Presence of Cardiovascular Structural Changes in Essential Hypertensive Patients With Coronary Microvascular Disease and Effects of Long-Term Treatment

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In asymptomatic essential hypertensive patients with angiographically normal coronary arteries and without left ventricular hypertrophy, dipyridamole-induced ischemic-like ST segment depression may be a marker of coronary microvascular disease. In this study we evaluated, first, whether this cardiac abnormality is linked to structural or functional vascular abnormalities, and second, the effect of antihypertensive treatment by 12-month administration of the angiotensin converting enzyme (ACE) inhibitor captopril (50 mg twice a day orally). In essential hypertensives with dipyridamole echocardiography stress test (DET) (DET+, n = 8) and without (DET−, n = 8) ST segment depression greater than 0.1 mV during intravenous dipyridamole infusion (0.84 mg/kg over 10 min), we studied the forearm blood flow (FFB, venous plethysmography, mL/100) modifications induced by intrabrachial acetylcholine (Ach) (0.15, 0.45, 1.5, 4.5, 15 μg/100 mL/min × 5 min each), an endothelium-dependent vasodilator, and by sodium nitroprusside (SNP) (1, 2, 4 μg/100 mL/min × 5 min each), a smooth muscle cell relaxant compound. Minimal forearm vascular resistances (MFVR), an index of arteriolar structural changes, were also calculated. Both Ach and SNP caused greater vasodilation in DET− as compared to DET+ while MFVRs were lower in DET− compared to DET+. After treatment, both DET+ and DET− patients showed a significant and similar reduction in blood pressure and left ventricular mass index, while vasodilation to acetylcholine and sodium nitroprusside was increased only in the DET+ group. In addition, forearm minimal vascular resistances were significantly reduced only in DET+ patients, who showed disappearance of dipyridamole-induced ischemic-like ST segment depression. In conclusion, these data confirm that essential hypertensive patients with microvascular coronary disease are characterized by the presence of structural changes in the forearm vascular bed. Our results also indicate that both cardiac and forearm vascular abnormalities can be reversed by antihypertensive treatment with an ACE inhibitor.


KEY WORDS: Hypertension (essential), endothelium, dipyridamole, antihypertensive treatment, cardiovascular hypertrophy regression.

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Hypertension is a well documented risk factor for the development of ischemic heart disease. Although the main mechanisms through which hypertension can affect coronary circulation are atherosclerosis and left ventricular hypertrophy, the occurrence of an impaired coronary reserve has been documented even in essential hypertensive patients with angiographically normal coronary arteries and without evidence of left ventricular hypertrophy.\textsuperscript{1-7} This abnormality has been located in the coronary microvascular bed and seems to be related to the presence of structural alterations in resistance vessels, determining a reduction in maximal vasodilatory capacity.\textsuperscript{5,9} However, essential hypertension is also characterized by impaired endothelial function,\textsuperscript{10-17} and this defect seems to be present in coronary arteries.\textsuperscript{14-17} Therefore the possibility exists that both a functional and structural defect could determine the dysfunction characteristic of coronary microvascular disease.

To investigate whether essential hypertensive patients with coronary microvascular disease are characterized by arterial functional or structural abnormalities, or both, the following experimental approach was used. First, we used the dipyridamole echocardiography stress test (DET),\textsuperscript{4-7} which can identify the presence of coronary microvascular disease when an ischemic-like electrocardiographic ST segment depression without detectable changes in left ventricular function is induced by dipyridamole. Second, in the forearm vascular bed of essential hypertensive patients with and without coronary microvascular disease we compared the vascular response to an endothelium-dependent and endothelium-independent vasodilator. In addition, minimal vascular resistances, an integrated index of vascular structural alterations, were also measured. The rationale of studying forearm vessels to evaluate coronary abnormality is related both to ethical aspects and the good correlation between alterations present in the two vascular districts observed in patients with either essential hypertension or syndrome X.\textsuperscript{18,19} Finally, the effect of antihypertensive treatment on the structural and/or functional cardiac and vascular abnormalities of these patients was also evaluated.

METHODS

Patients The study was performed using two groups (n = 8 for each) of patients with mild-to-moderate uncomplicated essential hypertension characterized by the presence or absence of ischemic-like electrocardiographic changes without echocardiographic transient regional dyssynergy during dipyridamole infusion ("DET+" and "DET--" group, respectively). Five out of eight DET+ patients were characterized by a history of chest pain. Patients were matched for age, sex, body weight, blood pressure at rest, left ventricular mass index, baseline hemodynamic and humoral characteristics, as shown in Table 1.

Ten patients had never been treated, while five patients reported a short history (no longer than 1 month) of pharmacological antihypertensive treatment. Three patients had been treated for a longer time, but without obtaining blood pressure normalization. Previous pharmacological treatment was diuretics, \(\beta\)-blockers or calcium entry blockers. Patients previously treated with ACE-inhibitors were excluded from the study. Each patient discontinued any pharmacological treatment at least 2 weeks prior to the study. Secondary causes of hypertension were excluded by the standard clinical and laboratory investigations. Hypercholesterolemic, diabetic and heavy smoker subjects were excluded. Each DET+ patient subsequently underwent a coronary angiography which showed normal or non-significant atherosclerotic lesions of epicardial coronary arteries and normal left ventricular function.

The study protocol was approved by the local ethical committee, according to institutional guidelines. All patients were aware of the investigational nature of the study and consented to it.

Resting Echocardiogram All patients had good quality resting one- and two-dimensional echocardiograms. Measurements were made by 2-D targeted M-mode tracings, obtained by parasternal long axis view. Left ventricular mass was calculated according to the Penn convention.\textsuperscript{20} Hypertrophy criteria were left ventricular mass index (LVMI) \(> 134\) g/m\(^2\) for men and \(> 110\) g/m\(^2\) for women.\textsuperscript{21}

Dipyridamole-Echocardiography Test Two-dimensional echocardiographic and 12-lead electrocardiographic monitoring was performed in combination with a dipyridamole infusion: 0.56 mg/kg over 4 min followed by 4 min of no dose and then 0.28 mg/kg over 2 min. The cumulative dose was therefore 0.84 mg/kg over 10 min.\textsuperscript{22} Aminophylline (240 mg), which promptly reverses the effects of dipyridamole, was readily available and was routinely given at the end of each test. During the procedure, blood pressure and electrocardiogram were recorded each minute. A commercially available wide angle phased array imaging system (Hewlett Packard Mod. 77020, 2.5 and 3.5 Mhz transducers) was used. Tracings were considered diagnostic for myocardial ischemia when there was a horizontal or downsloping ST segment shift of at least 0.1 mV 0.08 sec after the J point compared with baseline.

Forearm Blood Flow Study Studies were performed at 08:00 after overnight fasting, with the subjects lying supine in a quiet air-conditioned room (22 to 24°C). A
polyethylene cannula (21 gauge, Abbot, Sligo, Ireland) was inserted into the brachial artery under local anesthesia (2% lidocaine). The cannula was connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, CO) for determination of systemic mean arterial blood pressure (1/3 pulse pressure plus diastolic pressure), heart rate (model VSM1, Physiocontrol, Redmond, WA) and intra-arterial infusions. Forearm blood flow was measured by strain-gauge venous plethysmography (LOOSCO, GL LOOS, Amsterdam, The Netherlands). Circulation to the hand was occluded 1 min before each measurement of forearm blood flow by inflating a pediatric cuff around the wrist at suprasystolic blood pressure. Earlier work had determined the sensitivity and reproducibility of the method. Plethysmographic traces were read by the same observer (A.V.) who was not aware of the result of the DET test. Forearm volume was determined by the water-displacement method. Drugs used were infused through three-way stopcocks at concentrations that had no systemic effects.

**Experimental Design** Endothelium-dependent forearm vasodilation was evaluated by a dose-response curve to intraarterial acetylcholine, an endothelium-dependent vasodilator (cumulative increase in infusion rates: 0.15, 0.45, 1.5, 4.5, 15 μg/100 mL of forearm tissue/min for 5 min each dose). Endothelium-independent vasodilation was assessed by sodium nitroprusside (1, 2 and 4 μg/100 mL/min for 5 min each dose), a direct smooth muscle cell relaxant compound. In addition, minimal forearm vascular resistances (MFVR), an integrated index of vascular structural changes, were also evaluated by calculating the ratio between mean intraarterial blood pressure and maximal forearm vasodilation induced by 13 min of forearm ischemia plus 1 min of dynamic hand exercise. Ischemia was obtained by inflating the plethysmographic cuff (placed around the arm of the cannulated forearm) at 300 mm Hg. The sequence of the three experiments was randomized, and 30 min of recovery was allowed between each experimental intervention.

After completion of the above reported experimental protocol, patients were treated with the angiotensin converting enzyme (ACE) inhibitor captopril 50 mg twice daily. Patients then returned once monthly to our outpatient clinic for blood pressure evaluation. The goal of treatment was to reduce blood pressure to < 140/90 mm Hg. This target was achieved in 7/8 of each group by captopril alone, while in 1/8 of each group nifedipine (20 mg twice daily) was added. After 12 months of therapy patients underwent 2 weeks of pharmacological wash-out and repeated the same above reported protocol (prior plethysmographic study).

**Data Analysis** Data were analyzed in terms of changes in forearm blood flow. Because mean arterial blood pressure did not change significantly during the study, increments in forearm blood flow (FBF) were taken as evidence of local vasodilation. Results are expressed as mean ± SEM. Data were analyzed statis-
cally by the t test for paired or unpaired observations and by repeated measures of analysis of variance. Differences were considered statistically significant at a value of $P < .05$.

**Drugs**  Acetylcholine HCl (Farmigea S.p.A., Pisa, Italy) and sodium nitroprusside (Malesci, Milan, Italy) were obtained from commercially available sources. Acetylcholine was diluted freshly to the desired concentration by adding normal saline. Sodium nitroprusside was dissolved in glucosate solution and protected from light by aluminum foil.

**RESULTS**

Basal blood pressure values and echocardiography derived cardiac parameters were found to be similar in DET+ and DET− patients (Tables 1 and 2). Regional wall motion asynchrony during DET did not develop in any of the study subjects. In addition, other hemodynamic and humoral parameters were also similar in DET+ as compared to DET− (Table 1).

**Forearm Blood Flow Study**  Acetylcholine caused a dose-dependent increment in FBF which was found to be significantly ($P < .05$) reduced in DET+ (FBF from 3.9 ± 0.5 to a max of 17.8 ± 3.6 mL/100 cc/min) as compared to DET− patients (FBF: from 4.0 ± 0.7 to a max of 24.4 ± 4.1 mL/100 cc/min) (Figure 1). Similarly, dose-dependent vasodilation to sodium nitroprusside was also significantly ($P < .05$) reduced in the DET+ group (FBF from 3.9 ± 0.5 to a maximum of 18.3 ± 4.1 mL/100 cc/min) as compared to DET− subjects (FBF from 4.0 ± 0.9 to a maximum of 24.9 ± 4.1 mL/100 cc/min) (Figure 1).

Although basal FVRs were similar in both groups (responders: 30.2 ± 4.6 units, nonresponders: 29.2 ± 5.5 units), MFVRs were significantly ($P < .05$) higher in DET+ patients (2.8 ± 0.3 units) as compared to controls (2.29 ± 0.2 units) (Figure 2).

**Effect of Antihypertensive Treatment**  In all patients, antihypertensive treatment with the ACE inhibitor captopril (plus the calcium entry blocker nifedipine in one patient for each group) caused regression of arterial blood pressure values and a significant ($P < .05$) reduction in LVMI (Table 2). It should be noted that DET+ patients who previously showed ischemic-like electrocardiographic changes to dipyridamole infusion became negative (Figures 3 and 4). Moreover, in three out of five patients with chest pain, the symptom disappeared. Naturally the dipyridamole-echocardiography test was unmodified in the DET− group.

**Repeat of Forearm Blood Flow Study**  Forearm blood flow studies were repeated after 2 weeks of treatment washout. In this period blood pressure values increased from 139.2 ± 4.1 / 88.4 ± 3.4 to 163.4 ± 7.2 / 98.6 ± 2.6 mm Hg (DET+ group) and from 138.2 ± 5.7 / 87.6 ± 3.4 to 161.4 ± 8.4 / 99.1 ± 3.1 mm Hg (DET− group).

In DET+ patients, ($P < .05$) vascular response to both acetylcholine (FBF from 3.8 ± 0.6 to a maximum of 23.4 ± 3.9 mL/100 cc/min) and sodium nitroprusside (FBF from 3.7 ± 0.5 to a max of 24.1 ± 4.1 mL/100 cc/min) was found to be significantly ($P < .05$) increased as compared to pretreatment values (Figure 5). Similarly, MFVRs were also shown to be decreased ($P < .05$) by antihypertensive treatment (2.3 ± 0.2 units) (Figure 2).

In contrast, in DET− patients, antihypertensive therapy did not modify either the vasodilating response to acetylcholine (FBF from 3.9 ± 0.5 to a maximum of 25.1 ± 3.1 mL/100 cc/min) and sodium nitroprusside (FBF from 3.9 ± 0.6 to a maximum of 24 ± 3.9 mL/100 cc/min) (Figure 5) or MFVR (2.3 ± 0.2 units) (Figure 2).

It is important to observe that, after treatment, vascular response to acetylcholine and sodium nitroprusside and calculated MFVRs were no longer statistically different between DET+ and DET− groups (Figures 2 and 5).

**DISCUSSION**

The results of the present study indicate a reduction in the vasodilating effect of acetylcholine and sodium

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### TABLE 2. BLOOD PRESSURE VALUES AND LEFT VENTRICULAR MASS INDEX BEFORE AND AFTER 12 MONTHS OF TREATMENT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DET+ Group Before Treatment</th>
<th>DET+ Group After Treatment</th>
<th>DET− Group Before Treatment</th>
<th>DET− Group After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>168.4 ± 6.7</td>
<td>139.2 ± 4.1*</td>
<td>171.6 ± 5.9</td>
<td>138.2 ± 5.7*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>102.3 ± 4.1</td>
<td>88.4 ± 3.4*</td>
<td>101.4 ± 4.5</td>
<td>87.6 ± 3.4*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>65.3 ± 4.3</td>
<td>64.6 ± 3.7</td>
<td>62.6 ± 3.3</td>
<td>63.4 ± 3.9</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>122.7 ± 6.7</td>
<td>108.6 ± 5.2*</td>
<td>117.2 ± 6.4</td>
<td>102.2 ± 4.1*</td>
</tr>
</tbody>
</table>

* $P < .05$ or less versus before treatment.

Data are mean ± SD.

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVMI, left ventricular mass index.
Acetylcholine and increased minimal resistances in the forearm vascular bed of essential hypertensive patients with ischemic-like electrocardiographic ST segment depression during dipyridamole infusion, as compared to matched essential hypertensive patients with negative electrocardiographic response to dipyridamole. Thus, these data suggest the presence of structural changes in the forearm vascular bed of essential hypertensive patients with microvascular coronary disease.

The present investigation was designed assuming that dipyridamole-induced ischemic-like electrocardiographic changes not associated with left ventricular abnormalities can identify hypertensive patients with coronary microvascular disease.\(^4\)\(^-\)\(^7\) This possibility has been demonstrated in several pathological models, such as acute rejection after heart transplantation,\(^27\) hypertrophic cardiomyopathy\(^28\) or syndrome X,\(^29\) which have in common the pathological hallmark of a reduction in coronary flow reserve with angiographically normal coronary tree. In addition, the same pattern of response (an ischemic-like electrocardiographic ST depression without detectable changes in left ventricular function) has been demonstrated in both symptomatic\(^4\) or asymptomatic\(^6\) essential hypertensive patients, in the presence of an angiographically normal coronary artery tree. Although dipyridamole-induced ischemic-like electrocardiographic changes are more frequent in patients with left ventricular hypertrophy, they may also occur in the presence of normal myocardial mass.\(^5\) In essential hypertensive patients, DET shows a significantly higher feasibility and specificity in comparison to the exercise-electrocardiography stress test,\(^4\) while there is no significant difference between the sensitivity values. In addition it has been demonstrated that radionuclide angiography, although an excellent indicator of myocardial ischemia in normotensive patients, has a low predictive accuracy in hypertensive patients with chest pain.\(^30\) Even the 201-thallium exercise stress test, which shows very good sensitivity and specificity in normotensive patients, presents a significant drop in specificity in hypertensive patients.\(^31\)

Therefore we selected patients with essential hypertension who were "responders" to dipyridamole infusion but showed no evidence of left ventricular hypertrophy and had angiographically normal coronary arteries. As a control group we recruited hypertensive patients carefully matched for age, sex, blood pressure values and other clinical variables who were "non-

**FIGURE 1.** Line graphs show increase in forearm blood flow (FBF) induced by intraarterial acetylcholine (left) and sodium nitroprusside (right) in DET+ (○, n = 8) and DET− (●, n = 8) patients. Data are mean ± SEM expressed as percent increase above basal. *Significant difference between DET− and DET+ groups (P < .01).

**FIGURE 2.** Histograms represent basal (left) and minimal forearm vascular resistances (right) in DET+ (full columns) and DET− patients (empty columns) before (top) and after (bottom) 12 months of pharmacological antihypertensive treatment. *Significant difference between DET+ and DET− groups (P < .05).
responders” to dipyridamole infusion. It is important to observe that left cardiac mass index, although slightly greater in DET+ patients, was not statistically different between the two groups.

To study endothelial function, acetylcholine, a classical endothelium-dependent vasodilator, was infused in these patients. Curtailed response to this compound is considered as evidence of an impairment in endothelial function both in animals and humans. This paradigm is correct if intrinsic impairment of smooth muscle cell relaxing capacity is excluded by testing a vasodilator compound, such as sodium nitroprusside, which directly activates smooth muscle cells. Thus the present results indicating that the vasodilating effect of acetylcholine is blunted in DET+ patients as compared to DET− cannot be interpreted as evidence of impairment in endothelial function since, in the same patients, the response to sodium nitroprusside was also reduced. Therefore, the finding of an impaired response to vasodilators which act through endothelium-dependent and endothelium-independent mechanisms in the DET+ group suggests the presence of an intrinsic smooth muscle dysfunction in the forearm arterial wall of patients with supposed microvascular coronary disease. This interpretation is confirmed by detection of increased minimal forearm vascular resistances in DET+ patients as compared to DET− subjects. In this regard, a recent report by Tagawa et al demonstrated that nitric oxide plays a minimal role in vasodilation of peak reactive hyperemia in the human forearm, further supporting the possibility that minimal forearm vascular resistances can be considered an integrated index of arteriolar structural changes.

Considering the good correlation between vascular alterations in coronary and forearm arterial districts already documented both in essential hypertensive patients and in patients with syndrome X, it is possible to hypothesize that the vascular structural alterations found in forearm arterioles of DET+ essential hypertensive patients might also be present in the coronary microvascular bed, thus representing the pathophysiological substrate of coronary microvascular disease. In agreement with this hypothesis, Schwartzkopff et al reported that mean external arteriolar diameter, mean arteriolar wall area, percent medial wall area and mean periarteriolar fibrosis area of intramyocardial coronary arterioles are increased in essential hypertensive patients with impaired coronary flow reserve, increased minimal coronary resistance and angiographically normal coronary bed.

The second aim of the present investigation was to evaluate the effects of 12-month antihypertensive treatment with the ACE-inhibitor captopril on cardiovascular parameters in the same patients. To exclude possible direct pharmacological interaction of captopril and evaluate only the effects of treatment on cardiovascular structural alterations, the study was repeated after drug withdrawal for 2 weeks. We observed that in both DET+ and DET− patients pharmacological therapy induced a significant and similar reduction of blood pressure values (evaluated before drug withdrawal) and left ventricular mass index. In contrast, vasodilation to acetylcholine and sodium nitroprusside was greatly increased in the DET+ group only and was no longer statistically different from vascular response to the same agonists observed in the DET− group. In addition, forearm minimal vascular resistances were significantly reduced in the DET+ group, to values similar to those of DET− patients. Finally, it is noteworthy that dipyridamole-induced ischemic-like ST segment depression disappeared in those patients who were found positive prior to treatment. Therefore our data indicate that antihypertensive treatment with an ACE-inhibitor ameliorates forearm vascular structural abnormalities and normalizes the electrocardiographic response to dipyridamole infusion. This finding further supports the above reported hypothesis that vascular structural alterations represent the pathophysiological...
A representative example of tracings showing normal echocardiographic left ventricular function in basal conditions (Basal) and during dipyridamole stress (Dipyridamole) before and after pharmacological antihypertensive treatment.

FIGURE 4.

As far as the mechanism of action of captopril on cardiovascular structural changes is concerned, the present experimental design does not allow differentiation between a specific effect related to ACE-inhibition or an aspecific action determined by blood pressure reduction.

At least to our knowledge, few studies have evaluated the effect of different antihypertensive treatments on cardiovascular structural abnormalities. While available data seem to be discordant concerning the efficacy of diuretics and β-blockers, a general agreement exists on a favorable effect exerted by ACE-inhibitors and dihydropiridine calcium entry antagonists. Thus the finding that different kinds of treatment can determine a regression of vascular structural modifications suggests that blood pressure reduction, per se, could be the main mechanism responsible for this effect. However a specific action related to ACE inhibition cannot be excluded, especially considering the experimental evidence that treatment with ACE inhibitors, even administered at non-hypotensive doses, determines a positive effect both on development and regression of vascular structural alterations. These actions are related to inhibition either of the production of angiotensin II, which is a well-documented growth factor for smooth muscle cells and myocytes, or of the degradation of bradykinin, which, through prostacyclin and nitric oxide production, can inhibit cell proliferation.

Concerning the clinical relevance of the present observations, the crucial issue is whether dipyridamole-induced ST segment depression might merely be a nonspecific laboratory finding or on the contrary genuinely represents a marker of ischemia, at least under certain conditions. It is important to observe that DET+ patients are characterized by a reduced coronary reserve and by a higher risk of significant ventricular arrhythmias. Thus the possibility exists that electrocardiographic ischemic-like changes are the expression of a less severe (although still potentially clinically important) imbalance between subendocardial and subepicardial flow, the functional substrate of which is a restriction of coronary reserve resulting from an increase in resistances at the arteriolar level.

In conclusion, results of the present study indicate that essential hypertensive patients with ischemic-like...
electrocardiographic ST segment depression during dipyridamole infusion are characterized by the presence of decreased vasodilating response to endothelium-dependent and -independent agents and increased forearm minimal vascular resistances. In addition, both cardiac and forearm vascular abnormalities can be reversed by 12-month treatment with captopril. Taken together these data suggest that vascular structural changes are the morphological substrate of coronary microvascular disease which can affect patients with essential hypertension, and that this vascular alteration can be reversed by long-term treatment with an ACE inhibitor.

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