

# Calcium antagonist lercanidipine improves endothelium-dependent vasodilatation of the brachial artery in patients with proved coronary vasospasm

Silvija Janavičienė\*, Aleksandras Laucevičius, Eduardas Šubkovas,  
Žaneta Petrulionienė, Aleksandras Kibarskis, Ligita Ryliškytė

Centre of Cardiology and Angiology, Vilnius University Hospital Santariškių Klinikos; Heart Clinic, Vilnius University, Vilnius, Lithuania

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## Summary

**Objectives:** Some data show that endothelial function is impaired in coronary arteries as well as in the brachial arteries of patients with coronary vasospasm. Calcium antagonists could improve endothelium-dependent vasodilatation. The main aim of our study was to assess whether treatment with dihydropyridine calcium antagonist lercanidipine can improve endothelium-dependent vasodilatation in the brachial artery in patients with proved coronary vasospasm. The second aim was to assess, whether the improvement of endothelial function can relieve patients with coronary vasospasm of chest pain attacks.

**Design and Methods:** The study included 63 patients with coronarographically proved coronary vasospasm. Subjects were assigned to treatment with lercanidipine without changing current treatment for six months. An exercise stress test, flow-mediated dilatation (FMD), blood samples, the Seattle Angina Questionnaire were performed at baseline and after 6 months of treatment.

**Results:** There was a significant ( $p = 0.0004$ ) improvement of FMD ( $4.7 \pm 4.0$ – $8.1 \pm 4.2$ ) after 24-week treatment with lercanidipine compared with baseline FMD results. Also, the present study demonstrated a significant improvement of chest pain characteristics using the Seattle Angina Questionnaire ( $p = 0.0001$ ).

**Conclusion:** The present study has demonstrated that a six-month treatment with lercanidipine improves flow-mediated dilatation in the brachial artery in patients with coronary vasospasm. Improvement of endothelial function in the brachial artery under the treatment with lercanidipine is accompanied by the improvement of chest pain characteristics and stress-induced ischaemic changes.

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**Keywords:** coronary vasospasm, calcium antagonist, endothelium, atherosclerosis, vasodilatation

Coronary vasospasm plays a principal role in the pathogenesis of variant angina and is important in all forms of coronary heart disease, including myocardial infarction and sudden cardiac death [1]. Coronary vasospasm has been thought to correlate with severity of atherosclerosis and endothelial dysfunction [2]. The coronary vasomotor response to the infused NO agonists such as acetylcholine is an accepted method for assessing endothelium-dependent vasomotor function. Acetylcholine dilates normal coronary arter-

ies but produces vasoconstriction in atherosclerotic and diseased coronary arteries [3]. This vasoconstriction occurs early in angiographically normal arteries as well as in late stages of atherosclerosis [4]. The effect of statins and other cardiovascular drugs on coronary vasodilatation was found by the authors of the article [5]. However, the results of subsequent studies are not consistent in this relation [6,7]. Since the flow-mediated dilatation in the brachial artery is a non-invasive method, this technique could be more applicable in large studies for establishing endothelium dysfunction in coronary vasospasm [6].

A dysfunctioning endothelium loses its ability to exert a protective effect on the cardiovascular system by keeping vessels in a dilatory state, preventing platelets adhesion, smooth muscle cell

\* Corresponding address: Silvija Janavičienė, Centre of Cardiology and Angiology, Vilnius University Hospital Santariškių Klinikos, Santariškių str. 2, 08661 Vilnius, Lithuania.

E-mail: silvija.janaviciene@santa.lt (S. Janavičienė).

proliferation and migration, and adhesion molecule expression and therefore playing a major role in the development of atherosclerosis [8]. Hence, an important aim of treatment should be improving the endothelial dysfunction.

Calcium antagonists could improve endothelium-dependent vasodilatation, and this effect is related to the antioxidant activity that in turn can lead to the restoration of NO availability [9–14]. Probably, treatment with calcium antagonists prevents production of the hyperpolarizing factor [9]. Previous investigations studied the effect of calcium antagonists on coronary vasomotion [10,11] and on endothelium dysfunction in the peripheral arteries [12–16].

The main aim of our study was to assess whether treatment with dihydropyridine calcium antagonist lercanidipine can improve endothelium-dependent vasodilatation in the brachial artery in patients with proved coronary vasospasm. The second aim was to assess, if the improvement of endothelial function can relieve patients with coronary vasospasm of chest pain attacks.

## Design and Methods

The study included 63 patients with coronarographically proved coronary vasospasm. The diagnosis of coronary vasospasm was based on the following criteria:

1. Typical retrosternal chest pain.
2. No abnormalities on a 12-lead electrocardiogram (ECG) at rest in the absence of chest pain.
3. Normal left and right ventricular function, assessed by echocardiography.
4. Absence of other heart disease.
5. Normal coronary angiograms at visual analysis.
6. A positive selective coronary artery spasm provocative test with acetylcholine.

Additional criteria were:

1. Ischaemia-like ECG changes during the exercise stress test (horizontal or down sloping ST-segment depression > 0.1 mV).
2. A positive stress echo test.
3. Ischaemia-like ECG changes during chest pain at rest.
4. Prior myocardial infarction without left ventricular dysfunction.

Subjects were excluded from the study in cases of malignancy, kidney or liver failure, drug or alco-

hol abuse, systemic inflammatory disease or having contraindications to be treated with calcium antagonists.

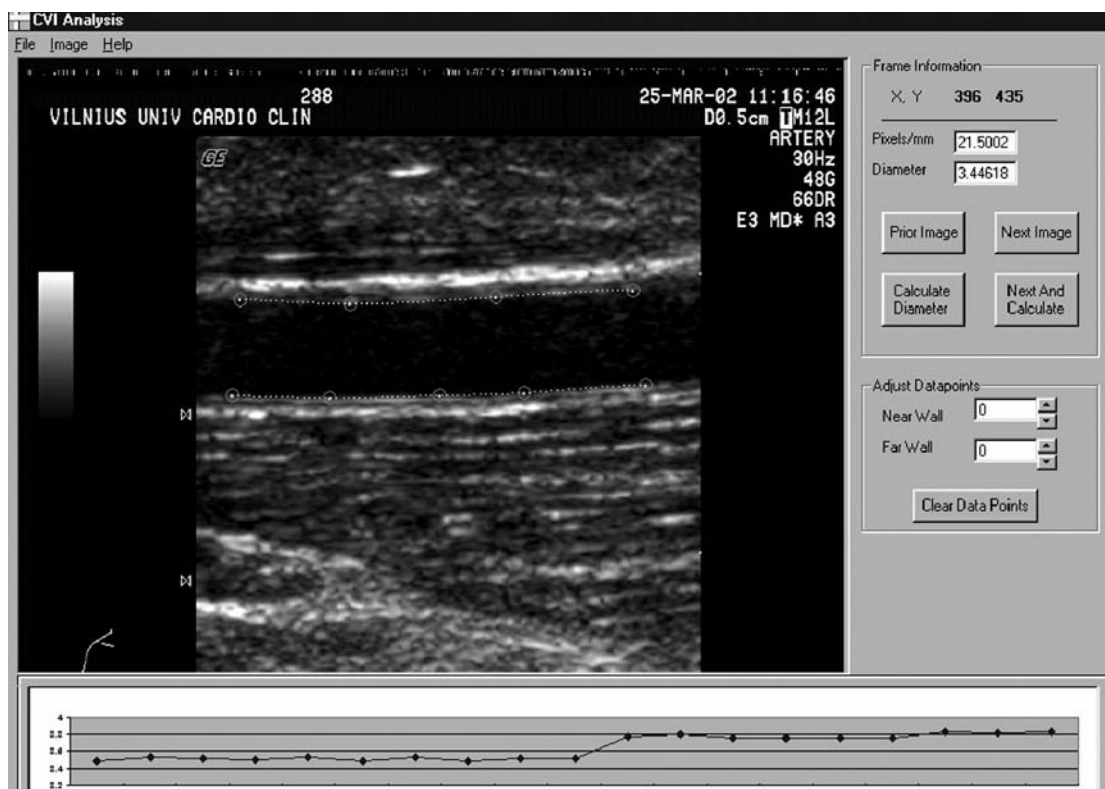
### *Flow-mediated dilatation of the brachial artery*

The endothelium-dependent flow-mediated dilatation (FMD) test in the brachial artery was performed using the method described by Celermajer et al [17] and adapted by the authors of the article [18] according to the international recommendation [19] and technical equipment used in our department.

For FMD, a B-mode of the right brachial artery was obtained in longitudinal section between 1 cm and 8 cm above the elbow using a 13 MHz linear array transducer and a LOGIQ 700 system (GE ultrasound). B-mode images were triggered to the ECG signal to obtain only end-diastolic frames and acquired on a computer using a commercial software program CVI Acquisition v.1.0.0.3 (1998 Information Integrity Inc.). Brachial artery diameter measurements were performed using a special software program CVI Analysis v.1.0.0.1 (1998 Information Integrity Inc.). Subjects were non-smoking, refrained from eating, drinking alcohol and coffee at least 12 hours prior to the study. The endothelium-dependent flow-mediated dilatation test in the brachial artery was performed in the supine position in a quiet, temperature controlled room (22–24°C). A cuff was placed around the forearm just below the elbow. Endothelium-dependent response was assessed as dilatation of the brachial artery to increased flow (flow-mediated dilatation, FMD). After 15–20 seconds of acquisition for measuring the basal diameter, the cuff was inflated for five minutes 100 mmHg above the systolic pressure and then deflated to induce reactive hyperemia. Brachial artery diameter measurements were performed after studying the acquired frames. The baseline vessel size was considered as the mean of the first 15–20 measures obtained during the first minute. FMD was calculated as the maximal percent increase in diameter above baseline (Figure 1).

### *Coronary artery spasm provocative test*

Coronary arteriography was obtained by a Sones technique using femoral approach with a 6F diagnostic catheter without any medication for at least 24 hours. A bipolar electrode catheter was inserted into the right ventricular apex through the femoral vein and was connected to a temporary pacemaker set at the rate of 50 beats/min to prevent bradycardia during acetylcholine (ACH) infusion. Acetylcholine was injected in incremental doses of 6, 20 and 60 mg into the left coronary artery over 2 minutes with



**Figure 1.** After 15–20 seconds of acquisition for measuring basal diameter, the cuff was inflated for five minutes 100 mmHg above the systolic pressure and then deflated to induce reactive hyperemia.

at least a 2 minutes interval between each injection. Then monoplane coronary arteriograms were taken to assess the lumen diameter of large epicardial coronary segments. When epicardial coronary spasm with coronary diameter reduction > 50% was provoked at any dose of ACH, 300 mcg of isosorbide dinitrate bolus was injected into the vessel and the provocation test was finished. During the study, arterial blood pressure and an ECG were continuously monitored. Lumen diameters of both the left descending and the circumflex coronary artery were evaluated during the end diastole by a quantitative coronary angiographic system (Medis Medical Imaging Systems, Neunen, Netherlands). The tip of the 6F diagnostic catheter was used for calibration. Measurements were done three times at 6 segments of the left coronary artery (proximal, middle and distal segments of the left anterior descending artery and the left circumflex artery). The segment that showed the largest constrictor response was used for the analysis. We defined epicardial coronary artery spasm as diameter reduction > 5% as compared with diameter on initial angiography (Figure 2).

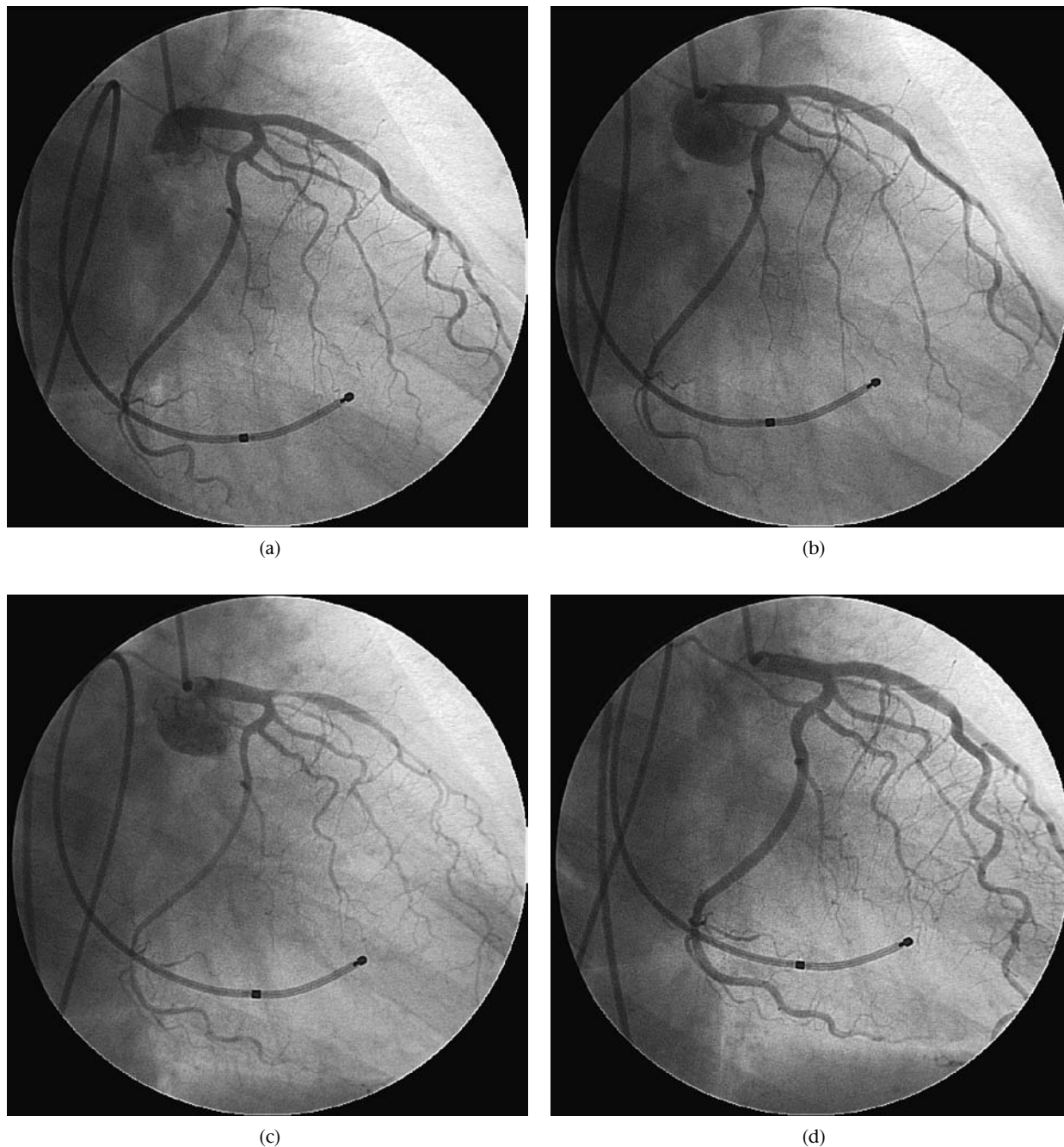
#### **Angina questionnaires**

The Seattle Angina Questionnaire (SAQ) to quantify the physical and emotional effects of

coronary artery disease [20] was used in this study. SAQ was translated into Lithuanian and adapted to national population. The questionnaire quantifies patient's symptom frequency, symptom stability, satisfaction with treatment and quality of life before and after treatment with lercanidipine. All parameters of angina were transferred into score. Analyses score ranged from 0 to 100. A higher score indicates better function.

#### **Study protocol**

Angiography and a coronary artery provocative test were performed in all subjects to enter the study. The assessment of flow-mediated dilatation of the brachial artery was performed in subjects with a positive acetylcholine test. All the studies were performed in the morning, after an overnight fast. A baseline exercise test was performed, blood samples for total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides, fibrinogen, serum glucose were collected. Urine samples were drawn to measure microalbuminuria. An oral glucose tolerance test was performed when glucose metabolism abnormalities were suspected [15]. Flow-mediated vasodilatation was performed in all the subjects entering the study. Subjects were assigned to treatment with lercani-



**Figure 2.** (a) – Coronary artery after the first dose of acetylcholine, injected into the left coronary artery, (b) – coronary artery after the second dose of acetylcholine, (c) – coronary artery after the third dose of acetylcholine, (d) – coronary artery after isosorbide dinitrate bolus.

dipine without changing current treatment for six months. Anginal symptoms were assessed using the Seattle Angina Questionnaires at the beginning and the end of the study. On follow-up, FMD and blood samples were collected after 1 and 3 months. Exercise stress test, FMD, blood samples were repeated after 6 months of treatment.

The study complied with the Declaration of Helsinki and local ethic/scientific committee approved the protocol.

**Results**

*Clinical characteristics of the patients before and after treatment*

53 patients completed the study without any side effects during the follow-up period. In total, 10 patients were withdrawn before the completion of the study: one patient with myocardial infarction on the 15th day of treatment, 9 patients – because of missing values. Baseline systemic demographic, hemodynamic character-

**Table 1.**

Baseline systemic, demographic, hemodynamic characteristics of patients with coronary vasospasm

Clinical characteristics	Baseline
Age, years	57.5 ± 9.3
Gender, M/F	18/35
Smokers, yes/no	13/40
BMI, kg/m <sup>2</sup>	29.9 ± 4.2
Hypercholesterolemia > 5.2 mmol/l, yes/no	31/22
Arterial hypertension (BP > 140/90), yes/no	38/15
Impaired glucose tolerance yes/no	11/32

BMI – body mass index; BP – blood pressure; F – female; M – male.

**Table 2.**

Clinical characteristics of patients with coronary vasospasm before and after 24-week treatment with lercanidipine

Clinical characteristics	Baseline	After 24 weeks of treatment	<i>p</i>
Systolic BP, mmHg	148 ± 22	139 ± 19	0.03
Diastolic BP, mmHg	89 ± 11	87 ± 13	0.49
HR, beats/min	76 ± 12	70 ± 10	0.02
Total cholesterol, mmol/l	6.2 ± 1.2	5.7 ± 0.9	0.05
HDL cholesterol, mmol/l	1.4 ± 0.3	1.3 ± 0	0.26
LDL cholesterol, mmol/l	4.2 ± 1.2	3.7 ± 0.9	0.04
Triglycerides, mmol/l	1.7 ± 1	1.9 ± 0	0.09
Glucose, mmol/l	5.8 ± 1.6	5.4 ± 0.7	0.29

BP – blood pressure; HDL – high-density lipoprotein; HR – heart rate; LDL – low-density lipoprotein.

istics of the patients are summarized in Table 1. During the course of the study, there were no deaths and no hospitalisation for chest pain. The mean age of the subjects studied was 57.5 ± 9.3 years. Study population consisted of 33.9% males and 66.1% females. Cardiovascular risk factors were present as follows: hypertension in 71.4%, hypercholesterolemia in 58.8%, impaired glucose tolerance or diabetes mellitus in 21%. The current treatment was not changed during the course of the study and was present as follows: beta-adrenoblockers (BAB) 34.9%, angiotensin converting enzyme (ACE) inhibitors 61.9%, diuretics 14.3%. The lipid lowering diet was recommended to all patients with dyslipidemia. All the patients were free of statins for the study period. Statistically significant reduction of blood pressure and decrease in total and LDL cholesterol levels were observed during the treatment (Table 2). No changes in smoking habits in 13 patients who smoked were observed during the treatment period.

### Seattle angina questionnaire results

The results of the Seattle Angina Questionnaire are presented in Table 3. At baseline, the num-

**Table 3.**

Seattle Angina Questionnaire at baseline and after 24-week treatment

Characteristic	Baseline	24-week treatment	<i>p</i>
Physical limitation	23 ± 18	40 ± 19	0.0001
Angina frequency	35 ± 28	53 ± 28	0.002
Quality of life	22 ± 17	39 ± 22	0.005
Summary score	44 ± 12	87 ± 20	0.0001

ber of chest pain episodes per month was 25. The average duration was 15 min. After 24-week treatment with lercanidipine, the number of chest pain episodes reduced up to 5 episodes per month with the average duration of 5 min.

### Results of flow-mediated dilatation of the brachial artery

The results of FMD of the brachial artery at baseline are summarized in Table 4. There was a significant (*p* = 0.0004) improvement of FMD (4.7 ± 4.0–8.1 ± 4.2) after 24-week treatment with lercanidipine compared with baseline FMD results (Figure 3). A statistically significant positive change in FMD was found after treatment with lercanidipine in patients with coronary vasospasm in the whole group of patients independently of the presence or absence of hypertension, hypercholesterolemia and treatment with betaadrenoblockers or ACE inhibitors.

## Discussion

Vasospastic angina – the occurrence of spasm at the site of angiographically normal arterial segments, is a less common finding than chronic stable angina [21]. Spontaneous remission is a frequent outcome in Western people with vasospastic angina and these patients could be asymptomatic during long-term treatment with calcium antagonists [22]. Calcium antagonists, that are effective in suppressing coronary vasospasm, act not only by interfering with the entry of calcium into smooth muscle cells and directly inducing smooth muscle relaxation [23–26], but also by improving the coronary artery endothelial function [9–15].

Many reports on patients with coronary vasospasm have identified impaired coronary flow responses to acetylcholine, which implies impairment of endothelium-dependent vasodilatation [27–29]. But the precise mechanism of endothelial dysfunction in patients with coronary vasospasm is still to be determined.

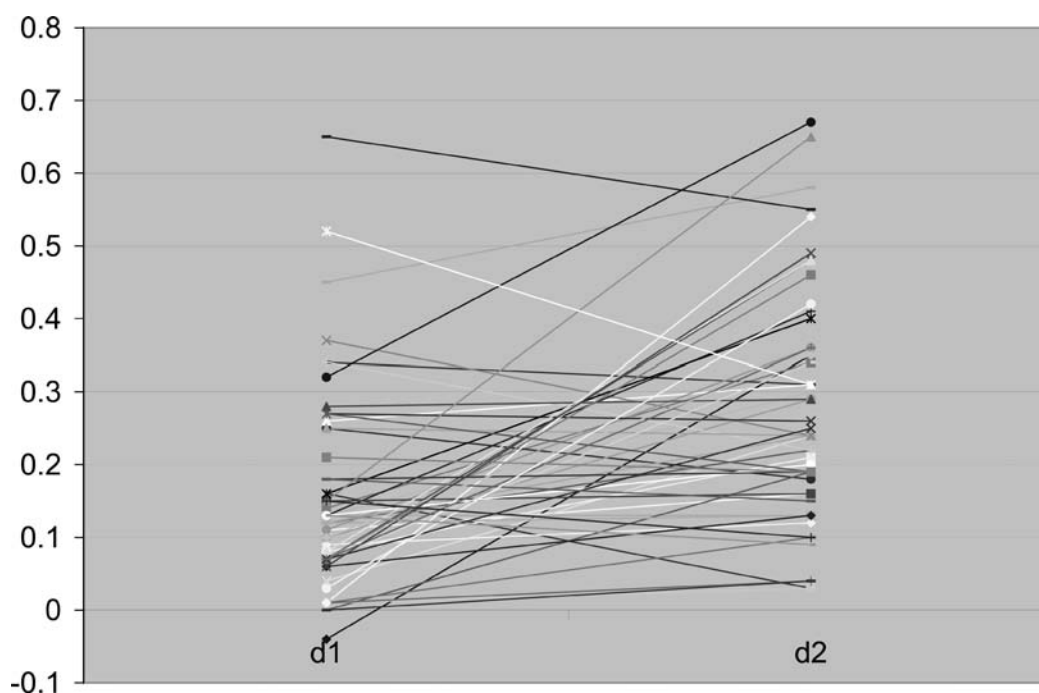
Previous studies had shown that basal NO availability may be decreased in spastic coronary

**Table 4.**

The results of the diameter and flow-mediated dilatation of the brachial artery before and after 24-week treatment with lercanidipine

	Diameter, mm		<i>p</i>	FMD, %		<i>p</i>
	Baseline	After 6 months		Baseline	After 6 months	
Men	3.9 ± 0.5	3.9 ± 0.7	0.94	2.7 ± 2.4	6.3 ± 2.6	0.0004
Women	3.2 ± 0.5	3.3 ± 0.4	0.46	5.8 ± 4.4	8.0 ± 4.8	0.005
With hypertension	3.3 ± 0.5	3.5 ± 0.5	0.37	5.2 ± 3.7	7.9 ± 4.1	0.001
Without hypertension	3.6 ± 0.7	3.5 ± 0.8	0.24	3.6 ± 4.5	6.5 ± 4.5	0.002
Hypercholesterolemia	3.2 ± 0.5	3.4 ± 0.5	0.47	5.6 ± 4.5	6.9 ± 3.8	0.004
Normocholesterolemia	3.6 ± 0.7	3.6 ± 0.7	0.77	3.8 ± 3.2	8.1 ± 4.6	0.0004
With diabetes mellitus	3.4 ± 0.4	3.4 ± 0.4	0.58	3.5 ± 0.2	3.8 ± 0.2	0.012
Without diabetes	3.5 ± 0.5	3.5 ± 0.3	0.55	3.6 ± 0.6	3.8 ± 0.4	0.002
Total	3.4 ± 0.6	3.5 ± 0.6	0.76	4.7 ± 4.0	8.1 ± 4.2	0.0004

FMD – flow-mediated dilatation.



**Figure 3.** Flow-mediated dilatation (FMD) of the brachial artery at baseline and after 24 weeks of treatment with lercanidipine. FMD changes of different patients: the increase of FMD was observed in 35 patients (66%), the decrease of FMD was observed in 17 patients (32%), no changes in FMD – in 1 patient (1.9%).

arteries, leading to increased basal coronary artery tone [30,31]. Moreover, the decrease in endothelial NO production after intracoronary injection of acetylcholine in patients with coronary vasospasm, may be responsible for the increase in the oxidative stress [32]. Furthermore, some data suggests that patients with coronary vasospasm might have increased plasma endothelin-1 (ET-1) concentration [33,34] or might have enhanced tendency to release ET-1 under stress circumstances [35]. Also, one of the possible mechanisms could be an increase of ICAM-1 and VCAM-1 levels in patients with coronary artery disease and coronary vasospasm compared with normal subjects [36]. These findings may suggest the presence of chronic inflammation with

the involvement of the endothelium in patients with chest angina pain and normal coronary angiograms.

Generalized endothelial dysfunction could be a common pathogenesis mechanism leading to the development of atherosclerosis. The reversal of endothelium dysfunction by pharmacological treatment could be important in the prevention of vascular diseases associated with atherosclerosis. In experimental hypertension models, endothelium dysfunction was shown to reverse with antihypertensive treatment [37–40], while in humans, the results of possible improvement of endothelium function by antihypertensive treatment are still inconsistent [13, 41–43]. The beneficial effect of calcium antag-

onists could be related to antioxidant properties, with consequent enhanced nitric oxide activity [44,45]. However, mechanisms of action by which calcium antagonists exert their anti-atherosclerotic effects have not been completely elucidated. Recent studies *in vitro* suggest that dihydropyridine calcium antagonists have antioxidant properties, antiproliferative effects on vascular smooth muscle cells [45], suppressive effect on adhesion molecule expression [45]. These activities are independent of calcium flux across voltage-operated calcium channels [44]. They are determined firstly by calcium antagonist's lipophilicity and then by their intrinsic antioxidant activity [46]. The data of studies with the animal models suggest, that dihydropyridine calcium antagonists increase the endothelial NO bioavailability, firstly through enhanced NO formation and secondly by prolonging the half-life of NO through antioxidant properties [46,47].

So, the results are not consistent on effects of calcium antagonists in humans. An improved relaxation after acetylcholine in small resistance arteries after 1 year of therapy with dihydropyridine calcium antagonist nifedipine was observed in one of the studies, while in this study no change was observed in patients treated with atenolol, despite of an equivalent hypotensive effect of both drugs [48]. Moreover, in essential hypertensive patients, dihydropyridine calcium antagonist lacidipine increased the response to acetylcholine and bradikinin when infused into the brachial artery and restored endothelium-dependent vasodilatation after 8 and 32 weeks of treatment [13]. In agreement with previous observations [12,15], the restoration of NO availability and inhibition of hyperpolarization was found after 3-month treatment with dihydropyridine calcium antagonist lercanidipine [12]. On the contrary, some studies demonstrated that treatment with calcium antagonist did not improve the endothelium-dependent vasodilatation in hypertensive patients [41–43].

The present study demonstrated that a six-month treatment with lercanidipine improves flow-mediated dilatation in the brachial artery in patients with coronary vasospasm. Furthermore, lercanidipine changes chest pain characteristics in patients with coronary vasospasm. The improvement of FMD and changes in chest pain characteristics after treatment with lercanidipine suggest that the mechanism of coronary vasospasm is very likely to be an endothelium dysfunction. The mechanism responsible for lercanidipine-induced improvement of endothelial function is very likely to be an oxidant effect [37–39]. Lercanidipine could have an effect on calcium-modulated potassium channels,

which, in turn, can be involved in the vasodilatory properties of the endothelium-dependent hyperpolarizing factor (EDHF) [49]. The possible contribution of the classic effect of calcium antagonist on voltage-gated L-type calcium channels, which are located in the smooth muscle but not in endothelial cells, is excluded by finding that treatment with lercanidipine did not change the response to glyceryl trinitrate in previous studies [12].

In our study we were able to show clearly a statistically significant positive effect of lercanidipine on flow-mediated dilatation in the brachial artery in patients with proved coronary vasospasm, independently whether they were hypertensive or not, had diabetes or dislipidemia or not. So, flow-mediated dilatation in the brachial artery could serve as a non-invasive tool to test the effect of calcium antagonist on endothelial function that could be used in everyday clinical practice. The positive dynamics of clinical signs under the treatment with lercanidipine in patients with vasospastic angina proves that the treatment with lercanidipine could be the treatment of choice in patients with vasospastic angina.

## Conclusions

1. Patients with normal coronary angiograms and positive stress tests for angina pectoris could be the patients with vasospastic angina.
2. Coronary vasospasm could be proved by a direct acetylcholine infusion into the coronary arteries.
3. Flow-mediated dilatation in the brachial artery could serve as a tool for monitoring the effect of calcium antagonist lercanidipine on endothelial function.
4. Improvement of endothelial function in the brachial artery under the treatment with lercanidipine is accompanied by the improvement of chest pain characteristics and stress-induced ischaemic changes.

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