Antianginal Effects of Lercanidipine on the Vasopressin or Methacholine Induced Anginal Model in Rats

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The antianginal effects of lercanidipine, a newly synthesized 1,4-dihydropyridine derivative calcium channel antagonist, were evaluated in experimental angina model rats and the effects were compared with those of nifedipine, benidipine and amlodipine. In the vasopressin-induced angina model, intravenous administration of lercanidipine dose-dependently suppressed vasopressin-induced ST-depression. Amlodipine barely suppressed it, while benidipine, at the same dose, completely suppressed it. Nifedipine had a potency between that of amlodipine and benidipine. Oral administration of lercanidipine showed similar effects to the intravenous administration test on ST change. High doses of amloidipine, benidipine and nifedipine suppressed ST-depression by almost 100%. In the methacholine-induced angina model, lercanidipine suppressed the ST elevation dose dependently. Amlodipine barely suppressed it, while benidipine at 30 µg/kg effected almost total suppression. Nifedipine had a potency between that of amloidipine and benidipine. Intraduodenal administration of benidipine also suppressed the ST-elevation dose dependently. Nifedipine, benidipine and amlodipine at 10 mg/kg all markedly suppressed the elevation. Lercanidipine was more potent than the other calcium channel antagonists tested. In conclusion, it was explicitly demonstrated that lercanidipine exerts potent protective effects on the ischemic electrocardiography (ECG) changes in a variety of putative angina pectoris models in rats. An antispasmyotic coronary vasodilating action may be involved in the mechanism. It is expected that lercanidipine will be useful as an antianginal agent.

Key words calcium channel antagonist; lercanidipine; angina pectoris; 1,4-dihydropyridine; antianginal effect

Angina pectoris is caused by an imbalance between the supply and demand for oxygen in the heart, and it is divided into Angina of Effort and Stable Angina depending on the cause of the anginal fit. The former is a situation of myocardial ischemia induced by a lack of oxygen, which is caused by an inability to increase blood flow due to coronary constriction. The latter is a situation of severe myocardial ischemia induced by coronary spasm or abnormal strain in periods of rest. Above all, in cases where anginal fit is frequent at daybreak or in the early morning, and a marked elevation of the ST segment from electrocardiography (ECG) is observed, it is called Variant Angina. ECG is used for an important diagnostic method for these diseases, because myocardial ischemia is closely reflected to changes on ECG such as elevation or depression in the ST segment. There are cases of these diseases where they occur alone or together; it cannot be classified simply, but regardless, a decrease in the oxygen demand and an increase in the myocardial blood flow are required in therapy.

The clinical treatment of angina pectoris is mainly based on three groups of drugs, nitrates, β-adrenoceptor antagonists and calcium antagonists, which lead to the depression of oxygen consumption or the elevation of oxygen supply. The antianginal effect of calcium antagonists may be explained by their coronary dilatory effect (leading to the elevation of oxygen supply), cardio depressive effect (leading to the decrease of oxygen consumption) or the reduction of cardiac afterload according to antihypertensive effect. Based on their chemical structure, the compounds selective for slow calcium channels are divided to three subgroups; dihydropyridine (ex. nifedipine), benzothiazepine (ex. diltiazem) and phenylalkylamine (ex. verapamil). Recently, a second or third generation of dihydropyridine derivatives has been developed with the aim of providing greater vascular selectivity and a longer duration of action in order to allow a once-a-day dosage.

A new 1,4-dihydropyridine derivative, lercanidipine (methyl 1,1-dimethyl-2-[N-(3,3-diphenylpropyl)-N-methyl-amino ethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) (Fig. 1), synthesized at the Recordati Research Laboratory, is characterized by high vascular selectivity, a slow-onset and a long duration of the antihypertensive effect. These characteristics may qualify lercanidipine as a useful drug for the treatment of essential hypertension and angina pectoris. Fifty eight nations have approved this drug for clinical use as an antihypertensive, and clinical trials have progressed in Japan. Clinical trials of the antianginal effect have also progressed in Europe, and previous data reveal the great potential of this drug for clinical use.

In this study, the possible target of the antianginal effect of lercanidipine was investigated using two models of angina pectoris with reference to nifedipine, benidipine and amlodipine.

Fig. 1. Chemical Structure of Lercanidipine
MATERIALS AND METHODS

Experimental Animals Male Donryu Strain rats were used for the vasopressin-induced angina model, and male Sprague-Dawley strain rats were used for the methacholine-induced angina models. All animals were obtained from Charles River Japan, Inc. (Kanagawa) and used for experiments at an age of 8—10 weeks. The animals were housed in rooms kept at a temperature of 23±2 °C and a relative humidity of 55±10% under a 12 h light–dark cycle with free access to food (Rodent Chow, NMF; Oriental Yeast Co., Tokyo) and water. All experiments were conducted according to the institution’s guidelines for the care and use of laboratory animals in research. The change of the lead-II electrocardiogram (ECG) was used as a parameter of ischemic changes in the heart. The amplitude of the ST-changes was determined using the method of Hiramatsu et al.9

Effects on Vasopressin-Induced Angina Model in Rats Donryu rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A polyethylene catheter filled with saline was inserted into the right femoral vein and right carotid artery (for the intravenous injection experiment) in the anesthetized rat. According to the method of Hiramatsu et al.,9 coronary vasospasm was induced with the intravenous injection of vasopressin (0.2 IU/kg). The standard limb lead II of the ECG was recorded with a bioelectrical amplifier (NEC-san-ei) and ECG-processor (Softron). The amplitude of the ST-segment was measured at intervals of 0.5 min for 5 min after the administration of vasopressin. The difference of the amplitude of the ST segment before and after vasopressin administration was presented as the depression of the ST segment (ΔST). Each drug was administered to anesthetized rats intravenously 2 min prior, or to conscious rats orally 1 h prior to the administration of vasopressin.

Effects on Methacholine-Induced Angina Model in Rats The methacholine-induced coronary vasospasm model was prepared as described previously.10 Briefly, Sprague-Dawley rats were anesthetized with pentobarbital (50 mg/kg i.v.) or urethane (1.0 g/kg s.c., in saline). The trachea and right femoral artery and vein were cannulated with a polyethylene catheter. For the bolus injection of methacholine into the ostia of the coronary artery, a polyethylene cannula was introduced through the exposed right carotid artery to a point near the aortic valve. The mean blood pressure (BPM) was measured by means of a cannula in the femoral artery with a pressure transducer connected to an amplifier. Single doses of methacholine (8.0 μg) in volumes of 10 μl were injected into the aorta over a period of 1 s, using an Eppendorff® pipette, before and 2, 15 and 30 min after intravenous administration, or 5, 30, 60 and 120 min after intraduodenal administration of each drug or vehicle. Drugs were given intravenously or intraduodenally while the rats were under anesthesia to observe the duration of the stable effects in the same rat. The standard limb lead II of the ECG was recorded with a bio-amplifier and ECG-processor. The difference between the amplitude of the ST segment after and immediately prior to the administration of methacholine was presented as the elevation of the ST segment. The effects of each drug on the ST elevation are expressed as a percentage of pre-administration values (for the characteristics of the ECG-processor, S wave elevation (ΔS) was considered to be the ST elevation.).

Drugs Lercanidipine was provided by Recordati (Milan, Italy). Nifedipine was purchased from Wako Pure Chemicals (Osaka, Japan). Vasopressin ([Arg⁸]-vasopressin) and methacholine were purchased from Sigma (St. Louis, MO, U.S.A.). Benidipine was purchased from Kyowa-Hakko (Coniel® Tablets; Tokyo, Japan). Amlodipine was purchased from Pfizer (Norvasc® Tablets; Tokyo, Japan). For the intravenous administration test, lercanidipine, nifedipine, benidipine and amlodipine (benidipine and amlodipine were extracted from tablets in our laboratory) were dissolved in ethanol (EtOH), and then diluted with propyleneglycol (PG) and saline (EtOH : PG : Saline = 1 : 1 : 8 at final ratio). For the oral administration test, these drugs were suspended in 0.5% Na-carboxymethylcellulose (CMC) solution. Vasopressin and methacholine were dissolved in saline.

Statistical Analyses Values are shown as the mean±S.E. The values of ECG changes after drug administration were compared with the control values after vehicle administration with Dunnett’s test, p values less than 0.05 were considered to be statistically significant.

RESULTS

Effects of Intravenous Administration on the Vasopressin-Induced ST-Depression Model After the intravenous injection of vasopressin (0.2 IU/kg), the amplitude of the ST-segment of the ECG was transiently increased, followed by a continuous depression (Fig. 2). The maximal ST-depression was approximately 200 μV in rats given vehicle for drugs (Fig. 3). The intravenous administration of lercanidipine (3—30 μg/kg) significantly and dose dependently inhibited vasopressin-induced ST-depression (Fig. 3). Benidipine (30 μg/kg) also showed significant protective effects against the vasopressin-induced ST-depression. Amlodipine had no effect, and nifedipine had a slight effect. In these doses, lercanidipine was more effective than nifedipine and amlodipine, and less effective than benidipine against the vasopressin-induced angina model.

BPM and heart rate changes are shown in Table 1. BPM were increased after vasopressin injection and peaked after
3 min in the control. Lercanidipine decreased BPm before vasopressin injection, and dose dependently inhibited the vasopressin-induced BPm increase. Similar to the case of ST-depression, benidipine inhibited the BPm increase more potently than lercanidipine. Amlodipine had no effect, and nifedipine had a slight effect.

Heart rates were decreased after vasopressin injection and peaked after 3 min in the control. Lercanidipine inhibited

Fig. 3. Effects of Intravenous Administration of Lercanidipine, Nifedipine, Benidipine and Amlodipine on Vasopressin (0.2 IU/kg i.v.)-Induced ST Depression of ECG in Anesthetized Rats
A: time course of ST-depression; ●: control, ○: 3 μg/kg, □: 10 μg/kg, △: 30 μg/kg. B: maximal depression of ST-segment 1: control, 2: lercanidipine-3 μg/kg, 3: lercanidipine-10 μg/kg, 4: lercanidipine-30 μg/kg, 5: nifedipine-30 μg/kg, 6: benidipine-30 μg/kg, 7: amlodipine-30 μg/kg. Each point represents the mean±S.E. of 6—8 rats. **p<0.01 vs. control value (Dunnett’s test).

Fig. 4. Effects of Oral Administration of Lercanidipine, Nifedipine, Benidipine and Amlodipine on Vasopressin (0.2 IU/kg i.v.)-Induced ST Depression of ECG in Anesthetized Rats
A: time course of ST-depression; ●: control, ○: 1 mg/kg, □: 3 mg/kg, △: 10 mg/kg. B: maximal depression of ST-segment 1: control, 2: lercanidipine-1 mg/kg, 3: lercanidipine-3 mg/kg, 4: lercanidipine-10 mg/kg, 5: nifedipine-1 mg/kg, 6: nifedipine-3 mg/kg, 7: benidipine-1 mg/kg, 8: benidipine-10 mg/kg, 9: amlodipine-1 mg/kg, 10: amlodipine-10 mg/kg. Each point represents the mean±S.E. of 5—7 rats. **p<0.01 vs. control value (Dunnett’s test).
Table 1. Effects of Intravenous Injection of Lercanidipine, Nifedipine, Benidipine and Amlodipine on Mean Blood Pressure and Heart Rate

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean BP (mmHg)</th>
<th>Mean HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min</td>
<td>3 min</td>
</tr>
<tr>
<td>Control</td>
<td>110.5±7.7</td>
<td>391.6±6.2</td>
</tr>
<tr>
<td>Lercanidipine</td>
<td>112.7±7.3</td>
<td>382.2±10.0</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>113.8±8.3</td>
<td>395.3±15.4</td>
</tr>
<tr>
<td>Benidipine</td>
<td>108.5±7.6</td>
<td>384.7±10.9</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>306.5±16.1</td>
<td>388.8±13.7</td>
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</tbody>
</table>

Table 2. Effects of Oral Administration on the Methacholine-Induced ST-Elevation Model

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>382.4±15.9</td>
</tr>
<tr>
<td>Lercanidipine</td>
<td>398.5±17.6</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>428.8±18.3</td>
</tr>
<tr>
<td>Benidipine</td>
<td>361.0±15.5</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>396.4±16.3</td>
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DISCUSSION

Lercanidipine, a newly synthesized 1,4-dihydropyridine type calcium antagonist, is characterized by high vascular selectivity, slow-onset and a long duration of its antihypertensive effect. In an isolated rabbit heart, this drug improved the posts ischemic recovery of the left ventricular function and prevented a coronary circulation disorder after ischemia. Another important aspect of the pharmacodynamics of angina pectoris is the absence of changes in heart rate vari-
Recently, Wirts et al. showed that lercanidipine has a partial agonistic effect on cardiac calcium channels along with its action having a marked voltage-dependence. These qualities lead to the vasoselectivity of this compound. These characteristics qualify lercanidipine as a useful drug in the treatment of essential hypertension and angina pectoris. Fifty-eight nations have approved this drug for clinical use as an antihypertensive, and clinical trials are progressing in Japan. Clinical trials for antianginal effect are also progressing in Europe, and previous data indicate the great potential of this drug for clinical use.

As for experimental models that reflect angina pectoris, various models, such as the vasopressin or isoproterenol-induced ST-depression models and the methacholine-induced ST-elevation model have been developed. The vasopressin-induced ST-depression model is that in which there occurs an imbalance between the oxygen supply and demand because of coronary constriction (depression of coronary blood flow) or systemic vascular constriction (increase of cardiac afterload) induced by the injection of vasopressin.
into the femoral vein of rats. In this case the coronary arteries do not completely occlude, therefore ischemia occurs mainly in the endocardium. The ST segment decreases in the same way as in Angina of Effort in humans. Karasawa et al. showed that the ST-depression induced by vasopressin injection is effectively inhibited by certain calcium antagonists but not by β-blockers. The methacholine-induced ST-elevation model is a model that induces severe transmural ischemia after the direct injection of methacholine into the coronary arteries. Then, the coronary trunks are transiently blocked, and similar to human Variant Angina, ST-elevations specific for transmural ischemia are observed.

In our experiment, the intravenous injection of vasopressin induced a transient ST-segment depression in rats and the degree of maximal ST-segment depression observed was similar to previous reports. As a result of the intravenous administration of lercanidipine, compared with nifedipine, benidipine and amlodipine in vasopressin-induced models, lercanidipine produced dose-dependent suppressive effects on the ST-depression, and its potency was higher than nifedipine and amlodipine. These effects occurred parallel to the suppressive effect on BPm, so it was suggested that these effects resulted from vasorelaxation on the basis of calcium channel antagonism. Although, in oral administration trials, no suppressive effect was recognized at 1 mg/kg in the control drugs treated group, effects were observed from 1 mg/kg in the lercanidipine treated group. With lercanidipine, the ST change was completely suppressed at 3 mg/kg, indicating that the utility of this drug is sufficiently higher than the control drugs.

In addition, lercanidipine showed a tendency to effect the suppression of heart rate decreases associated with ischemic changes caused by vasopressin treatment. This characteristic is considerably different from the other control drugs (intravenous administration). Benidipine had the highest suppressive effect on ST-depression, but because of its high affinity for cardiac calcium channels, the drug itself strongly represses cardiac function (Table 1). However, lercanidipine has a high vascular selectivity and a low cardio suppressive effect in comparison with nifedipine or amlodipine. Therefore, this high vascular selectivity and cardio suppressive effect are the reason for the greater repressive effect on the heart rate decline than the other control drugs.

In the Stable Angina model, lercanidipine suppressed the ST-elevation induced by methacholine injection dose dependently. The potency of this effect was higher than nifedipine or amlodipine, similar to the vasopressin-induced model (intravenous administration). The main mechanism may be the repression of coronary spasm by a calcium channel antagonistic effect. However, even if amlodipine has a coronary re-laxation effect at a very low concentration (10^{-9} mol/l) in the in vitro study, such effectiveness was not observed in this study. The pharmacokinetic profile of amlodipine after oral administration is characterized by a higher hepatic distribution and slow redistribution into the systemic circulation. However for high plasma clearance in rat, plasma amlodipine after intravenous administration may be eliminated immediately. This may be the mechanism whereby the effectiveness was recognized only with oral administration, but the details are unclear.

Recently, the anti-ischemic action and the effects of lercanidipine on the autonomic function in patients with stable effort angina in double-blind clinical trial was reported. In this report, lercanidipine was effective in reducing ischemia in patients with angina of effort. Furthermore, it does not cause adrenergic activation, which is the main mechanism hypothesized by which the negative effect on cardiovascular mortality assigned to short-acting DHP-calcium antagonists (ex. nifedipine) is explained.

In conclusion, it was explicitly demonstrated that lercanidipine exerts potent protective effects on the ischemic ECG changes in the putative angina pectoris model in rats. An antispasmodic coronary vasodilating effects may be involved in the mechanism. It is expected that lercanidipine will prove useful as an antianginal agent.

REFERENCES