Comparative Effects of Azelnidipine and Amlodipine on Myocardial Function and Mortality After Ischemia/Reperfusion in Dogs

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Received October 12, 2010; Accepted April 4, 2011

Abstract. Effects of azelnidipine were examined and compared with those of amlodipine on stunned myocardium in dogs. The left anterior descending (LAD) coronary artery was ligated for 20 min and subsequently released for 60 min. A vehicle, azelnidipine (0.3 mg/kg), or amlodipine (0.3 or 1 mg/kg) was injected intravenously 20 min before LAD ligation. The heart rate increased after a depressor response in the presence of amlodipine, while it decreased despite a decrease in arterial pressures in the presence of azelnidipine. After reperfusion, the coronary flow (CF) significantly increased in the presence of azelnidipine, but did not change with amlodipine after reperfusion. A positive inotropic effect was observed after treatment with both calcium antagonists. Ischemia significantly decreased the percentage of segment shortening (%SS) in all groups. Treatment with both calcium antagonists significantly increased %SS after reperfusion, although high-energy phosphate levels did not improve in the presence of calcium antagonists 60 min after reperfusion. Mortality with azelnidipine was significantly lower than that with 0.3 mg/kg amlodipine immediately after reperfusion. In conclusion, improvement in myocardial stunning after pretreatment with azelnidipine is associated with an increase in CF after reperfusion. The negative chronotropic action may have contributed to decreased mortality due to reperfusion arrhythmias. Azelnidipine is more beneficial than amlodipine and may provide an additional advantage to patients with angina and hypertension.

Keywords: azelnidipine, calcium antagonist, reflex sympathetic hyperactivity, myocardial stunning, mortality

Introduction

Myocardial stunning is a mechanical dysfunction that occurs after a brief period of ischemia and is characterized by the absence of irreversible damage (1, 2). Intracellular calcium plays a key role in myocardial contraction and relaxation, and administration of calcium antagonists attenuates myocardial stunning (3–5). Some investigators have reported that nifedipine, a short-acting dihydropyridine calcium antagonist, increases the risk of ischemic heart disease because of its effects on reflex responses (6, 7). For example, nifedipine activates the sympathetic nervous system and causes reflex tachycardia after a rapid decrease in blood pressure. Therefore, administration of amlodipine, a long-acting calcium antagonist, is recommended in patients with hypertension and angina (8). However, Kuramoto et al. (9) have reported that the heart rate (HR) increases after short-term treatment with amlodipine but not azelnidipine. Azelnidipine, a third-generation dihydropyridine calcium antagonist, has a higher vascular selectivity with few adverse reactions (10). We (11) demonstrated that myocardial contractile dysfunction recovered and HR decreased with azelnidipine treatment after a brief period of ischemia, but the mechanism of action remains unclear.

In this study, we compared the effects of azelnidipine with those of amlodipine on stunned myocardium in dogs.
Materials and Methods

The animal experimental plan used here has been approved by the Committee of the Laboratory Animal Center and conforms to the Guiding for the Care and Use of Laboratory Animals published by Hokkaido Pharmaceutical University School of Pharmacy (published, 1998; revised, 2001 and 2007).

Preparation of animals

Pentobarbital (30 mg/kg) anesthetized adult mongrel dogs of both sexes, weighing 7.0 – 22.5 kg (n = 57) were used. The surgical procedure and measurements of hemodynamics were performed according to the method described previously (12). Myocardial contraction was evaluated using a pair of ultrasonic crystal probes implanted in the left anterior descending (LAD) coronary artery region of the left ventricle. Percentage of segment shortening (%SS, an index of myocardial contraction) was calculated using the equation %SS = [(diastolic segment length – systolic segment length) / diastolic segment length] × 100. %SS values were normalized to the respective pre-injection value (100% dimethyl sulfoxide; DMSO, azelnidipine, or amlodipine) of %SS. Arterial blood pressure, HR, left ventricular end systolic pressure (LVESP), and left ventricular end diastolic pressure (LVEDP) were monitored on a polygraph (360 System; Nihondenki Sanei, Tokyo). Coronary flow (CF) was measured using an ultrasonic flow probe (T-106; Transonic Systems, Inc., Ithaca, NY, USA).

Experimental protocol

After the control observations, DMSO (n = 9) and 0.3 mg/kg of azelnidipine (n = 12), 0.3 mg/kg of amlodipine (n = 9), or 1 mg/kg of amlodipine (n = 4), dissolved in DMSO, was injected into the left femoral vein at a volume of 0.1 ml/kg. LAD coronary artery was ligated 20 min after administration of the drug or vehicle and perfused 20 min after ischemia. Hemodynamic changes and %SS were measured for a further 60 min. A full-thickness transmural sample of the myocardium was taken from the previous ischemic region 60 min after reperfusion. The myocardial sample was immediately frozen with freezing clamps previously chilled in liquid nitrogen. The frozen tissue samples were used for biochemical analysis (13).

Biochemical analysis of tissue energy metabolites

The frozen myocardial samples were pulverized in liquid nitrogen and extracted with 6% perchloric acid. ATP, ADP, AMP, creatine phosphate (CrP), and lactate levels in the neutralized perchloric extracts were determined by standard enzymatic procedures (13). Total adenine nucleotide (TAN) was calculated as follows: [ATP] + [ADP] + [AMP]. The energy charge potential (ECP) was calculated from ATP, ADP, and AMP levels to evaluate the myocardial energy condition as follows: ([ATP] + 0.5[ADP]) / [TAN].

Statistical analyses

All values are expressed as means ± S.E.M. Differences in hemodynamics within groups were compared using paired Student’s t-test. Significant differences in %SS after administration of the drug, during ischemia, and during 60-min of reperfusion between the groups were evaluated by repeated measure ANOVA. Myocardial metabolite levels were compared using Fisher’s exact test. Differences in mortality between groups were analyzed using the chi-square (χ²) test. P < 0.05 was considered statistically significant.

Results

Mortality

The mortality rate is summarized in Table 1. Initially, 57 dogs were used; however, 23 died because of ventricular fibrillation (VF) that appeared immediately after reperfusion. The details about the survival are as follows: DMSO-treated, 8 of 17 dogs; azelnidipine-treated, 2 of 14 dogs; 0.3 mg/kg amlodipine–treated, 10 of 19 dogs; and 1 mg/kg amlodipine–treated, 3 of 7 dogs. Therefore, only 34 dogs completed the protocol and were used to calculate hemodynamic, %SS, and myocardial metabolite data for analyses. Significant differences in mortality rate between dogs treated with DMSO and drugs were absent. However, mortality of dogs treated with azelnidipine was significantly lower than that of dogs treated with 0.3 mg/kg amlodipine after reperfusion (Table 1).

Hemodynamics and %SS changes

Changes in systolic and diastolic pressure, HR, CF, LVESP, and LVEDP are shown in Fig. 1. %SS values were normalized to the respective drug pre-injection values (Fig. 2).

Systolic and diastolic pressures slightly increased after

| Table 1. Mortality analysis immediately after reperfusion |
|----------------|----------------|
| Mortality rate |
| DMSO | 47.1% |
| Azelnidipine | 14.3%* |
| Amlodipine (0.3 mg/kg) | 52.6% |
| Amlodipine (1 mg/kg) | 42.9% |

*P < 0.05 vs. amlodipine (0.3 mg/kg).
Fig. 1. Effects of azelnidipine and amlodipine on hemodynamic changes. Either DMSO (vehicle; open circle, n = 9), 0.3 mg/kg azelnidipine (closed triangle, n = 12), 0.3 mg/kg amlodipine (open triangle, n = 9), or 1 mg/kg amlodipine (open square, n = 4) was injected intravenously at time zero. LAD was ligated 20 min after the administration of DMSO or drugs and was subsequently released 20 min after coronary occlusion. Data are expressed as means ± S.E.M. *P < 0.05, compared with time zero for each drugs.
DMSO treatment. The systolic pressure during ischemia and reperfusion did not change compared with DMSO pre-injection values. In dogs treated with azelnidipine, systolic pressure significantly decreased after drug treatment, and this depressor response continued during ischemia. In dogs treated with 0.3 mg/kg amlodipine, systolic pressure decreased temporarily after drug treatment, and depressing response was observed during ischemia. In dogs treated with 1 mg/kg amlodipine, no significant change in the systolic pressure was observed from the beginning to end of the experiment, although the values appeared to be a little higher than pre-injection values.

Diastolic pressure increased after DMSO treatment, and it was restored to the control level during reperfusion. In dogs treated with azelnidipine and both doses of amlodipine, a diastolic pressure–lowering effect was maintained until the end of the experiment.

HR slightly but significantly decreased with DMSO treatment, and this continued throughout the experiment. In dogs treated with azelnidipine, HR significantly decreased, and this response was maintained until the end of the experiment. In the dog treated with 0.3 mg/kg amlodipine, HR significantly increased after drug treatment, and this response was maintained until the end of the experiment. HR temporarily increased after 1 mg/kg amlodipine.

CF in the LAD region did not change after DMSO treatment and immediately decreased to 0 ml/min after LAD ligation. In dogs treated with azelnidipine and both doses of amlodipine, CF significantly increased before ischemia and immediately decreased to 0 ml/min after LAD ligation. CF significantly increased 5 min after reperfusion in dogs treated with DMSO, azelnidipine, and 0.3 mg/kg amlodipine, whereas no reactive hyperemia was observed in dogs treated with 1 mg/kg amlodipine 5 min after reperfusion. In dogs treated with azelnidipine, CF increased significantly 20 min after reperfusion, but not in those with both doses of amlodipine.

LVEDP did not change after amlodipine, azelnidipine, or DMSO treatment. LVEF significantly increased during ischemia and then changed to pre-ischemic values after reperfusion in all groups. %SS was not modified by DMSO treatment during pre-ischemia, whereas %SS significantly increased with azelnidipine and both doses of amlodipine. In all groups, %SS values were lower during ischemia than those at 0 min. %SS failed to recover in dogs treated with DMSO after reperfusion, whereas it significantly increased after reperfusion in dogs treated with azelnidipine- and both doses of amlodipine. In particular, %SS values increased more significantly from pretreatment values in dogs treated with 1 mg/kg amlodipine.

Myocardial energy metabolic changes

Energy and carbohydrate metabolite values in 60 min–reperfused hearts are summarized in Table 2. ATP values were slightly lower in dogs treated with azelnidipine and both doses of amlodipine than those in dogs treated with DMSO, but the difference was not statistically significant. ADP and TAN values were significantly lower in dogs treated with azelnidipine and both doses of amlodipine than those in dogs treated with DMSO. AMP and ECP values were not significantly different between dogs treated with DMSO and both the drugs. CrP levels were significantly lower in the hearts of dogs treated with 1 mg/kg amlodipine than those in hearts of dogs treated with DMSO. AMP and ECP values were not significantly different between dogs treated with DMSO and both the drugs. Significant differences in CrP levels were absent between dogs treated with DMSO and both the drugs, except for those treated with 1 mg/kg amlodipine. Lactate levels in the hearts of dogs treated with azelnidipine were significantly higher than those in the hearts of dogs treated with DMSO; however, significant differences in lactate levels were absent between dogs treated with DMSO and amlodipine.
Discussion

Nifedipine, a short-acting dihydropyridine calcium antagonist, has been reported to cause reflex sympathetic stimulation and tachycardia when blood pressure decreases after the administration of the drug (14, 15). Amlodipine, a long acting calcium antagonist, is characterized by a higher vascular selectivity and smaller positive inotropic effect; therefore, amlodipine is recommended for patients with hypertension and angina (8). In this study, HR increased after intravenous injection of 0.3 mg/kg amlodipine. Furthermore, it temporally increased after treatment with 1 mg/kg amlodipine (Fig. 1). Because blood pressure decreased immediately after an intravenous injection of both doses of amlodipine, this drug may contribute to reflex sympathetic hyperactivity. We speculate that the intravenous injection of amlodipine is more likely to provoke reflex sympathetic stimulation than its oral administration because 1 mg/kg of amlodipine is not as high as the clinically relevant dose (1.25 – 10 mg) (16).

In dogs treated with azelnidipine, HR significantly decreases despite decrease in systolic and diastolic pressures. Why did azelnidipine decrease HR when it was not decreased by either dose of amlodipine? Koike et al. (17) have reported that azelnidipine not only has an affinity for the L-type Ca²⁺ channels (high-voltage activated), but also for the T-type Ca²⁺ channels (low-voltage activated). Li et al. (18) have reported that the low voltage–dependent activation/inactivation and slow deactivation of T-type Ca²⁺ channels may play a physiological role in carrying the depolarizing current at low membrane potentials. They have speculated that these channels may play a crucial role in triggering high voltage–activated channels (pacemaker function) by modulating the rate of repetitive firing (low-threshold spikes). Cohen et al. (19) have reported that the T-type Ca²⁺ channel blockage has an anti-tachy-arrhythmic effect. In this study, therefore, HR may have been modulated by the T-type Ca²⁺ channel blockage after treatment with azelnidipine. In contrast, it has been demonstrated that amlodipine has low affinity for T-type Ca²⁺ channels (20). Kuramoto et al. (9) have demonstrated that the pulse rate decreases after azelnidipine treatment, whereas it significantly increases after amlodipine treatment. Thus, amlodipine may not be able to suppress the positive chronotropic effect by reflex sympathetic hyperactivity.

In the pre-ischemic condition, coronary vasodilatory effects were observed after treatment with either azelnidipine or both doses of amlodipine (Fig. 1). In dogs treated with 1 mg/kg amlodipine, reactive hyperemia was not observed 5 min after reperfusion. The increase in CF was observed 20 min after reperfusion in dogs treated with azelnidipine, but not in those treated with both doses of amlodipine (Fig. 1). We (21) have demonstrated that a strong coronary vasodilator is not always effective in treating ischemic myocardium because a potent coronary vasodilator may cause coronary steal, thereby worsening ischemia-induced myocardial injury. Generally, calcium antagonists mainly provoke vasodilatory effects in a normal arteriole. Both myocardium and coronary artery damage may result either from ischemia, reperfusion, or both.

LVESP significantly increased after reperfusion in the presence of amlodipine. Nakaya et al. (14) have demonstrated that left ventricular pressure increases in dogs after nifedipine treatment. In this study, reflex sympathetic hyperactivity occurred after an initial depressor response to amlodipine treatment, and we speculated that enhanced LVESP is a common response to L-type cal-

### Table 2. Myocardial metabolites values in 60-min reperfused hearts following 20-min ischemia

<table>
<thead>
<tr>
<th></th>
<th>Vehicle DMSO (n = 9)</th>
<th>Azelnidipine 0.3 mg/kg (n = 12)</th>
<th>Amlodipine 0.3 mg/kg (n = 9)</th>
<th>Amlodipine 1.0 mg/kg (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>3.852 ± 0.276</td>
<td>3.405 ± 0.200</td>
<td>3.444 ± 0.107</td>
<td>3.100 ± 0.248</td>
</tr>
<tr>
<td>ADP</td>
<td>1.222 ± 0.232</td>
<td>0.739 ± 0.078*</td>
<td>0.691 ± 0.018*</td>
<td>0.679 ± 0.046*</td>
</tr>
<tr>
<td>AMP</td>
<td>0.291 ± 0.026</td>
<td>0.286 ± 0.021</td>
<td>0.321 ± 0.022</td>
<td>0.357 ± 0.023</td>
</tr>
<tr>
<td>TAN</td>
<td>5.364 ± 0.460</td>
<td>4.429 ± 0.239*</td>
<td>4.455 ± 0.124*</td>
<td>4.136 ± 0.276*</td>
</tr>
<tr>
<td>ECP</td>
<td>0.835 ± 0.011</td>
<td>0.852 ± 0.010</td>
<td>0.850 ± 0.005</td>
<td>0.830 ± 0.011</td>
</tr>
<tr>
<td>CrP</td>
<td>11.015 ± 1.589</td>
<td>9.462 ± 0.567</td>
<td>8.717 ± 0.504</td>
<td>7.347 ± 0.404*</td>
</tr>
<tr>
<td>Lac</td>
<td>1.050 ± 0.257</td>
<td>1.824 ± 0.318*</td>
<td>1.110 ± 0.112</td>
<td>1.666 ± 0.290</td>
</tr>
</tbody>
</table>

TAN, total adenine nucleotide; ECP, energy charge potential; CrP, creatine phosphate; Lac, lactate. Myocardial samples were obtained 60 min after reperfusion following 20-min ischemia. Vehicle, azelnidipine, or amlodipine was given intravenously. 20 min before the onset of ischemia. Values are means ± S.E.M. (μmol/g wet tissue). *P < 0.05 vs. vehicle group.
cium antagonists. However, in dogs treated with azelnidipine, LVESP did not increase significantly after reperfusion; therefore, this response may be partially associated with T-type Ca\(^{2+}\) channel antagonized effects. Either HR or systemic pressure augmentation or both contribute to increases in preload and afterload, thus leading to an increase in myocardial oxygen demand. It has been speculated that the rate-pressure product is an indirect index of myocardial oxygen demand in clinical study (22). HR and systemic blood pressure decreased with azelnidipine treatment, which affected afterload or preload decrease, and it may be correlated to an LVESP immovable response. HR increased after treatment with amlodipine; this response may be concerned with partial increase of afterload and it may contribute to increased LVESP.

Myocardial mechanical dysfunction during reperfusion following brief periods of ischemia is known as stunning (1, 2). In this study, treatment with either azelnidipine or both doses of amlodipine significantly enhanced the recovery of myocardial stunning compared to treatment with DMSO (Fig. 2). Some investigators have reported that myocardial stunning is associated with decreased ATP levels (23), Ca\(^{2+}\) overload (24), and oxygen-derived free radical formation (1). However, in this study, ADP and TAN levels were significantly lower in the heart in the presence of azelnidipine and both doses of amlodipine than in the presence of DMSO 60 min after reperfusion (Table 2). Gross et al. (25) have demonstrated that myocardial segment function is recovered by amlodipine treatment 60 min after reperfusion following a 45 min-ischemia. A 45-min ischemia causes irreversible myocardial tissue damage and necrosis. Therefore, we selected a 20-min ischemia in which the ischemia-induced myocardial damage is reversible and myocardial necrosis does not occur. They have speculated that myocardial segment function recovers due to augmentation of TAN levels, which is caused by collateral blood flow progression in ischemic heart treated with amlodipine. The collateral blood flow may be necessary for recovery in ischemic irreversible damaged hearts, that is, myocardial infarction. In this study, myocardial segment function was recovered by amlodipine as well as azelnidipine treatment 60 min after reperfusion following 20-min ischemia (reversible damaged heart), although TAN levels were not augmented in the ischemic area with both drug treatments (Table 2). The mechanism of %SS recovery observed with both calcium antagonists on stunning in this study may be a different from that in the myocardial infarction model used by Gross et al. This may be because %SS-enhancement was observed 60 min after reperfusion in this study despite TAN levels being lower than with DMSO treatment. Myocardial contractility increased immediately after treatment with either azelnidipine or both doses of amlodipine before ischemia was induced. We (11) have demonstrated that the increase in myocardial contractility after azelnidipine treatment is inhibited by pretreatment with propranolol and atropine. Therefore, this positive inotropic effect after treatment with azelnidipine or both doses of amlodipine may also be a reflexive response to decreases in diastolic pressure through regulation by the sympathetic nervous system. The observed reflex sympathetic hyperactivity may have continued during ischemia and reperfusion because myocardial contractility was a little higher after treatment with a high dose of amlodipine than after that with DMSO during ischemia. Moreover myocardial contractility dysfunction resolved immediately after reperfusion in the dogs treated with amlodipine in a dose-dependent manner. Therefore, in the calcium antagonist–treated groups, the significant recovering effect of myocardial contractile dysfunction may have been associated with reflex sympathetic hyperactivity, although TAN values in the hearts of dogs treated with azelnidipine and both doses of amlodipine were significantly lower after reperfusion. Increasing CF after azelnidipine injection was sustained during reperfusion. This increase in CF may have partially contributed to the improvement in myocardial contractility after reperfusion. The main cause of functional recovery by amlodipine may be reflex sympathetic hyperactivity because CF is not sustained after reperfusion in dogs treated with amlodipine. The accelerated reflex sympathetic hyperactivity associated with calcium antagonists could be disadvantageous to patients with ischemic heart disease because there is an oxygen demand in the damaged heart. Therefore, in this study, the decrease in TAN levels can be attributed to reflex sympathetic hyperactivity.

VF immediately after reperfusion leads to death. Mortality immediately after reperfusion was lower in dogs treated with azelnidipine than in dogs treated with both doses of amlodipine (Table 1: statistically significant vs. 0.3 mg/kg amlodipine–treated dogs). The observed accelerated reflex sympathetic hyperactivity caused an increase in both myocardial contractility and HR after the depressor response. HR increase is concerned with ventricular tachycardia and VF, which is caused by norepinephrine release from cardiac sympathetic nerve endings as ischemia-induced reperfusion injury (26). Therefore, the negative chronotropic action of azelnidipine may have contributed to decreased mortality due to VF immediately after reperfusion.

In conclusion, pre-treatment of dogs with either azelnidipine or amlodipine improved myocardial contractile dysfunction during reperfusion following a brief period of ischemia. In this study, the limitation of stunning with azelnidipine may be partially associated with increased
CF after reperfusion and constant decreased HR. Azelnidipine is more beneficial than amlodipine and its negative chronotropic action would provide an additional advantage to patients with hypertension and angina.

Acknowledgment

Azelnidipine was kindly supplied by Daiichi-Sankyo Co., Tokyo, Japan.

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