have beneficial effects on the ischemic myocardium. These drugs increase coronary blood flow and decrease myocardial oxygen demand by reducing afterload, preload and cardiac contractility. Furthermore, calcium...
antagonists may exert a direct myocardial protective effect by inhibiting calcium uptake by ischemic myocardial cells, \textsuperscript{10} although this is not yet fully clear.\textsuperscript{11)-13) The purpose of our study was to examine the effects of nicardipine administered directly into the coronary artery on energy metabolism and contraction of the hypoxic myocardium in an \textit{in vivo} perfusion model.

\textbf{Materials and Methods}

Thirteen mongrel dogs weighing 10 to 20 Kg were anesthetized with 25 mg/Kg pentobarbital. Artificial respiration with a Harvard pump was used to maintain the arterial blood oxygen and carbon dioxide tension at around 100 and 30 mmHg, respectively, throughout the experiments. A left thoracotomy was performed at the fifth intercostal space. After pericardotomy, the left anterior descending coronary artery (LAD) was dissected just distal to the first diagonal branch. The tip of a bypass tube from the left subclavian artery was connected at this site.\textsuperscript{10),14),15) A brief interruption of the blood supply (less than 60 sec) was necessary for inserting the tip of the bypass tube into the LAD (Fig. 1). A bypass tube with a diameter of 4 mm contained an electromagnetic flow probe (Nihon Koden Co.) for measurement of the LAD blood flow and the flow rate of artificial perfusates. The tube had a three-way stopcock for hypoxic perfusion and for measurement of the shunt pressure. A pair of microcrystals (Schuessler Co.) was implanted in the subendocardium to evaluate serial changes in the regional contraction of the hypoxic myocardium. Aortic pressure and left ventricular pressure were measured through a catheter inserted via a femoral artery and a catheter in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Schematic representation of experimental model. AOF = aortic flow; CBF = coronary blood flow; LVP = left ventricular pressure; AOP = aortic pressure. Shaded area means the myocardium perfused with LAD.}
\end{figure}
the apex of the left ventricle. Aortic blood flow was measured with an electromagnetic flow probe around the ascending aorta.

Reactive hyperemia after a 15-sec LAD occlusion was validated by observing at least 200% of the control flow. After hemodynamic stabilization, control data were recorded with a simultaneous limb lead ECG on a polygraph. Infusion of the hypoxic solution was begun at the same rate as LAD blood flow during the control period with a tubing pump (Cole-Parmer Instrument Co.).

Two kinds of hypoxic solution were used for infusion: the original Krebs-Henseleit solution (KHS) (KCl 3.8 mM, KH₂PO₄ 1.2 mM, MgSO₄ 1.2 mM, NaCl 118 mM, NaHCO₃ 25 mM, glucose 10 mM) gassed with 95% N₂ and 5% CO₂ was perfused in 6 dogs (Group I), and hypoxic KHS containing 0.2 mg/dl of nicardipine in 7 dogs (Group II). The oxygen content of the hypoxic solution was less than 0.1 vol%. The returning coronary venous blood and the hypoxic solution were drained through a catheter inserted into the great cardiac vein to minimize transfer of the drug into the systemic circulation.

After 5 min of perfusion with the hypoxic solution, the center of the hypoxic myocardium and a region of nonhypoxic myocardium were biopsied transmurally by means of a cylindrical knife mounted on an electrical drill. The tissue sample was compressed and frozen quickly with Wollenburger type forceps precooled in liquid nitrogen. The entire procedure took about 15 sec. Tissue contents of adenosine triphosphate (ATP), diphosphate (ADP) and monophosphate (AMP) were measured by high-performance liquid chromatography.¹⁶

A paired t-test was used for statistical evaluation of changes in the measured variables in each animal group. Differences in measured variables among animal groups were analyzed with the non-paired t-test. A 'p' value of less than 0.05 was considered to be statistically significant.

Results

The mean perfusion rates were 22.2±7.7 and 24.0±13.3 ml/min in Groups I and II, respectively. In 2 dogs in Group II, the systemic nicardipine concentration was negligible.

Mean values and standard deviation for the hemodynamic parameters in the 2 groups are summarized in Table I. There were no statistical differences in any of these indices during the control period. The 5-min regional hypoxic perfusion did not result in significant alterations in heart rate, aortic pressure or aortic flow. Left ventricular end-diastolic pressure increased in the 2
Table I. Hemodynamic Parameters Before and After a 5-min Hypoxic Perfusion Period

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th></th>
<th>Group II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 5 min</td>
<td>Control 5 min</td>
<td>Control 5 min</td>
<td>Control 5 min</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>169±32</td>
<td>163±33</td>
<td>168±25</td>
<td>171±25</td>
</tr>
<tr>
<td>AOP (mmHg)</td>
<td>114±26</td>
<td>119±27</td>
<td>100±24</td>
<td>96±27</td>
</tr>
<tr>
<td>AOF (ml/min)</td>
<td>1437±696</td>
<td>1729±794</td>
<td>1232±304</td>
<td>1729±793</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4.8±2.5</td>
<td>9.5±3.2*</td>
<td>2.3±2.4</td>
<td>5.7±2.8†</td>
</tr>
</tbody>
</table>

* p<0.01 compared with control values.
† p<0.05 compared with Group I at 5 min.

HR = heart rate; AOP = mean aortic pressure; AOF = aortic flow; LVEDP = left ventricular end-diastolic pressure.

Table II. The Serial Changes in Regional Myocardial Contraction Induced by Hypoxic Perfusion

Changes in EDLc (mm)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>30 sec</th>
<th>1 min</th>
<th>2 min</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10</td>
<td>10.6±0.6</td>
<td>11.1±0.7</td>
<td>11.6±1.0</td>
<td>11.7±0.9</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>10.2±0.5</td>
<td>10.9±0.9</td>
<td>11.7±0.8</td>
<td>11.8±1.7</td>
</tr>
</tbody>
</table>

Change in %SS (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>30 sec</th>
<th>1 min</th>
<th>2 min</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>21.1±6.8</td>
<td>6.7±5.1</td>
<td>0.7±6.8</td>
<td>−5.2±7.5</td>
<td>−4.3±7.7</td>
</tr>
<tr>
<td>Group II</td>
<td>23.3±12.3</td>
<td>9.6±7.8</td>
<td>7.0±10.8</td>
<td>1.1±9.3</td>
<td>1.7±8.3</td>
</tr>
</tbody>
</table>

EDLc = corrected end-diastolic length; %SS = percent segment shortening.

Table III. Adenine Nucleotide Contents from Hypoxic and Nonhypoxic Myocardium

<table>
<thead>
<tr>
<th></th>
<th>TAN (µmol/g)</th>
<th>ATP (µmol/g)</th>
<th>ADP (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhypoxic Myocardium (n=13)</td>
<td>5.77±0.65</td>
<td>4.59±0.59</td>
<td>0.98±0.19</td>
</tr>
<tr>
<td>Hypoxic Myocardium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (n=6)</td>
<td>3.68±0.59**</td>
<td>2.23±0.45**</td>
<td>1.25±0.45**</td>
</tr>
<tr>
<td>Group II (n=7)</td>
<td>4.23±0.47**</td>
<td>2.84±0.43**†</td>
<td>1.20±0.13**</td>
</tr>
</tbody>
</table>

† p<0.05 compared with hypoxic myocardium in Group I.
** p<0.01 compared with nonhypoxic myocardium.

groups, probably due in part to the transfer of hypoxic solution into the systemic circulation throughout the 5-min perfusion period.

The changes in regional myocardial shortening induced by hypoxia are shown in Table II. There was no difference in the normalized end-diastolic segment length (EDLc) between the 2 groups after the perfusion period.
Nicardipine apparently did not affect the course of lengthening of the hypoxic myocardium in end-diastole. The regional contraction deteriorated and there was no difference in the percent segment shortening (%SS) between the 2 groups.

The mean values and standard deviations of myocardial adenine nucleotide contents of the hypoxic and nonhypoxic myocardium are shown in Table III. The total adenine nucleotide (TAN) and ATP were reduced and ADP contents were increased in comparison with those in the nonhypoxic myocardium in both groups. However, the degree of reduction in ATP content in Group II was significantly less than that in Group I.

**DISCUSSION**

Several experimental studies have shown that calcium antagonists have beneficial effects on ischemic myocardium. However, reports that they may reduce the myocardial infarction size are equivocal. Endo reported that nicardipine administered intravenously before or shortly after coronary artery occlusion limited the infarct size for 6 hr in open-chest dogs. However, when administration was delayed, the infarct size was not reduced. By contrast, Alps et al reported that intravenous nicardipine treatment 1 hr after ligation limited infarct size significantly in baboons. The metabolic preservation, observed in Group II in this study, has been noted in some clinical studies and experimental studies, but was not confirmed in others. The acute beneficial effects of nicardipine on lactate metabolism were noted in Rousseau’s clinical study as an increased coronary blood flow and a decreased rate pressure product. However, Rousseau reported that long-term antianginal therapy improved lactate metabolism; plasma levels of nicardipine (18±13 ng/ml) were relatively low and changes in global coronary blood flow were negligible. In an experimental myocardial ischemic model, Sunamori et al reported that nicardipine maintained ATP levels and CP without increasing coronary blood flow. These and other results cast some doubt on the hypotheses that coronary vasodilation or a reduction in myocardial oxygen demand is the main mechanism of action of the calcium antagonists on the ischemic myocardium.

This study showed that direct administration of nicardipine into a coronary artery can produce a high concentration in the local myocardium with negligible systemic blood concentration. The 5-min hypoxic perfusion in 2 groups did not result in significant changes in the systemic hemodynamics. Thus the preservation of ATP by intracoronary administration of nicardipine was not related to a reduction in oxygen consumption of the whole heart.
due to hemodynamic changes. However, protection of energy metabolism by a direct calcium blocking action of nicardipine cannot be ruled out.

Nicardipine has been shown in clinical\textsuperscript{18,29} and experimental\textsuperscript{91,28} studies to have a minor effect on cardiac contractility. In our study, the sequential changes in the regional myocardial contraction induced by hypoxia were the same for the 2 groups. It has recently become possible to administer a thrombolytic agent or several drugs directly to the ischemic myocardium with the advent of percutaneous transluminal coronary revascularization. This study indicates that intracoronary administration of nicardipine may be clinically useful as a supplemental method for protecting the ischemic myocardium without producing systemic hemodynamic deterioration during intracoronary thrombolytic therapy or coronary angioplasty. However, the clinical implications of this study should be taken with caution.

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