Differential Effects of Calcium-Channel Blockers on Vascular Endothelial Function in Patients With Coronary Spastic Angina

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Background: The effects of the 3 classes of L-type calcium-channel blockers (CCBs) on vascular endothelial function have not been clarified in patients with coronary vasospasm.

Methods and Results: Twenty-five normotensive patients (age 64.0 ± 1.4 years) with coronary vasospasm were randomly treated for 3 months with benidipine, diltiazem, and verapamil, which belong to the dihydropyridine, benzothiazepine, and phenylalkylamine classes of CCBs, respectively. Endothelium-dependent flow-mediated dilatation (FMD), endothelium-independent nitroglycerin-induced dilatation in the brachial arteries, and plasma cyclic guanosine 3',5'-monophosphate (cGMP), a nitric-oxide-related product, were assessed before and after treatment. At baseline, the patients with vasospasm had significantly lower FMD as compared with normal subjects ($n=8$). Blood pressure did not differ among the 3 groups before and after treatment. Benidipine significantly increased FMD (from 4.7 ± 0.6 to $7.4 \pm 1.1\%$, $P<0.05$) and plasma cGMP levels. In contrast, neither diltiazem nor verapamil affected FMD and cGMP levels. None of the treatments affected nitroglycerin-induced dilatation.

Conclusions: Benidipine, but not diltiazem or verapamil, improves endothelial dysfunction beyond blood pressure lowering effects in patients with coronary vasospasm. Upregulation of the nitric oxide–cGMP system by benidipine may partly contribute to the improvement. The dihydropyridine class may be more beneficial for vascular endothelial function than the non-dihydropyridine classes of CCBs. (Circ J 2009; 73: 713–717)

Key Words: Benidipine; Calcium-channel blockers; Coronary artery disease; Endothelial function; Vasospasm

Coronary vasospasm plays an important role in the pathogenesis of not only variant angina but also coronary heart disease in general, including acute coronary syndrome.^{1–4} In humans, coronary vasospasm is induced by acetylcholine, histamine, ergonovine, or serotonin, all of which cause vasodilation by virtue of the release of nitric oxide (NO) when the endothelium is intact.^{5–9} Therefore, vascular endothelial dysfunction is thought to be important in the pathogenesis of coronary spasm. Recent studies have shown that endothelial NO activity is decreased in patients with coronary spastic angina (CSA), leading to impaired flow-mediated dilation (FMD) in systemic arteries as well as coronary arteries.^{10–12} Thus, protecting vascular endothelial function is critical in treating coronary spasm.

Calcium-channel blockers (CCBs) are recommended as first-line therapy in angina patients with coronary spasm.¹³ Recent studies have demonstrated that certain CCBs improve vascular endothelial dysfunction.^{14,15} There are 3 classes of voltage-dependent L-type CCBs: dihydropyridines, benzothiazepines and phenylalkylamines, broadly classified according to chemical structure.^{16,17} The cardiovascular profile,

including vasoselectivity, inhibition of vascular smooth muscle contraction and reduction of myocardial contractility, varies with the different types of CCB.¹⁸ However, it is still unknown whether in patients with CSA the beneficial effects on vascular endothelial function are common among the CCBs.

We designed the present study to compare the effects of the 3 classes of L-type CCBs on endothelial-dependent FMD of the brachial arteries in patients with CSA. For comparison, we used benidipine, diltiazem and verapamil, which belong to the dihydropyridine, benzothiazepine, and phenylalkylamine classes of L-type CCBs, respectively.

Methods

Study Population

From October 2001 to December 2004, 84 patients were diagnosed with CSA by performing spasm provocation tests. Of these, the present study included 25 consecutive patients with CSA (mean age 64.0 ± 1.4 years, 8 men) in whom spontaneous angina occurred at rest. All the patients with CSA had angiographically normal coronary arteries and showed angiographically documented coronary spasm associated with ischemic ST segment changes after intracoronary injection of acetylcholine, as previously reported.⁵ Coronary spasm was defined as an abnormal contraction ($\geq 90\%$) of an epicardial coronary artery associated with an attack of chest pain and ischemic ECG changes (ST depression >0.1 mV or ST elevation >0.2 mV in more than 2 leads). We defined multivessel coronary spasm as spasm of 2 or 3 of the 3 major epicardial coronary arteries (right coronary artery (RCA), left anterior descending (LAD) or left circum-

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Table 1. Clinical Characteristics of Study Subjects

	Controls (n=8, 2 men)	CSA (n=25, 8 men)
Age (years)	57.7±4.3	64.0±1.4
Body mass index (kg/m ²)	22.8±0.8	24.1±0.8
Systolic blood pressure (mmHg)	124±6	131±3
Diastolic blood pressure (mmHg)	77±3	76±3
Heart rate (beats/min)	73±4	70±2
Total cholesterol (mg/dl)	190±10	208±6
Triglycerides (mg/dl)	103±16	122±9
HDL-cholesterol (mg/dl)	50±4	55±4

Values are expressed as mean±SEM.

CSA, coronary spastic angina; HDL, high-density lipoprotein.

flex artery). The study also included 8 control subjects (mean age 57.7±4.3 years, 2 men). The control subjects underwent diagnostic cardiac catheterization for evaluation of chest pain. They had angiographically normal coronary arteries and did not show coronary spasm after intracoronary injection of acetylcholine. Control subjects were studied to compare the baseline data with those in patients with CSA. All the subjects were normotensive (<160/100 mmHg), did not have either diabetes (fasting blood sugar <110 mg/dl and HbA_{1c} <5.8%) or a smoking habit, and had a plasma total cholesterol <240 mg/dl. These risk factors for atherosclerosis have been shown to be associated with impaired endothelium-dependent vasodilation. All the female patients were postmenopausal and were not on hormone replacement therapy. None of the study subjects had a history of previous myocardial infarction, congestive heart failure or other serious diseases. Written informed consent was given by all participants, and the hospital's Ethics Committee approved the study protocol.

Study Protocol

Consecutive patients with CSA were randomized to 1 of 3 CCB groups: benidipine 8 mg/day (2 men, 7 women; age 63.5±2.2 years), diltiazem 200 mg/day (4 men, 4 women; age: 65.0±2.2 years) and verapamil 120 mg/day (2 men, 6 women; age: 62.3±2.9 years). Medication was continued for 3 months. No other medications affecting vascular tone or endothelial function, such as nitrates, angiotensin-converting enzyme inhibitors, angiotensin II receptor blocker, aspirin or hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), were administered to any of the study patients. Measurement of blood pressure, pulse rate and

flow-dependent vasodilation, and blood sampling were performed before and after 3 months' treatment. All medications except CCBs were withdrawn for at least 24 h before the measurements were taken.

Vascular Function

FMD and dilatation by nitroglycerin were assessed according to a method described previously!¹⁹ In brief, the diameter of the right brachial artery was measured by high-resolution ultrasound cardiography (Aplio, Toshiba, Tokyo, Japan). To produce reactive hyperemia, blood flow to the forearm was prevented by inflation of the cuff on the arm to at least 50 mmHg above systolic pressure for 5 min. The diameter was measured from the anterior to the posterior interface between the media and adventitia, and it was calculated from 3 cardiac cycles synchronized with the R-wave peaks on ECG. Measurement at 60 s after cuff release showed the maximal dilatation. The diameter change was expressed as the percent change relative to diameter during the initial resting scan (%FMD); 15 min later, a resting scan was recorded and a sublingual nitroglycerin spray (300 µg, Toa Eiyo Co, Tokyo, Japan) was administered and 3 min later, the last scan was performed. The diameter change was expressed as percent dilatation by nitroglycerin. All arterial diameters were measured by an observer who was unaware of the subject grouping.

Blood Sampling

Blood was sampled on the morning of the ultrasound examination before and after treatment. Serum total cholesterol, triglyceride and high-density lipoprotein cholesterol levels were measured. The concentration of plasma cyclic guanosine 3',5'-monophosphate (cGMP) was also measured by radioimmunoassay (SRL Co, Tokyo, Japan).

Statistical Analysis

The data were compared using ANOVA or unpaired t-test. A value of P<0.05 was considered significant. All data are expressed as mean±SEM.

Results

Comparison of Patients and Control Subjects

There were no differences between groups when compared for age, body mass index, systolic and diastolic blood pressures, heart rate, total cholesterol, triglyceride and high-density lipoprotein-cholesterol (Table 1). Brachial artery

Figure 1. Percent increase in vessel diameter induced by flow-mediated dilatation (FMD, **a**) or sublingual nitroglycerin (NTG, **b**) treatment in control and patients with coronary spastic angina (CSA). Values are mean±SEM.

Table 2. Clinical Characteristics of Patients With CSA

	Benidipine group (n=9, 2 men)		Diltiazem group (n=8, 4 men)		Verapamil group (n=8, 2 men)	
	Before	After	Before	After	Before	After
Age (years)	63.5±2.2	–	65.0±2.2	–	62.3±2.9	–
Body mass index (kg/m ²)	26.0±2.2	–	24.0±1.1	–	23.4±1.7	–
Systolic blood pressure (mmHg)	131±5	123±7	135±5	134±7	127±4	124±6
Diastolic blood pressure (mmHg)	79±4	78±6	76±5	84±6	72±4	80±4
Heart rate (beats/min)	67±5	67±5	69±3	65±4	74±2	69±2
Total cholesterol (mg/dl)	205±14	–	195±4	–	216±8	–
Triglycerides (mg/dl)	119±21	–	106±12	–	136±18	–
HDL-cholesterol (mg/dl)	59±8	–	48±9	–	57±6	–
Involved artery in provocation test						
Left main artery	0 (0%)	–	0 (0%)	–	0 (0%)	–
LAD	7 (78%)	–	6 (75%)	–	6 (75%)	–
LCX	4 (44%)	–	4 (50%)	–	3 (38%)	–
RCA	7 (78%)	–	7 (88%)	–	5 (63%)	–
Multivessel spasm	6 (67%)	–	5 (63%)	–	4 (50%)	–

Values are mean±SEM.

LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery. Other abbreviations see in Table 1.

Figure 2. Percent increase in vessel diameter induced by flow-mediated dilatation (FMD, **a**) or sublingual nitroglycerin (NTG, **b**) in patients with coronary spastic angina before and after 3-month treatment with benidipine, diltiazem or verapamil. Values are mean±SEM.

FMD was significantly impaired in patients with CSA compared with control subjects ($4.8\pm0.6\%$ vs $9.9\pm2.1\%$, $P<0.05$; **Figure 1**). In contrast, the difference in nitroglycerin-induced dilatation between the patients and control group was not significant ($11.8\pm0.9\%$ vs $15.6\pm2.9\%$, NS; **Figure 1**).

Effects of CCBs on Vascular Function

At baseline, blood pressure, heart rate, total cholesterol, triglyceride and high-density lipoprotein cholesterol did not differ among the 3 treatment groups. After 3 months' treatment, systolic and diastolic blood pressures did not differ. None of the CCBs administered caused significant changes in blood pressure after treatment (**Table 2**). Both diltiazem and verapamil decreased heart rate after treatment, but the decreases showed no significant differences (**Table 2**). Of the patients among the 3 treatment groups, the LAD and RCA were commonly involved in the spasm provocation tests (**Table 2**). Throughout the study, 2 patients in each of the diltiazem and verapamil groups had an anginal attack during the treatment. None of the patients in the benidipine

group had anginal attacks during the treatment.

Brachial artery FMD at baseline did not differ among the 3 groups. Benidipine significantly increased FMD after treatment compared with baseline ($4.7\pm0.6\%$ to $7.4\pm1.1\%$, $P<0.05$). In contrast, there were no significant differences between the pre- and post-treatment FMD values in the diltiazem ($4.7\pm1.3\%$ to $6.3\pm0.8\%$, NS) and verapamil groups ($5.8\pm0.8\%$ to $6.2\pm0.8\%$, NS) (**Figure 2a**). None of the CCBs affected nitroglycerin-induced dilatation (benidipine: $13.0\pm2.2\%$ to $12.5\pm1.4\%$, NS; diltiazem: $12.1\pm1.6\%$ to $12.6\pm1.4\%$, NS; verapamil: $11.9\pm0.8\%$ to $11.3\pm0.7\%$, NS) (**Figure 2b**).

Effects of CCBs on Plasma cGMP Levels

Plasma cGMP levels were significantly elevated after treatment with benidipine (2.3 ± 0.4 pmol/ml to 3.4 ± 0.5 pmol/ml, $P<0.05$), whereas the changes from baseline were not significant with diltiazem (2.8 ± 0.3 pmol/ml to 2.7 ± 0.4 pmol/ml, NS) or verapamil (3.7 ± 0.9 pmol/ml to 3.0 ± 0.4 pmol/ml, NS) (**Figure 3**).

Figure 3. Plasma cyclic guanosine 3',5'-monophosphate (cGMP) levels in patients with coronary spastic angina before and after 3-month treatment with benidipine, diltiazem or verapamil. Values are mean $\pm$ SEM.

Discussion

CCBs are widely used to treat patients with CSA. The coronary smooth muscle of patients with vasospastic angina is hypercontractile to various stimuli such as histamine,²⁰ ergonovine,⁷ serotonin⁸ etc. In addition to an altered contractile response of coronary smooth muscle, impaired endothelial function, especially NO function, is associated with coronary vasospasm.^{21,22} In the present study, we showed that endothelium-dependent FMD was decreased in vasospastic angina patients compared with control subjects, thus confirming the impaired vascular endothelial function in these patients and in agreement with previous reports.^{11,12} Furthermore, of the 3 CCBs, only benidipine, a dihydropyridine, provided a significant improvement in endothelium-dependent FMD and plasma cGMP concentration in patients with coronary vasospasm. Diltiazem and verapamil, non-dihydropyridine-type CCBs, had no effect on FMD or cGMP. These findings suggest that different types of CCBs have different effects on vascular endothelial function in patients with CSA.

Endothelium-dependent FMD of the brachial artery evaluated by high-resolution ultrasound has been studied extensively in recent years.¹⁹ It is believed to reflect vascular NO function, because an increase in wall shear stress due to the increase in blood flow activates endothelial NO synthase and subsequent production of NO. NO evokes vasorelaxation by activating guanylate cyclase, thereby increasing cGMP concentration. Thus, endothelium-dependent FMD can be imaged and quantified as an index of vasomotor function. Endothelial dysfunction can be regarded as a systemic disorder and thus measured in different vascular beds. The method for measuring FMD used in the present study is clinically attractive because it is noninvasive and allows repeated measurements over time for studying the effectiveness of various interventions that may affect vascular health. A growing body of evidence suggests that endothelial dysfunction is associated with cardiovascular events, so assessment of FMD may be useful for predicting the likelihood of such events.²³

This is the first report to show different effects of CCBs on vascular endothelial function in patients with CSA. Although a number of reports have focused on endothelial function, a lack of consensus exists concerning the utility of calcium antagonists in the management of endothelial dysfunction.²⁴ Of the 3 CCBs studied, only benidipine significantly improved endothelium-dependent FMD and cGMP. Benidipine is a dihydropyridine CCB developed in Japan. It has a slow-onset, long-lasting action with high affinity and selectivity for blood vessels²⁵ and has been widely used to treat angina pectoris and hypertension.

The precise mechanism by which benidipine improves endothelial dysfunction is presently unclear. To our knowledge, L-type calcium channels have not been demonstrated in vascular endothelial cells. Accordingly, the effect of benidipine on endothelial function is most likely a calcium-channel-independent action. The fact that diltiazem and verapamil, the other L-type CCBs, showed no such effect lend support for that possibility. In this study, which included normotensive patients, none of the 3 CCBs had significant effects on blood pressure. Furthermore, blood pressure at the end of 3 months of treatment did not differ among the 3 groups. Therefore, the blood-pressure-lowering effect of benidipine is of little importance in the improvement of endothelial function and increase in cGMP, to which some secondary mechanisms may contribute.

There are some possible explanations for the observation that only benidipine improved endothelial function. The first is direct enhancement of NO production. The increase in plasma cGMP supports the hypothesis that benidipine improves NO function. Previous studies using animal models have demonstrated that benidipine increases endothelial NO synthase gene expression and enhances NO production.^{26–28} A recent study has shown that benidipine improves decreases in tetrahydrobiopterin, an essential cofactor of NO synthase, in the plasma and kidneys, leading to reduction of proteinuria in a rat model of type II diabetes.²⁸ Preservation of tetrahydrobiopterin levels by benidipine may be partly involved in enhancement of NO production. In contrast to benidipine, apparent enhancement of NO production has not been reported for diltiazem¹⁵ or verapamil.²⁹ The second possible explanation is antioxidant. Numerous reports have referred to the antioxidant effect of benidipine³⁰ which is more potent than that of diltiazem or verapamil.³¹ Decreased free radicals contribute to the prolongation of NO. This antioxidant effect of benidipine may contribute to the improvement of endothelial function. The third reason is differences in the cardiovascular profile, including vasoselectivity, inhibition of vascular smooth muscle contraction and reduction of myocardial contractility, among CCBs.¹⁸ More particularly, phenylalkylamine and benzothiazepines have much in common, showing a similar selectivity for heart and vessels and a relatively large reduction in myocardial contractility, whereas dihydropyridines are characterized by a high degree of vasoselectivity. Previous studies comparing the impact of benidipine on the myocardium and coronary arteries indicated that the compound exhibited vasoselectivity, more remarkable than either diltiazem or verapamil.²⁵ This vasoselectivity combined with the aforementioned mechanisms is likely to improve endothelial function.

In the present study, anginal attacks were completely suppressed only by benidipine during the treatment. A retrospective cohort study in Japanese patients demonstrated a significant improvement in prognosis with benidipine compared with other CCBs, as well as its utility in long-term

treatment of CSA.³² The beneficial effect of benidipine on vascular endothelial function as observed in the present study may be associated with an improved prognosis for patients with CSA.

Study Limitations

The prevalence of CSA is commonly higher in male patients than in female patients. In the present study, however, female subjects with CSA were predominant in the benidipine and verapamil groups. Many male smokers diagnosed with CSA were excluded from the study, which may explain the higher proportion of women. The small number of patients analyzed and the lack of data concerning other dihydropyridine CCBs hinder us from determining whether the favorable effect on endothelial function is peculiar to benidipine or common among dihydropyridine CCBs. This should be further investigated and future studies should also focus on the association between the time course of FMD change and cardiovascular events.

Conclusions

Benidipine, but not diltiazem or verapamil, protects against vascular endothelial dysfunction in patients with CSA. This property is independent of its blood-pressure-lowering effect and may be partly due to upregulation of the NO–cGMP system. Thus, CCBs have differential effects on vascular endothelial function in patients with CSA. Dihydropyridine-type CCBs may be more beneficial to vascular endothelial function than non-dihydropyridine-type CCBs, but it needs to be clarified in future studies whether this beneficial effect is common among the dihydropyridine-type CCBs.

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