Background The present study was designed to investigate whether orally administered benidipine and manidipine protect the myocardium from ischemia–reperfusion injury.

Methods and Results Each drug (1, 3 or 10 mg/kg) was administered orally once daily for 1 week. The isolated rat heart model (Langendorff perfusion) was used, and each heart was subjected to global ischemia at 37°C for 40 min followed by reperfusion. Post-ischemic recovery of left ventricular (LV) function (measured as developed pressure (LVDP), dP/dt max and end-diastolic pressure) was compared with a control group. Creatine kinase (CK) leakage was also measured. Post-ischemic recovery of LVDP and LV dP/dt max were significantly increased by 3 mg/kg benidipine (LVDP: 87.5±10.1 vs 64.6±11.9%; LV dP/dt max: 97.8±10.4 vs 70.2±15.7%; p<0.05). CK leakage was significantly lower than in the control group (39.4±7.5 vs 61.1±9.8 IU per 15 min per kg; p<0.05). Manidipine produced significant recoveries in LVDP and LV dP/dt max at a dose of 1 mg/kg (LVDP: 93.7±16.5% vs 53.4±9.5%; dP/dt max: 104.2±21.9% vs 55.5±15.5%; p<0.05). CK leakage was also significantly reduced at the same dose (50.0±18.3 vs 80.1±14.0 IU per 15 min per kg; p<0.05).

Conclusions Orally administered benidipine and manidipine exerted significant cardioprotective effects against ischemia–reperfusion injury. (Circ J 2004; 68: 241–246)

Key Words: Benidipine; Ischemia–reperfusion; Manidipine; Oral administration

Orally Administered Benidipine and Manidipine Prevent Ischemia–Reperfusion Injury in the Rat Heart

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The purpose of the present study was to examine whether oral administration of the long-acting calcium antagonists, benidipine or manidipine, was effective in the prevention of ischemia–reperfusion injury.
grade perfusion was initiated with Krebs-Henseleit bicarbonate buffer equilibrated with 95% O₂ and 5% CO₂ at 37°C at a constant perfusion pressure of 100 cm H₂O. Through a left atrial incision, a latex balloon connected to a pressure transducer was inserted into the left ventricular (LV) cavity for measurement of the LV isovolumic pressure. The balloon was inflated to obtain a LV end-diastolic pressure (LVEDP) of 4–8 mmHg. The LVEDP was identified as the lowest value from the LV pressure curve. After attachment of a thin wire to the right atrium, all hearts were paced at 330 beats/min. The LV developed pressure (LVDP) and LV dP/dt were continuously monitored using a polygraph and a computer analysis system (LEG-1000 Nihon Kohden, Tokyo, Japan).

Experimental Protocol

For stabilization, Langendorff perfusion was performed for 20 min (n=6 in each group). Hearts were then subjected to global ischemia for 40 min at 37°C after cardiac arrest, which was achieved by clamping the aortic cannula and injecting St Thomas Hospital cardioplegic solution (in mmol/L: NaCl 110, NaHCO₃ 10, KCl 18, MgCl₂ 1.2, CaCl₂·2H₂O 1.2; pH adjusted to 7.8). The cardioplegic solution was infused at 37°C at a constant perfusion pressure of 100 cm H₂O for 3 min. After a period of ischemia, reperfusion was performed for 60 min. The LVDP, LV dP/dt max and LVEDP were measured every 10 min. Post-ischemic recovery of LVDP or LV dP/dt max after 60 min of reperfusion was expressed as a percentage of the pre-ischemic value.

Drugs

Benidipine hydrochloride (Kyowa Hakko Kogyo, Tokyo, Japan) and manidipine hydrochloride (Takeda Chemical Industries, Osaka, Japan) were suspended in 0.3% sodium carboxymethyl cellulose aqueous solution. The variable dose solution was injected into the stomach (1 ml) once daily for 1 week. There were no significant changes in body weight among the groups, and no rats died during the drug administration period. Four h after the last administration, blood samples were taken to measure the plasma drug concentrations by LC/MS/MS for benidipine hydrochloride and by high performance liquid chromatography for manidipine hydrochloride.

Assay for Creatine Kinase (CK)

CK leakage was assessed in the coronary effluent samples collected during the first 15 min of the reperfusion period. The total volume of the coronary effluent was measured during this 15-min period. A mixture of this sample (1 ml) and N-acetylcysteine (20 μmol/L) was stored at 4°C. The values for CK leakage were expressed as the total activity (IU) for the 15-min collection period normalized to the body weight (kg).

Statistical Analysis

All data are expressed as the mean±standard deviation. Multiple comparisons against a control group were made using a one-way analysis of variance followed by a Dunnett’s test. A value of p<0.05 was considered to be significant.

Results

Preischemic Cardiac Conditions

The pre-ischemic parameters of cardiac function in each group following the stabilization period are summarized in Table 1. In the benidipine-treated group, no significant changes were observed in LVDP or LV dP/dt max during the pre-ischemic perfusion. In the group receiving 10 mg/kg manidipine, the LVDP and LV dP/dt max were significantly depressed.

Plasma Concentrations of the Drugs

The mean plasma concentrations of benidipine and manidipine after 1 week of oral administration are shown in Table 2. Dose-dependent increases in the benidipine plasma concentrations were detected, with the infusions of 3 mg/kg and 10 mg/kg benidipine producing significantly greater concentrations than the 1 mg/kg infusion. The plasma concentration of manidipine increased significantly with administration of 10 mg/kg compared with 1 mg/kg.

Benidipine Hydrochloride Study

The percentage of post-ischemic recovery of LVDP was

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### Table 1 Cardiac Function During the Pre-Ischemic Period

<table>
<thead>
<tr>
<th>Drug</th>
<th>LVDP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>+LV dP/dt max (mmHg/sec)</th>
<th>−LV dP/dt max (mmHg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>138±22</td>
<td>6.5±1.5</td>
<td>3,900±551</td>
<td>2,483±440</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>150±11</td>
<td>6.7±1.5</td>
<td>3,983±458</td>
<td>2,783±394</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>135±32</td>
<td>4.9±1.1</td>
<td>3,883±267</td>
<td>2,417±598</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>121±15</td>
<td>6.8±0.8</td>
<td>3,267±547</td>
<td>2,150±295</td>
</tr>
</tbody>
</table>

Data are presented as the mean±SD (n=6 in each group). LVDP, left ventricular developed pressure; LVEDP, left ventricular end diastolic pressure; +LV dP/dt max maximum first derivative of left ventricular pressure during systole; −LV dP/dt max maximum first derivative of left ventricular pressure during diastole. *p<0.05 vs Control group.

### Table 2 Plasma Concentration of the Drugs (ng/ml)

<table>
<thead>
<tr>
<th>Drug</th>
<th>1 mg/kg</th>
<th>3 mg/kg</th>
<th>10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benidipine</td>
<td>0.14±0.08</td>
<td>0.60±0.18*</td>
<td>0.67±0.17*</td>
</tr>
<tr>
<td>Manidipine</td>
<td>0.65±0.19</td>
<td>1.62±0.33</td>
<td>15.2±1.82*</td>
</tr>
</tbody>
</table>

Data are presented as the mean±SD (n=4 in each group). *p<0.05 vs 1 mg/kg group.
significantly improved with the administration of 3 mg/kg or 10 mg/kg benidipine (control 64.6±11.9% to 87.5±10.1% and 97.4±22.3% with 3 mg/kg and 10 mg/kg benidipine, respectively) (Fig 1a). The dose-dependent changes in LV dP/dt max were similar to the changes in LVDP (Fig 1b), with significant improvements in LV dP/dt with 3 mg/kg or...
10 mg/kg benidipine (control 70.2±10.4% to 97.8±10.4% and 101.1±23.1% with 3 and 10 mg/kg, respectively). Benidipine also significantly reduced the post-ischemic LVEDP (control 34.7±16.5 mmHg to 11.2±4.4 and 22.6±12.6 mmHg with 3 and 10 mg/kg benidipine, respectively) (Fig 1c). CK leakage was also significantly reduced by 3 mg/kg and 10 mg/kg benidipine compared with the control group (control 61.1±9.8 IU per 15 min per kg to 39.4±7.5 IU per 15 min per kg and 46.4±12.0 IU per 15 min per kg with 3 mg/kg and 10 mg/kg benidipine, respectively) (Fig 1d).

Manidipine Hydrochloride Study

Post-ischemic recovery of LVDP increased significantly with manidipine administration (control 53.4±9.5% to 93.7±16.5%, 89.6±21.0% and 106.0±27.3% with 1, 3 and 10 mg/kg manidipine, respectively) (Fig 2a). The percentage recovery of LP dP/dt was also significantly improved with manidipine administration (control 55.4±15.5% to 104.2±21.9%, 92.8±29.3% and 99.8±27.6% with 1, 3 and 10 mg/kg manidipine, respectively) (Fig 2b). The post-ischemic LVEDP was significantly lower with 1 mg/kg manidipine than in the control group (10.5±4.4 mmHg vs 47.5±21.4 mmHg) (Fig 2c). However, 3 and 10 mg/kg manidipine produced dose-dependent increases in LVEDP. The CK leakage was significantly lower with 1 mg/kg manidipine than in the control group (80.2±14.0 vs 50.0±18.3 IU per 15 min per kg) (Fig 2d). However, the decreases in CK leakage were not significant with 3 or 10 mg/kg manidipine.

Relationship Between Pre-Ischemic Rate–Pressure Product and Post-Ischemic LVDP

To assess the relationship between the effects on pre-ischemic myocardial oxygen demand and their effects on post-ischemic recovery of contractile function, the LVDP after 60 min of reperfusion was plotted against the rate–pressure product immediately before ischemia. There was no correlation between the 2 parameters in each drug (Fig 3a, b).

Discussion

The present study clearly shows that oral administration of the long-acting dihydropyridine derivative calcium antagonists, benidipine and manidipine, produces significant improvements in LV performance and CK leakage after global ischemia in isolated rat hearts. These results indicate that 1 week of oral administration of benidipine and manidipine exerts a significant cardioprotective effect against ischemia–reperfusion injury.

It is difficult to evaluate the recovery of LV function when cardiac function is depressed before ischemia, and thus functional recovery of the heart (LVDP and LV dP/dt) was expressed as a percentage of the pre-ischemic control values. Benidipine did not produce any negative inotropic effects before ischemia, and improved post-ischemic LV functional recovery at a dose of 3 or 10 mg/kg, demonstrating its cardioprotective effects. The observation that enzyme leakage was minimized with 3 mg/kg benidipine suggests that this would be the optimal dose for cardiac protection. On the other hand, manidipine exerted negative inotropic effects in a dose-dependent manner, and pre-ischemic cardiac functions were significantly depressed with 10 mg/kg. Although significant recovery of heart function was demonstrated with all doses of manidipine, the reduction in enzyme leakage was not significant other than with 1 mg/kg. The finding that 3 mg/kg or 10 mg/kg manidipine did not reduce enzyme leakage indicates an absence of cardioprotective effects at these doses. Thus, from these results we suggest that a significant cardioprotective effect was only obtained at 1 mg/kg of manidipine.

Fujimura et al. and Yao and Karasawa have also reported cardioprotective effects of benidipine using isolated perfused hearts. However, their methods of administration were different. Fujimura et al added benidipine to the cardioplegic solution in a rabbit heart model, whereas Yao and Karasawa infused benidipine during pre-ischemic perfusion in a rat heart model. Both studies concluded that benidipine provides significant myocardial protection. Yao and Karasawa used 2 doses of benidipine (1 and 10 nmol/L), and detected a significant improvement at 10 nmol/L. Fujimura et al used 1 nmol/L of benidipine added to the cardioplegic solution in both normothermic and hypothermic conditions. Fujimura et al reported that a higher concentration (100 nmol/L) of benidipine significantly depressed post-reperfusion cardiac function, and suggested a bell-shaped dose–response curve for benidipine-mediated...
ated cardiac protection, as seen with other calcium antagonists. Therefore, it is considered that benidipine acts according to a bell-shaped dose–response manner similar to other short-acting calcium antagonists such as nifedipine and diltiazem. Kobayashi et al reported that the anti-hypertensive effects of benidipine do not correlate with the plasma concentration; however, we considered it to be important for evaluating the drug efficacy and thus conducted a dose–response study.

The concentration of a drug can be precisely calculated when it is added to a cardioplegic solution or a perfusion solution, but precise drug concentrations cannot be determined with oral administration. To compare the drug concentrations with the previous studies mentioned, we measured the plasma concentrations of both benidipine and manidipine (Table 2). The molecular weight of benidipine is 542. In our study, oral administration of 3 mg/kg benidipine produced a plasma concentration of 0.6 ng/ml, giving a calculated concentration of approximately 1.1 nmol/L. Our results showing that 3 mg/kg benidipine is the effective dose for ischemia–reperfusion injury are in agreement with a previous study.

It has been reported that the anti-hypertensive effects persist for 8 h after oral administration of 1 mg/kg benidipine. The actions of this drug persisted longer than expected from the blood concentration, because of slow partitioning of the drug into deep membrane compartments and tight binding to receptors. The long-acting Ca²⁺ antagonists are partitioned into the lipid membrane before they reach the dihydropyridine receptors and then bind to the receptors through the lipid membrane. This explains why the effects of the drugs are independent of their plasma concentrations. Moreover, high affinity receptor binding has already been shown by the slow dissociation rates of these compounds from specific sites on the dihydropyridine receptors.

To the best of our knowledge, there have been no studies on the cardioprotective effects of manidipine against ischemia–reperfusion injury. Compared with benidipine, there are several differences in the pharmacokinetics of manidipine. The half-life of manidipine is longer than that of benidipine, and the effective dose against hypertension differs from that of benidipine. In spite of these differences, we have shown that the cardioprotective effects of manidipine are similar to those of benidipine except for the optimal dose. The plasma concentration of manidipine at the optimal dose (1 mg/kg) is the same as that of benidipine. The differences between the pharmacokinetics of the 2 drugs probably influenced the tendency for a cardioprotective effect. Here, we have demonstrated that two Ca²⁺ antagonists with different structures exert cardioprotective effects against ischemia–reperfusion injury.

However, the precise mechanism for their cardioprotective effects is unknown despite numerous studies. There have been some reports explaining the mechanism, such as that the effects are expressed via bradykinin- and nitric oxide-dependent pathways, the action of ATP-sensitive K⁺ channels, ATP storage in the myocardium before ischemia, the suppression of the myocardial oxygen requirement before ischemia and so on. In our study, calcium antagonists were effective for cardioprotection, but it is unclear how they act. We assessed the relationship between the LVDP after 60 min of reperfusion and the rate–pressure product immediately before the global ischemia (Fig 3), but there was no correlation between the rate–pressure product before the ischemic insult and the post-ischemic contractile function because the cardiac performance improved largely without pre-ischemic depression of the heart at the optimal dose and the function at higher dosages fell off remarkably. Adachi et al reported that the double product in the pre-ischemic period correlated with the post-ischemic contractile function, but our data did not reveal this correlation. Benidipine and manidipine both have a tendency to depress cardiac contraction in the dose-dependent manner of cardiac drugs, but there was no significant relationship between cardiac depression and cardioprotection in the present study. The details of the mechanism are unknown and further studies are needed to clarify the mechanism of the cardioprotective effects.

Before the experiment, we wanted to determine the ideal period for oral administration and in the present study we used 1 week of pre-ischemic oral administration. Kobayashi et al have reported the absorption and distribution following repeated administration of ¹⁴C-labeled benidipine. In both plasma and tissues, the radioactivity levels increased after repeated oral administration once daily for the period of 1 week, and the activity reached a steady state between 1 and 2 weeks. For these reasons, we chose a 1-week period for oral administration of both drugs. Unfortunately, the length of time needed for manidipine to reach a stable level is unknown. Further studies are needed to clarify whether the administration period affects the cardioprotective effects of these drugs.

In conclusion, the 1,4-dihydropyridine derivative calcium antagonists, benidipine and manidipine, taken orally are effective in reducing ischemia–reperfusion injury in the isolated rat heart model. The 2 drugs are commercially available and are used clinically as anti-hypertension agents. We expect that these calcium antagonists will become available for use prior to open heart surgery.

References


