Original articles

Long-term changes in the response of conductance and resistance coronary vessels to endothelium-dependent and independent vasodilators

A double-blind placebo-controlled study of the effect of a 6-month treatment with cilazapril

Carlo Di Mario, Sipke Strikwerda, Robert Gil, Aida Azar, Alexis Rames*, Patrick W. Serruys

*Intracoronary Imaging Laboratory and Cardiac Catheterization Laboratory, Division of Cardiology, Thoraxcenter, Erasmus University, Rotterdam, The Netherlands, *Department of Cardiovascular Research, F. Hoffmann-La Roche, Basel, Switzerland*

Key words:
Acetylcholine; Angiotensin-converting enzyme inhibitors; Atherosclerosis; Doppler ultrasound; Endothelium-derived relaxing factor; Quantitative angiography.

Background. **The aim of this study was to assess the effect of a 6-month treatment with the inhibitor of the angiotensin-converting enzyme cilazapril on the response of conductance and resistance vessels to endothelium-dependent and independent vasodilators, in a randomized placebo-controlled parallel group single center study.**

Methods. **Quantitative angiographic and Doppler flow time-averaged peak velocity measurements were performed in an artery with < 30% diameter stenosis after sequential selective intracoronary injection of papaverine (7 mg), acetylcholine (0.036, 0.36 and 3.6 μg/ml at 2 ml/min) and isosorbide dinitrate. Repeated assessment was performed after a 6-month treatment with cilazapril 20 mg/day or placebo. Thirty-four patients were enrolled in the study undergoing elective percutaneous coronary interventions for stable angina. Main outcome measures were percent differences from baseline and absolute measurements of mean coronary cross-sectional area, coronary flow time-averaged peak velocity and flow resistance in the initial study and at follow-up for the placebo and the treated group.**

Results. **No significant differences between the placebo and the treated group were observed in the modifications of cross-sectional area after acetylcholine and isosorbide dinitrate and in the response of time-averaged peak velocity to papaverine. After the maximal concentration of acetylcholine a high but statistically not significant increase in flow and a decrease in flow resistance were observed in the treated group (medians: 45% increase vs 4% increase for coronary flow, and 44% decrease vs 1% increase for flow resistance in the cilazapril and in the treated group, respectively, p = NS).**

Conclusions. **In patients with coronary artery disease, a 6-month treatment with 20 mg of cilazapril/day did not modify the response to endothelium-independent and dependent vasodilators of epicardial arteries without any significant stenoses but induced a consistent, although not significant, increase in flow and decrease in flow resistance after acetylcholine.**

(Ital Heart J 2000; 1 (10): 674-683)

This study was supported by a Research Grant by F. Hoffmann-La Roche, Basel, Switzerland.

Received January 26, 2000; revision received July 21, 2000; accepted July 27, 2000.

Address:

Dr. Carlo Di Mario

Cardiologia Interventistica
Centro Cuore Columbus
Via Buonarroti, 48
20145 Milano

Introduction

Experimental reports suggest that angiotensin-converting enzyme (ACE)-inhibition can normalize structural and functional changes of the vascular system in the presence of arterial hypertension and atherosclerosis and can prevent intimal hyperplasia after vascular injury. In spontaneously hypertensive rats[1] or after chronic beta-adrenergic stimulation with isoproterenol[2] a prolonged therapy with the long-acting ACE-inhibitor cilazapril induces a complete normalization of the medial thickness of coronary, renal, carotid, and mesenteric arteries. The reduced local release of the powerful vasoconstrictive and mitogenic agent angiotensin II consequent to the inhibition of the tissue-based (vascular) ACE may prevent the intimal thickening[3,4] and avoid infiltration of blood-borne monocytes-macrophages, factors responsible for a reduced subendothelial diffusion and increased inactivation and degradation of the labile nitric oxide[5]. An alternative mechanism can be the stimulation to the production of nitric oxide, endotheli-

um-derived hyperpolarizing factor and prostacyclin because of the reduced breakdown of circulating bradykinin and increased effect of the local vascular kallikrein-kinin systems[6,7].

The clinical experience with ACE-inhibitors in this field is more limited, with most studies performed in peripheral arteries showing conflicting results on conductance and resistance arteries and variations in response to different agents[8-10]. The only randomized trial testing the effect of ACE-inhibition on coronary endothelial function was limited to assess changes in the epicardial vasculature[11].

The novelty of this study is that we assessed the response of both conductance and resistance coronary arteries to endothelium-dependent and independent vasodilators in patients with coronary artery disease after a 6-month treatment with the ACE-inhibitor cilazapril in a randomized placebo-controlled parallel-group double-blind study.

Methods

Patient population. Thirty-four patients (26 men, 8 women, mean age 58 ± 9 years) candidates for elective single-vessel percutaneous revascularization procedure because of the presence of stable angina pectoris, with at least one normal or near-normal major coronary artery (no diameter stenoses > 30%) were enrolled in the study and gave witnessed informed consent.

Clinical exclusion criteria were: systolic blood pressure < 100 mmHg and maintenance therapy with diuretics, ACE-inhibitors or lipid lowering drugs. Angiographic exclusion criteria were the presence of abnormal wall dynamics in the territory of distribution of the studied artery and the presence of visible collaterals from the studied vessels.

Catheterization procedure. All vasoactive medication, with the exclusion of short-acting sublingual nitrates, was withheld at least 48 hours before catheterization. No sublingual, intravenous or intracoronary nitrates were used in the 6 hours before the catheterization procedure. Before catheterization, 10 000 IU of heparin, 250 mg of acetylsalicylic acid and 5-10 mg of diazepam were administered intravenously. The artery to be studied was instrumented using a 9F giant lumen (inner lumen 0.084") Amplatz or Judkins guiding catheter (left coronary artery) or a 7F Judkins diagnostic catheter (right coronary artery). A 0.018" Doppler angioplasty guidewire was then advanced to a normal or near-normal straight proximal segment of the artery to be studied and a stable flow time-averaged peak velocity (APV) signal was obtained. A 3.6F flexible infusion catheter (Tracker 25, Target Therapeutics, San José, CA, USA) was then inserted over the Doppler wire into the proximal segment of the coronary artery in order to obtain a selective injection into the left anterior descending or left circumflex artery. For the right coronary artery a selectively engaged 7F diagnostic catheter was used for the injection. A venous sheath was placed in the femoral vein to facilitate the insertion of a right ventricular pacing catheter, if necessary.

After the baseline acquisition of APV, heart rate and aortic blood pressure, the measurements were repeated 30 s after a bolus injection of 7 mg of papaverine diluted in 1.5 ml of isotonic saline solution. After a recovery period of 8 min, new basal measurements were performed followed by a cineangiogram suitable for quantification. Scalar concentrations of acetylcholine (Dispersa, Wintherthur, Switzerland) at 37°C (0.036, 0.36, 3.6 μg/ml) were infused at a flow rate of 2 ml/min using a precision pump-injector (Mark V, Medrad, Pittsburgh, PA, USA). Using these dilutions and flow rates and assuming a coronary blood flow of 80 ml/min in the studied artery, intracoronary blood concentrations of 10^{-8}, 10^{-7} and 10^{-6} M were estimated. Five minutes after the beginning of the infusion of each concentration, APV and hemodynamic measurements were acquired and a new cineangiogram was performed. Five minutes after the end of the series of acetylcholine infusions, a new baseline APV was acquired and, 1 min after a bolus injection of 3 mg of isosorbide dinitrate, a new cineangiogram was performed.

The mean duration of the study from the arterial puncture to the beginning of the revascularization procedure was 54 ± 15 min and the patients underwent the planned angioplasty procedure.

Treatment allocation and follow-up evaluation. Patients were randomly assigned to placebo or cilazapril. The trial medication was given for the first time in the evening after successful revascularization and consisted of either cilazapril (10 mg bid) or placebo. The study medication or any other vasoactive treatment was withheld at least 48 hours before the follow-up catheterization.

The follow-up assessment of endothelial function was performed according to the previously described protocol.

Data acquisition and analysis. Data on all compliant patients who completed the follow-up period were entered for analysis. A patient was judged compliant when he consumed more than 80% of the trial medication with no interruption longer than 5 days. A 0.018" flexible and steerable angioplasty guidewire mounting a 12 MHz piezoelectric transducer at the tip was used (FloWire, Endosonics Inc., Rancho Cordova, CA, USA). APV (mean of 2 beats)[12,13] (Fig. 1) and coronary flow reserve, defined as the ratio between APV at the peak effect of the papaverine injection and APV at baseline conditions, were measured. The catheter, filmed before filling with contrast medium, was used as a scaling device. The same angiographic view was maintained during the study, avoiding foreshortening or superimposition of side branches on the arterial segments of interest. A previously validated[14] cine-film-based system (CAAS System, Pie Medical Data, Maastricht, The Netherlands) was used to measure the

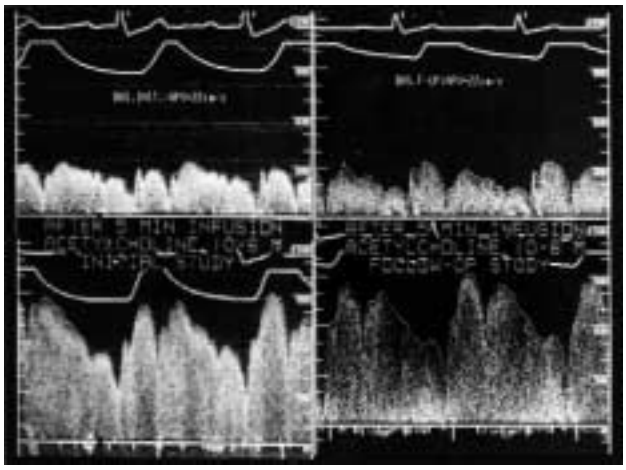

Figure 1. *Flow time-averaged peak velocity (APV, cm/s) measurement in a proximal right coronary artery at baseline* (upper panels) *and after 5 min of infusion of the maximal acetylcholine concentration (10^{-6} M) of acetylcholine* (lower panels). *Note the similar pattern and value of APV in the initial and follow-up assessment. APV scale: 120 cm/s in all panels. BAS.INIT. = basal assessment initial study; BAS.F-UP = basal assessment follow-up.*

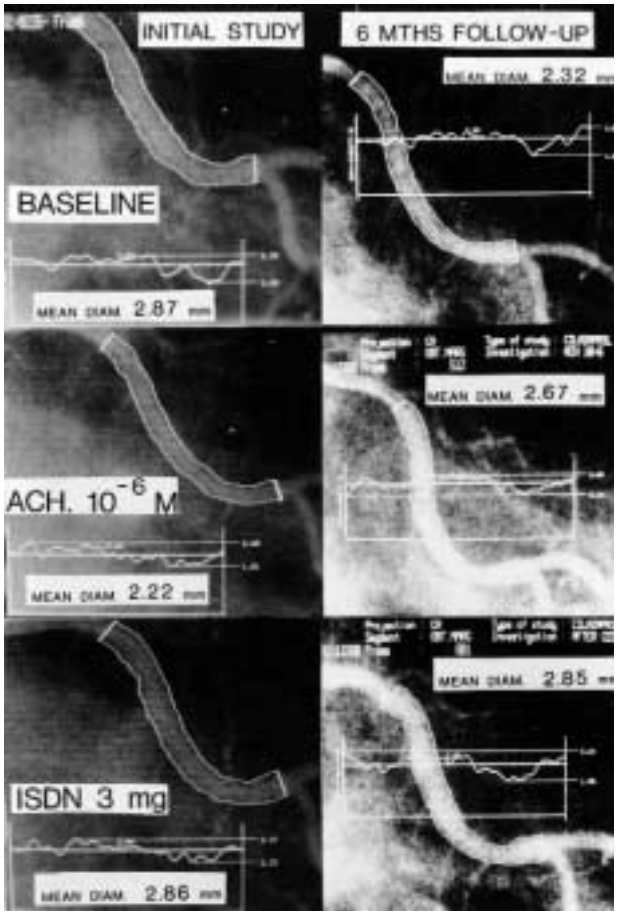

Figure 2. *Quantitative angiographic measurements of the mean diameter of a proximal segment of the left circumflex artery. Two side branches are used as landmarks to facilitate consistent repeated measurements of the same segment throughout the procedure. Note the variability of the baseline measurements, reflecting the variable resting coronary tone* (upper panels), *the reversal at follow-up of the sharp decrease in mean coronary diameter (-22%) observed in the initial study after the maximal concentration of acetylcholine (ACH 10^{-6} M)* (mid panels), *and the similar measurements of mean coronary diameter after vasodilation with isosorbide dinitrate (ISDN)* (lower panels).

mean diameter over a 2-3 cm long proximal/mid and distal coronary segment, using easily visible side branches as anatomical landmarks to allow for the analysis of the same segments in the various phases of the protocol and in the follow-up assessment (Fig. 2). Cross-sectional area (CSA) was calculated from the corresponding diameter assuming a circular arterial cross-section. In order to determine coronary flow and flow resistance, a user-defined reference diameter was measured at the site of the Doppler sample volume.

Statistical analysis. The study was powered to detect a statistically significant decrease in CSA reduction after acetylcholine in the treated group assuming an average CSA reduction of 30% after the maximal concentration of acetylcholine in the initial assessment, similar in the two randomization arms, no changes in the placebo group at 6 months and a 50% decrease in area reduction in the cilazapril group. Demographic and hemodynamic data were expressed as mean (SD). The difference between absolute measurements in the initial and follow-up study of CSA, blood flow APV and derived parameters was calculated for the treated and the placebo group. Since a non-Gaussian distribution of these differences was observed, results were presented as medians ± one quartile. To facilitate comparison, normalization was performed and, by protocol, the results were expressed as a percentage of the half-sum of the initial and follow-up measurements. Statistical significance was tested using a non-parametric test (Wilcoxon rank score).

Results

The placebo and the cilazapril group had similar baseline demographic characteristics (Table I). Angiographically visible irregularities were observed in the majority of the arteries studied (13 in the cilazapril and 11 in the placebo group, p = NS). Two patients in the placebo group and 1 in the cilazapril group did not complete the study. Reasons for exclusion were: withdrawal of consent (n = 1), hypotension (n = 1), severe angina 6 weeks after balloon angioplasty requiring surgical revascularization (n = 1).

A moderate but significant increase in heart rate and a decrease in blood pressure were observed both in the initial study and in the follow-up study after papaverine and isosorbide dinitrate. Heart rate and aortic pressure did not show any significant difference between initial and follow-up assessment, with the exception of a higher heart rate at follow-up in the cilazapril group after papaverine (77 ± 13 vs 70 ± 13 b/min, $p < 0.05$). When the measurements of the placebo and cilazapril group were compared, lower aortic pressures were observed in the treated than in the placebo group, with a statistically sig-

Table I. Clinical and biohumoral characteristics of the treatment groups.

	Cilazapril (n=17)	Placebo (n=17)	p
Sex (F/M)	3/14	5/12	NS
Age (years)	57.2 ± 9.5	57.1 ± 9.7	NS
Weight (kg)	80.2 ± 7.7	80.4 ± 8.7	NS
Height (cm)	172 ± 9	175 ± 8	NS
History of arterial hypertension	6 (35%)	6 (35%)	NS
Current or previous smokers	13 (76%)	11 (65%)	NS
Wall irregularities	13 (76%)	11 (65%)	NS
Studied artery			
LAD	4 (24%)	3 (18%)	NS
LCX	7 (41%)	7 (41%)	NS
RCA	6 (35%)	7 (41%)	NS
Complete follow-up study	16 (94%)	15 (88%)	NS
Beta-blockers/nitrates follow-up	10/16 (62%)	9/15 (60%)	NS
Re-PTCA	7/16 (44%)	6/15 (40%)	NS
Initial total cholesterol			
mmol/l	6.2 ± 0.8	6.5 ± 1.2	NS
mg/dl	240 ± 31	251 ± 46	
Follow-up total cholesterol			
mmol/l	6.4 ± 0.7	6.3 ± 1.5	NS
mg/dl	247 ± 27	244 ± 58	
Initial hemoglobin			
mmol/l	8.77 ± 0.64	8.75 ± 0.66	NS
mg/dl	14.1 ± 1.0	14.1 ± 1.1	
Follow-up hemoglobin			
mmol/l	8.84 ± 0.56	8.77 ± 0.60	NS
mg/dl	14.2 ± 0.9	14.1 ± 1.0	

LAD = left anterior descending artery; LCX = left circumflex artery; PTCA = coronary angioplasty; RCA = right coronary artery.

nificant difference in the initial assessment after the maximal concentration of acetylcholine (106 ± 13 vs 115 ± 13 mmHg, $p < 0.02$), after isosorbide dinitrate (100 ± 11 vs 110 ± 12 mmHg, $p < 0.02$), and in the follow-up assessment at the peak effect of papaverine (100 ± 12 vs 111 ± 16 mmHg, $p < 0.05$).

Cross-sectional area. The individual data of the CSA of the proximal segment at baseline and the percent changes at the end of acetylcholine infusion in the placebo and in the treated group are reported in tables II and III. Baseline and follow-up CSA was smaller in the treated group than in the placebo group but the difference was not statistically significant.

All the acetylcholine concentrations induced a progressive decrease in CSA in the initial assessment, both in the placebo and in the cilazapril group. At follow-up, both groups showed a smaller percent area reduction from baseline after acetylcholine. A significant increase in CSA was observed after isosorbide dinitrate in both groups, with no significant difference between the two groups and between the initial and follow-up assessment. At the peak effect of acetylcholine 10^{-6} M, both groups showed a trend towards a smaller reduction in CSA, more evident in the placebo group but without any significant differences.

Time-averaged peak velocity, coronary flow and flow resistance. Tables IV and V present the percent changes from baseline in APV between initial and follow-up assessment in the placebo and in the cilazapril group. A similar APV was present in the placebo and in the cilazapril group in the initial assessment (23 ± 10 cm/s placebo vs 24 ± 7 cm/s cilazapril, p = NS). Basal APV at follow-up remained unchanged in the cilazapril group but decreased to 21 ± 5 cm/s in the placebo group (p = NS), possibly because of the moderate increase observed in CSA. APV showed a significant increase in both groups after papaverine, with a coronary flow reserve of 3.00 in the placebo group and 2.81 in the cilazapril group. This difference, not statistically significant, is the consequence of the above-mentioned reduction of baseline APV at follow-up in the placebo group. If changes in absolute APV at peak hyperemia were considered, the medians of the changes from baseline were similar in both groups (Fig. 3).

Flow APV remained unchanged at the lowest acetylcholine concentration. A moderate increase was ob-

Table II. Cross-sectional area and percent difference from baseline of the proximal segment in the initial and follow-up assessment (placebo group).

No.	Baseline (mm^2)		ACH 10^{-8} (% change)		ACH 10^{-7} (% change)		ACH 10^{-6} (% change)		ISDN (% change)	
	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up
1	4.37	5.94	-2.5	-4.3	28	-3.6	-0.8	-2.9	67	34.6
2	6.47	4.23	-41.8	-18.1	-45.4	-5.1	-40.2	32.4	-0.7	50.9
3	5.94	8.29	-12.7	-16.5	-1.4	-17	-10.6	-7.8	32	-7.8
4	7.07	6.79	-1.3	-1.3	-9.7	5.5	-23.7	-6.7	4.0	17.7
5	4.15	2.60	NA	-14.8	-26.6	9	-64	11.3	-22.9	7.8
6	6.65	8.45	-0.7	-15.8	6.3	-19.1	-4.1	-20.7	-0.7	-10.7
7	5.68	6.29	-12.9	-14.3	-10.8	-15.6	-19.7	-14.3	21.1	15.4
8	5.27	4.99	-22.5	6.4	-25.9	3.2	-36.1	-10.8	2.3	8.1
9	6.88	8.35	22	12	12.5	2.5	13.2	-22.5	45.5	2.5
10	1.81	2.19	-11.5	18.8	13.6	-1.2	-3.9	7.3	111.4	65.7
11	4.79	5.85	-5.6	-3.6	-37	-10	-40.2	-11.4	13.4	-7.9
12	7.84	7.07	3.8	-6.5	7.7	-11	7.7	1.3	-4.4	15.9
13	16.76	14.25	-7.6	2.8	-4.7	-12.3	-9.7	-15.7	0	26.4
14	7.07	7.21	-3.3	-12.8	-14.1	-17.6	-1.3	12.9	3.4	18.6
15	7.12	8.35	-3.9	-15.3	-9.1	-4.2	-18.9	-0.6	39.9	20.6
Mean	6.52	6.72	-7.2	-5.5	-7.8	-6.4	-16.8	-3.2	20.7	17.2
SD	3.2	2.9	14*	11	20	9*	21*	15	34*	21*

ACH = acetylcholine; ACH 10^{-8} = $p < 0.05$ for initial assessment; ACH 10^{-7} = $p < 0.02$ for follow-up assessment; ACH 10^{-6} = $p < 0.005$ for initial assessment; ISDN = isosorbide dinitrate, $p < 0.02$ for initial and $p < 0.01$ for follow-up assessment; NA = not analyzable. * significant difference with baseline measurement.

Table III. Cross-sectional area and percent difference from baseline of the proximal segment in the initial and follow-up assessment (cilazapril group).

No.	Baseline (mm^2)		ACH 10^{-8} (% change)		ACH 10^{-7} (% change)		ACH 10^{-6} (% change)		ISDN (% change)	
	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up
1	2.80	2.38	-15.2	-18.6	-23.8	-40.7	-30.1	-66.3	-5.2	23
2	6.24	6.93	0	4.8	-21.4	-0.7	-30	-23.9	3.6	2.7
3	4.83	3.02	-31.7	20.3	-24.8	-5.0	-35.6	21.4	9.9	64
4	12.57	10.87	4.0	-15.5	6.6	-24.1	14.5	-32.8	10.8	-7.4
5	2.19	3.87	-27.7	-9.7	-17.1	-40.7	-17.1	-46.1	32.2	-10.5
6	2.16	1.81	-4.8	4	-4.8	-7.7	-8.2	-49.5	20.2	32.5
7	8.04	6.60	-1.2	-11.4	-3.1	-6.8	-14.4	-2.7	-4.9	0.7
8	7.60	6.60	-20.7	-11.4	-30.6	-6.8	-38.9	-2.7	20.2	0.7
9	3.70	4.41	-22.5	0	-23.3	11.3	-35.7	-5.8	-20.1	67.8
10	7.69	6.11	-35.2	12.5	-24.5	6.5	-40.2	-20.3	1.9	-2.1
11	3.87	3.90	1.8	-19.6	-13.9	-15.5	-8.8	-15.5	3.6	9.2
12	4.08	4.37	-30.5	-8.3	-39.7	-17.0	-62.8	-35.2	8.0	1.7
13	5.81	6.20	0	-2.9	-0.7	2.9	-21.5	-20.2	38.4	43
14	4.79	3.73	-16.3	4.6	-22.1	-6.3	-46.3	-16.7	17.7	10.3
15	6.51	7.11	-7.5	-3.9	-6.1	-17.7	-10.8	-14.7	39.4	19.5
16	3.70	6.24	-5.4	-24.5	-13.3	-15.6	-49	**	40.3	0.7
Mean	5.41	5.26	-13.3	-5	-16.4	-11.5	-27.2	-22.1	13.5	16
SD	2.69	2.26	13*	12	12*	15*	19*	22*	18*	24*

ACH = acetylcholine; ACH 10^{-8} = $p < 0.0005$ for initial assessment; ACH 10^{-7} = $p < 0.0001$ for initial and $p < 0.01$ for follow-up assessment; ACH 10^{-6} = $p < 0.0001$ for initial and $p < 0.001$ for follow-up assessment; ISDN = isosorbide dinitrate, $p < 0.005$ for initial and $p < 0.02$ for follow-up assessment. * significant difference with baseline measurement; ** occlusive spasm of the artery (excluded from analysis of mean and SD).

Table IV. Time-averaged peak velocity and percent difference from baseline at the Doppler site in the initial and follow-up assessment (placebo group).

No.	Baseline (cm/s)		Papaverine (% change)		ACH 10^{-8} (% change)		ACH 10^{-7} (% change)		ACH 10^{-6} (% change)	
	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up
1	20	16	100	275	0	0	5	25	60	106.2
2	22	22	304	304	31.8	31.8	36.4	36.4	122.7	263.6
3	21	25	233	276	9.5	12	66.7	16	152.4	76
4	20	16	225	312	-5	0	0	0	85	43.7
5	24	26	117	196	8.3	-7.7	16.7	42.3	91.7	350
6	24	15	121	353	0	0	-4.2	26.7	0	113.3
7	23	19	196	342	-8.7	10.5	-4.3	36.8	191.3	115.8
8	17	16	312	281	11.8	0	47.1	6.25	82.3	262.5
9	21	18	171	255	-9.5	0	61.9	44.4	228.6	238.9
10	55	29	64	117	-27.3	0	-36.4	-3.4	118.2	-10.3
11	15	23	127	48	0	0	46.7	-4.3	160	30.4
12	9	12	378	175	11.1	0	0	25	66.7	108.3
13	18	19	239	253	5.5	0	-5.5	21	44.4	79
14	36	24	192	254	-2.8	4.2	13.9	33.3	55.5	75
15	21	30	214	80	9.5	10	14.3	6.7	42.8	23.3
Mean	23	21	200	235	-2	4	17	21	100	125
SD	10	5	87*	93*	13	9	29*	16*	62*	105*

ACH = acetylcholine; ACH 10^{-7} = $p < 0.05$ for initial and $p < 0.0002$ for follow-up assessment; ACH 10^{-6} = $p < 0.0001$ for initial and $p < 0.0005$ for follow-up assessment; Papaverine = $p < 0.0001$ for initial and follow-up assessments. * significant difference with baseline measurement.

Table V. Time-averaged peak velocity and percent differences from baseline at the Doppler site in the initial and follow-up assessment (cilazapril group).

No.	Baseline (cm/s)		Papaverine (% change)		ACH 10^{-8} (% change)		ACH 10^{-7} (% change)		ACH 10^{-6} (% change)	
	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up	Initial	Follow-up
1	22	16	100	137	-4.5	31.2	-18.2	81.2	-9.1	375
2	19	20	152	190	-15.8	0	15.8	30	73.7	120
3	26	34	203	135	-3.8	-17.6	-3.8	-35.3	-11.5	-5.9
4	14	20	192	215	35.7	-5	35.7	10	85.7	155
5	32	24	75	200	-9.4	4.2	-6.2	45.8	0	141.7
6	19	24	321	175	0	-4.2	84.2	-12.5	131.6	**
7	28	23	153	213	0	4.3	-3.6	30.4	53.6	87
8	39	23	207	213	0	4.3	5.1	30.4	66.7	87
9	25	24	208	341	0	4.2	28	29.2	92	91.7
10	27	33	144	106	0	-3	33.3	0	166.7	81.8
11	21	21	109	114	-23.8	4.8	14.3	4.8	-19	4.8
12	11	22	372	127	27.3	4.5	281.8	95.4	709.1	154.5
13	28	22	132	163	0	-13.6	-3.6	-9.1	3.6	63.6
14	23	29	182	169	-26.1	27.6	-17.4	65.5	-21.7	65.5
15	20	20	245	245	5	15	10	-5	60	45
16	30	34	93	82	-6.7	5.9	46.7	20.6	163.3	**
Mean	24	24	181	177	-1.4	3.9	31	24	97	105
SD	7	5	81*	63*	16	13	72	35*	175*	92*

ACH = acetylcholine; ACH 10^{-7} = $p < 0.02$ for follow-up assessment; ACH 10^{-6} = $p < 0.05$ for initial and $p < 0.001$ for follow-up assessment; Papaverine = $p < 0.0001$ for initial and follow-up assessment. * significant difference with baseline measurement; ** occlusive spasm of the artery (excluded from analysis).

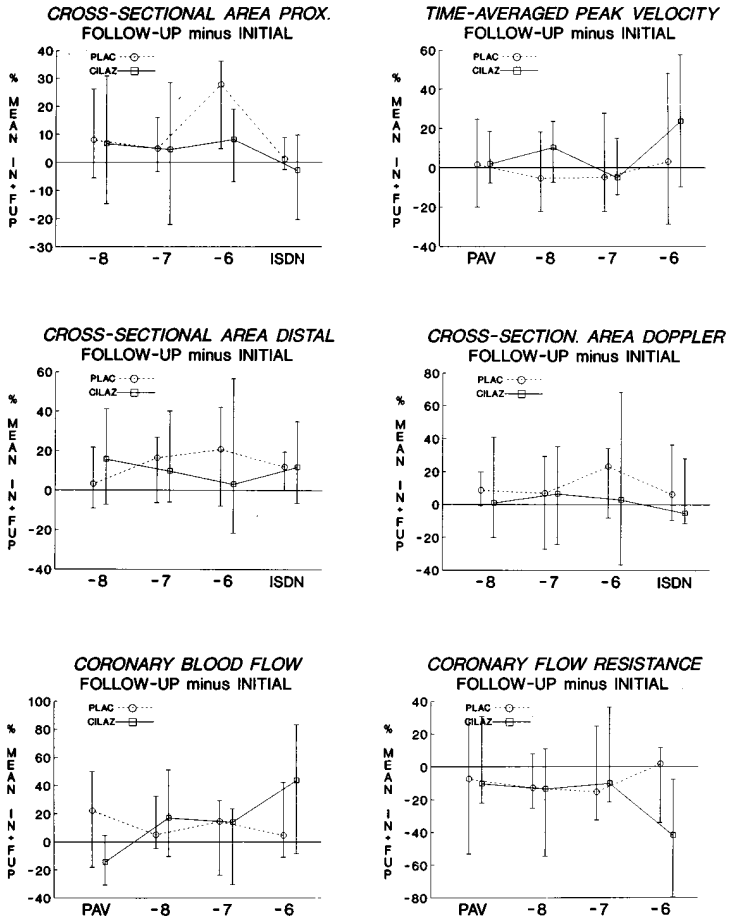

Figure 3. *Percent median measurements ± 25th and 75th percentile of the difference between follow-up and initial study. After acetylcholine, larger cross-sectional areas were measured in the cilazapril (CILAZ) and, more consistently, in the placebo (PLAC) group. Similar measurements of flow time-averaged velocity, coronary flow and flow resistance were present after the maximal concentration of acetylcholine while, in the cilazapril group, the infusion of acetylcholine 10^{-6} M induced a larger increase in flow time-averaged velocity and coronary flow and decrease in flow resistance at follow-up than in the initial study. Due to large individual variations, none of these differences were statistically significant. -8, -7, -6 = logarithm of the concentration of acetylcholine. ISDN = isosorbide dinitrate; PAV = papaverine; PROX = proximal segment.*

served at the peak effect of the intermediate acetylcholine concentration (between 17 and 31% increase), while flow APV doubled after the highest acetylcholine concentration. When the absolute velocities after acetylcholine 10^{-6} M were compared, the median flow APV increase was higher in the cilazapril than in the placebo group (Fig. 3).

In the initial assessment coronary flow showed a moderate increase after maximal acetylcholine concentration in the placebo group (p = NS) and no changes in the cilazapril group (Fig. 4). In the follow-up assessment, most patients in the cilazapril group showed a higher flow after acetylcholine 10^{-6} M than in the initial assessment. The median flow increase from the initial to the follow-up assessment was 45% in the treated group (all patients included) and 4% in the placebo group (p = NS) (Fig. 3).

Coronary flow resistance after maximal acetylcholine concentration remained unchanged in the placebo group (+2%) but showed a high decrease in the cilazapril group, with half of the arteries showing a decrease in flow resistance from the initial assessment of 44% or more (Fig. 3).

Discussion

This study is the first attempt to assess the changes in coronary dimensions and flow measured invasively in the catheterization laboratory after long-term treatment with ACE-inhibitors.

In hypertensive patients treated for 4 months with 10 mg of cilazapril and studied with plethysmography, the minimal forearm resistance showed a 16 ± 20% reduction[8] but no changes were observed after the injection of the endothelium-dependent vasodilator acetylcholine. Using serial subcutaneous gluteal biopsies in mildly hypertensive patients treated with atenolol or cilazapril for 1 year, a significant reduction in the media-lumen ratio of the small arteries studied (200-400 μm) was observed in the cilazapril but not in the atenolol group, despite the treatments being equally effective in lowering elevated blood pressure[9]. In this study, the *in vitro* arterial relaxation after acetylcholine of the excised arterioles was also improved in the cilazapril but not in the atenolol group. Brachial artery endothelial function has recently been assessed in 80 patients with angiograph-

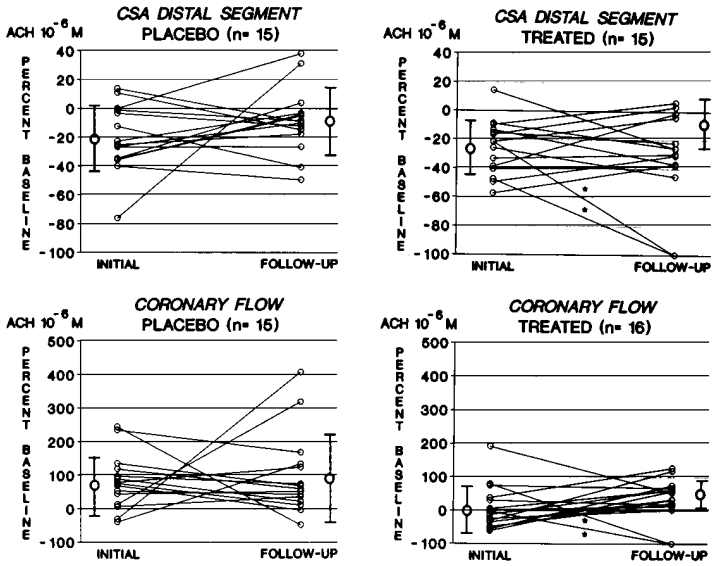

Figure 4. Upper panels: *individual changes in cross-sectional area (CSA) of the distal segment at the end of the infusion of the maximal concentration of acetylcholine (ACH 10-6 M) expressed as a percent of the baseline measurement and divided into placebo* (left panel) *and cilazapril* (right panel) *groups.* Lower panels: *changes in coronary flow in the same condition. * complete occlusion in the treated group* (right panels).

ic evidence of coronary artery disease before and after 8 weeks of treatment with two ACE-inhibitors (quinalapril and enalapril), the ACE losartan and the calcium antagonist amlodipine[10]. Flow mediated vasodilation was improved only after treatment with quinapril and the improvement was limited to those patients with I/D and I/I ACE genotype. Similar variations in response to different ACE-inhibitors have been observed in patients with heart failure (quinapril but not enalapril effective)[15].

A recent study with a similar design in terms of duration of treatment and vessels assessed (TREND, Trial on Reversing Endothelial Dysfunction) (untreated coronary arteries with < 40% diameter stenosis)[11] has shown a protective effect of quinapril, with a reversal of the vasoconstrictive effect induced by acetylcholine at baseline in the treated group and no changes in the placebo group.

In our study patients had different clinical characteristics, including higher average total cholesterol (6.4 mmol/l in this study and 5.2 mmol/l in the TREND trial) and incidence of systemic hypertension (35 and 47%). Recent reports have shown an improvement of coronary flow reserve and response to acetylcholine after treatment with 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors[16-19], confirming the importance of hypercholesterolemia in the impairment of the endothelial response. The higher maximal concentration of acetylcholine (10^{-4} M) and the larger cohorts in the two groups of the TREND study increased the possibility of detecting a difference between the treated and the placebo group. The most important difference between this study and the TREND study, however, was the higher dose and different agents used (cilazapril 20 mg/day vs quinapril 40 mg/day). The vascular tissue specificity of the lipophilic agent quinapril may justify the different results observed. This study, the first to assess coronary flow and resistances in the coronary system after ACE-inhibitors, was powered only to detect CSA changes. Despite the large improvement in flow and flow resistance after acetylcholine in the cilazapril group this trial remains inconclusive. A merit of this trial is the identification in the placebo group of large individual differences in the long-term response, probably reflecting variability of measurement with a technique very sensitive to orientation of probe and hemodynamic conditions at the time of assessment[20]. Probably because of this large unexpected variability in the placebo group, the striking increase in maximal flow and decrease in minimal flow resistance in the cilazapril group did not confirm the improvement previously shown in the coronary arteries with long-term treatment with the same agent in peripheral arteries[8]. A possible explanation for the lack of changes in epicardial vessels and the trend towards an improvement in resistance vessels emerges from experimental and clinical studies showing that the specific inhibitor of the nitric oxide synthesis N^G-monomethyl-L-arginine completely impairs the response to acetylcholine in the epicardial but not in the resistance vessels[21,22]. These findings suggest that other mechanisms of endothelium-mediated vasodilation are prevalent in the microvasculature[23,24], thus explaining the different changes in the response to acetylcholine after a long-term treatment with cilazapril in conductance and resistance vessels.

Study limitations. The uneven distribution of coronary atherosclerotic wall thickening detected with intravascular ultrasound is associated with a progressive impairment of endothelium-mediated vasodilation[25,26].

This angiographic study could not exclude the difference in severity of atherosclerotic involvement in the placebo and in the treated group, suggested by the smaller angiographic CSA in the treated group, and could not study subsets of patients with less severe atherosclerotic changes, more likely to respond to the active treatment.

The presence of the guiding catheter may have reduced the maximal flow response by inducing a partial obstruction during hyperemia. To avoid this phenomenon, the injection of papaverine in the left anterior descending and left circumflex artery was performed through a subselective infusion catheter after withdrawal of the guiding catheter from the ostium and, in the right coronary artery, a small catheter, tapered at the tip (diagnostic 7F) was used for injection.

This study tried to include a real-world population of patients with coronary artery disease, including factors known to impair left ventricular function such as hypertension or high cholesterol levels. Even if no changes in these parameters were present at follow-up, it is conceivable that in these patients an improvement of endothelium-mediated response cannot be achieved without effective removal of these underlying causes.

This study was initiated before the data on the importance of the polymorphism of ACE genotype were known[27] and did not screen the patients included according to the different genotype. As shown in the previously quoted report[10], it might be possible that only some genotypes respond to ACE-inhibition.

We did not try to adjust the intracoronary acetylcholine concentration to the measured coronary flow, which may explain differences in the individual vessel response. We used a method of acetylcholine administration well established by previous studies upon which power analysis of this study was calculated[28,29].

In conclusion, in patients with coronary artery disease, a 6-month treatment with 20 mg of cilazapril/day did not modify the response to endothelium-independent and dependent vasodilators of epicardial arteries without any significant stenoses but induced a consistent, although not significant, increase in flow and decrease in flow resistance after acetylcholine.

References

1. Clozel JP, Kuhn H, Hefti F. Decreases of vascular hypertrophy in four different types of arteries in spontaneously hypertensive rats. Am J Med 1989; 87 (Suppl 6): 92S-95S.
2. Roux S, Clozel JP, Fischli W, Kuhn H. Isoproterenol impairs the rat coronary vasculature: effects of angiotensin-converting enzyme inhibition. J Cardiovasc Pharmacol 1992; 19: 525-31.
3. Osterrieder W, Muller RKM, Powell JS, Clozel JP, Hefti F, Baumgartner HR. Role of angiotensin II in injury-induced neointima formation in rats. Hypertension 1991; 18 (Suppl 2): II60-II64.
4. Clozel JP, Muller RKM, Dipl N, Roux S, Filschi W, Baumgartner HR. Influence of the status of renin-angiotensin system on the effect of cilazapril on neo-intima formation after vascular injury in rats. Circulation 1993; 88: 1222-7.
5. Clozel M, Kuhn H, Hefti F, Baumgartner HR. Endothelial dysfunction and subendothelial monocytes macrophages in hypertension: effect of angiotensin converting enzyme inhibition. Hypertension 1991; 18: 132-41.
6. Auch-Schwelk W, Bossaller C, Claus M, Graf K, Grafe M, Fleck E. Local potentiation of bradykinin-induced vasodilatation by converting-enzyme inhibition in isolated coronary arteries. J Cardiovasc Pharmacol 1992; 20 (Suppl 9): S62-S67.
7. Hecker M, Bara AT, Busse R. Angiotensin-converting enzyme inhibitor unmasks endogenous kinin production by bovine coronary artery endothelium. Eur Heart J 1993; 14 (Suppl 1): 161-3.
8. Kiowski W, Linder L, Nuesch R, Martina B. Effects of angiotensin converting enzyme inhibition on endothelial vasodilator function in primary human hypertension. Eur Heart J 1993; 14 (Suppl C): 5-9.
9. Schiffrin EL, Deng LY, Larochelle MRC. Effects of a beta-blocker or a converting enzyme inhibitor on resistance arteries in essential hypertension. Hypertension 1994; 23: 83-91.
10. Anderson TJ, Elstein E, Haber H, Chardonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol 2000; 35: 60-6.
11. Mancini GBJ, Henry GC, Macaya C, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing Endothelial Dysfunction) Study. Circulation 1996; 94: 258-65.
12. Di Mario C, Roelandt JRTC, de Jaegere P, Linker DT, Oomen J, Serruys PW. Limitations of the zero crossing detector in the analysis of intracoronary Doppler. A comparison with fast Fourier transform of basal, hyperemic, and transstenotic blood flow velocity measurements in patients with coronary artery disease. Cathet Cardiovasc Diagn 1993; 28: 56-64.
13. Di Mario C, Gil R, Sunamura M, Serruys PW. New concepts for interpretation of intracoronary velocity and pressure tracings. Br Heart J 1995; 7: 453-61.
14. Di Mario C, Haase J, den Boer A, Reiber JHC, Serruys PW. Videodensitometry versus edge detection for the assessment of in vivo intracoronary phantoms. Am Heart J 1992; 124: 1181-9.
15. Horning B, Arakawa N, Haussmann D, Drexler H. Differential effects of quinaprilat and enaprilat on endothelial function of conduit arteries in patients with chronic heart failure. Circulation 1998; 98: 2842-8.
16. Treasure CB, Klein L, Weintraub WS, et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995; 332: 481-7.
17. Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995; 332: 488-93.
18. Baller D, Notohamiprodjo G, Gleichmann U, Holzinger J, Weise R, Lehnamm J. Improvement in coronary flow reserve determined by positron emission tomography after 6 months of cholesterol lowering therapy in patients with early stages of coronary atherosclerosis. Circulation 1999; 99: 2871-5.
19. Guethlin M, Kasel AM, Coppenrath K, Ziegler S, Delius W, Schwaiger M. Delayed response of myocardial flow reserve to lipid lowering therapy with fluvastatin. Circulation 1999; 99: 475-81.
20. Di Mario C, Gil R, Serruys PW. Long-term reproducibility of coronary flow measurements in patients with coronary artery disease. Am J Cardiol 1995; 73: 1177-80.

21. Sudhir K, MacGregor JS, Amidon TM, Gupta M, Yock PG, Chatterjee K. Differential contribution of nitric oxide to regulation of vascular tone in coronary conductance and resistance arteries: intravascular ultrasound studies. Am Heart J 1994; 127: 858-65.
22. Lefroy DC, Crake T, Uren NG, Davies GJ, Maseri A. Effect of inhibition of nitric oxide synthesis on epicardial coronary artery caliber and coronary blood flow in humans. Circulation 1993; 88: 43-54.
23. Chen G, Suzuki H, Weston AH. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br J Pharmacol 1988; 95: 1165-74.
24. Komaru T, Lamping KG, Eastham CL, Harrison DG, Marcus ML, Dellsperger KC. Effect of an arginine analogue on acetylcholine-induced coronary microvascular dilatation in dogs. Am J Physiol 1991; 262: H2001-H2007.
25. Zeiher AM, Schachinger V, Hohnloser SH, Saurbier B, Just H. Coronary atherosclerotic wall thickening and vascular reactivity in humans. Circulation 1994; 89: 2525-32.
26. Zeiher AM, Schachinger V, Minners J. Long-term cigarette-smoking impairs endothelium-dependent coronary artery vasodilator function. Circulation 1995; 92: 1094-100.
27. Ueda S, Meredith PA, Morton JJ, Connell JM, Elliott HL. ACE (I/D) genotype as a predictor of the magnitude and duration of the response to an ACE-inhibitor drug (enalaprilat) in humans. Circulation 1998; 98: 2148-53.
28. Zeiher AM, Drexler H, Wollschlager H, et al. Coronary vasomotion in response to sympathetic stimulation in humans: importance of the functional integrity of the endothelium. J Am Coll Cardiol 1989; 14: 1181-90.
29. Zeiher AM, Drexler H, Wollschläger H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with impaired coronary blood flow regulation in patients with early atherosclerosis. Circulation 1991; 84: 1984-92.