Abstract—To compare the effect of antihypertensive drugs on endothelium-dependent vasodilation in the peripheral conduit arteries of patients with essential hypertension, in a prospective, randomized, parallel group study, endothelial function was assessed in 168 hypertensive patients before and after 6-month treatment with randomly assigned nifedipine GITS (30 to 60 mg, n = 28), amlodipine (5 to 10 mg, n = 28), atenolol (50 to 100 mg, n = 29), nebivolol (5 to 10 mg, n = 28), telmisartan (80 to 160 mg, n = 29), and perindopril (2 to 4 mg, n = 28). If necessary, hydrochlorothiazide (25 mg) was added to each compound. We evaluated brachial artery flow-mediated, endothelium-dependent dilation (high-resolution ultrasound) compared with endothelium-independent response to glyceryl trinitrate (25 μg/s). Brachial artery diameter was measured by automatic computerized analysis. Forty healthy subjects were evaluated as a control group. Oxidative stress production was evaluated by measuring plasma malondialdehyde and plasma lipoperoxides; plasma antioxidant capacity was assessed as ferric-reducing antioxidant power. Hypertensive patients showed a significantly (P < 0.01) lower flow-mediated dilation (5.2 ± 1.9%) as compared with healthy control subjects (7.1 ± 2.6%). Response to glyceryl trinitrate was similar in control subjects and patients. At baseline, blood pressure, diameter, flow-mediated dilation, and response to glyceryl trinitrate were similar in the different treatment groups. All treatments similarly reduced blood pressure, but only perindopril increased flow mediated dilation (from 5.1 ± 2 to 6.4 ± 2.4%; P < 0.01) without modifying the response to glyceryl trinitrate. Perindopril but also telmisartan nifedipine and amlopidine reduced oxidative stress and increased plasma antioxidant capacity. In patients with essential hypertension, ACE inhibitors appear to be the only compounds able to improve conduit artery endothelium-dependent vasodilation. (Hypertension. 2003;41:1281-1286.)

Key Words: antihypertensive therapy □ arteries □ endothelium □ hypertension, essential □ vasodilation

Endothelium plays a primary role in the modulation of vascular tone and structure. The major endothelium-derived relaxing factor is nitric oxide (NO), which is derived from l-arginine by the activity of the enzyme NO-synthase. An additional important endothelium-derived relaxing substance is the not-yet-identified hyperpolarizing factor.

Essential hypertension is characterized by impaired endothelium-dependent vasodilation, since oxidative stress causes a reduction in NO availability. In these conditions, the reduced vascular response to endothelial agonists is no longer NO-dependent but is sustained by alternative pathways, including hyperpolarization.

It has been suggested that in patients with essential hypertension, endothelial dysfunction could act as a promoter of atherosclerosis. Moreover, endothelial dysfunction and oxidative stress are independent predictors of cardiovascular events. Thus, it is conceivable that reversing endothelial dysfunction could represent an adjunctive target for antihypertensive treatment. However, it has been widely demonstrated that mere blood pressure reduction is not sufficient to improve or restore endothelial dysfunction. This beneficial effect can be achieved only by specific mechanisms not shared by all drug classes.

In patients with essential hypertension, a large body of evidence has well characterized the effect of antihypertensive drug classes on endothelium-dependent vasodilation in the peripheral microcirculation, but little evidence is available in peripheral conduit arteries. This aspect is important because endothelium is an autocrine-paracrine organ, and drug effects could differ according to which vascular district is evaluated.

Thus, in this study, we addressed the effect of chronic (6 months) antihypertensive treatment with the main antihypertensive drug classes, including ACE inhibitors, calcium antagonists, AT-1 receptor antagonists, and β-blockers, on flow-mediated endothelium-dependent dilation (FMD) of the brachial artery (BA) and oxidative stress production in an extensive sample of patients with essential hypertension.

Methods

The study population included 40 (27 men) normotensive control subjects (51.7 ± 9.3 years; blood pressure [BP], 119.4/6.9/...
TABLE 1. Clinical Characteristics of Normotensive Subjects and Essential Hypertensive Patients Participating in the Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Subjects (n=40)</th>
<th>Essential Hypertensive Patients (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.7±11.3</td>
<td>51.6±9.8</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>24/16</td>
<td>111/69</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118.9±7.8</td>
<td>152±9*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77.8±4.4</td>
<td>98±6*</td>
</tr>
<tr>
<td>Heart rate, beats/minute</td>
<td>70±11</td>
<td>69±12</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.1±2.3</td>
<td>27.0±1.9</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.88±0.18</td>
<td>0.91±0.16</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>92.1±10.3</td>
<td>96.7±8.5</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>207.2±23.0</td>
<td>216.5±32.3</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>55.8±14.7</td>
<td>51.9±19.9</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>127.1±28.1</td>
<td>135.9±42.9</td>
</tr>
<tr>
<td>MDA, μmol/L</td>
<td>3.8±2.8</td>
<td>5.4±3.0*</td>
</tr>
<tr>
<td>LOOH, μmol/L</td>
<td>3.5±1.8</td>
<td>4.5±2.0*</td>
</tr>
<tr>
<td>FRAP, mmol/L</td>
<td>685±343</td>
<td>399±206*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. HDL indicates high density lipoprotein; LDL, low density lipoprotein; MDA, plasma malondialdehyde; LOOH, plasma lipoperoxides; and FRAP, ferric-reducing antioxidant power. *P<0.01.

78.9±5.1 mm Hg) and 180 (121 men) matched untreated patients with essential hypertension (52.6±9.8 years; BP, 154.1±10.5/101.2±6.3 mm Hg). Patients were recruited from June 1999 to December 2001 among outpatients and enrolled in the study if they were never treated or had a history of discontinuous treatment. All were nonsmokers or smoked fewer than 5 cigarettes per day, with alcohol consumption <50 mg/dl, absence of diabetes mellitus and renal function impairment, and total cholesterol <240 mg/dl (Table 1).

The protocol was approved by the Ethics Committee of the University of Pisa, and all patients gave written informed consent to the study.

Experimental Procedure
Vascular ultrasound scans were performed in the morning, with subjects supine, in a quiet air conditioned room (22° to 24°C). A B-mode scan of the right BA was obtained in longitudinal section between 5 and 10 cm above the elbow, using a 7.0-MHz linear array transducer and a standard AU5 Armonic system (ESAOTE Biomedica) as described. Briefly, the transducer was held at the same point throughout the scan by a stereotactic clamp. End-diastolic frames (ECG-triggered) were acquired every second on a personal computer with the use of a commercial software program (microVIDEO DC30/plus, Pinnacle Systems GmbH). Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow.

Experimental Protocol
Endothelium-dependent vasodilation was assessed as dilation of the BA induced by increased flow (FMD). After 1 minute of acquisition to measure basal diameter, the cuff was inflated for 5 minutes at 250 mm Hg and then deflated to induce reactive hyperemia (RH). Endothelium-independent dilation was obtained by administration of a low dose (25 μg) of sublingual glyceryl trinitrate (GTN), a dose previously tested to obtain a vasodilation similar to FMD. Vascular reactivity was assessed at baseline and after 6 months of treatment according to a randomized, single-blind, parallel-group study design. Study drugs included 2 different calcium antagonists, nifedipine GITS (30 to 60 mg daily) and amlodipine (5 to 10 mg daily), 2 different β-blockers, atenolol (50 to 100 mg daily), and nebivolol (5 to 10 mg daily) and drugs interfering with the renin-angiotensin system, such as the ACE inhibitor perindopril (2 to 4 mg daily) and the AT1-receptor antagonist telmisartan (80 to 160 mg daily).

BP values were determined by an automatic digital device (Omron HEM-705CP) as the mean of 3 measurements obtained at 3-minute intervals. Each drug was started at the lower dose; after 1 month of treatment, patients whose BP values were found to be >140 to 90 mm Hg were given the higher dose of the corresponding compound. Moreover, after a further month of treatment, patients not yet normalized by single drug administration were treated by adding a diuretic (hydrochlorothiazide 12.5 mg). Additional visits were scheduled every month up to the end of the 6-month treatment period.

To investigate the direct effect of antihypertensive treatment on oxidative stress and antioxidant capability, venous samples were collected at baseline and after 6-month drug treatment. Oxidative stress was evaluated through measurement of plasma malondialdehyde (MDA) by spectrophotometric assay and plasma lipoperoxides with a colorimetric method. Antioxidant capacity was measured as plasma total antioxidant capability by measuring ferric-reducing antioxidant power (FRAP; spectrophotometric assay).

Data Analysis
The final analysis was performed in 168 patients, with 12 patients having been excluded because they required additional drugs beyond those specified in the study.

BA measurements were performed on acquired frames by the computerized edge detection system. Baseline BA diameter was the mean of measures obtained during the first minute. FMD and response to GTN were calculated as the maximal absolute increase or percent increase (MPI) in diameter above baseline. FMD was also calculated as area under the curve (AUC) of percent change in diameter after RH.

Blood flow volume was calculated by multiplying Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr²). Flow velocity was measured at baseline and within 15 seconds after cuff release. RH was calculated as percent increase in flow after cuff release as compared with baseline flow.

Data were analyzed with the use of an NCSS statistical package. Descriptive data are expressed as mean±SD. The hypertensive and control groups were compared by a 2-sample Student t test for parametric values and a Wilcoxon signed-rank test for nonparametric values. A value of P<0.05 was considered statistically significant.

FMD and Oxidative Stress Variability
The variability of FMD was tested in the control subjects by repeating the vascular study 6 months after baseline determination. MPI variability was 14%, with a mean difference of 0.9% between the first and second measurements. According to these results, the patient number of the present study has 80% power at the 5% level to detect a 1.5% improvement in FMD after therapy.

In our laboratory, oxidative stress variability was assessed in 40 healthy subjects. Intrasyllable variability was found to be 3% for FRAP, 7% for lipoperoxides (LOOH), and 9% for MDA; interassay variability was 12% for FRAP, 17% for LOOH, and 19% for MDA.

Results
Basal systemic demographic, hemodynamic, and humoral characteristics for normotensive subjects and patients with essential hypertension are summarized in Table 1. Age, sex, plasma cholesterol, glycemia, and smoking history were similar between the 2 study groups, who differed in BP measurements (Table 1). Hypertensive patients showed significantly (P<0.01) reduced FMD (0.26±0.09 mm; MPI, 5.2±1.9%; AUC, 392±120 U) as
compared with healthy control subjects (0.36±0.13 mm; MPI, 7.1±2.6%; AUC, 550±175 U) (Figure 1). No difference was found in RH (normotensive subjects, 540±257%; hypertensive subjects, 478±224%; P=NS). Response to GTN was similar in healthy control subjects (0.35±0.15 mm; MPI, 7.0±3.1%) and hypertensive patients (0.36±0.14 mm; MPI, 7.2±2.8%; P=NS) (Figure 1).

Oxidative stress was higher in hypertensive patients as compared with control subjects (Table 1).

When hypertensive patients were randomly assigned to subgroups according to treatment, no difference in clinical characteristics was observed among the different treatment groups (Tables 2 and 3). At baseline, BA diameter (Table 3), FMD, and response to GTN (Figure 2) were similar in the differently treated groups.

All treatments similarly reduced BP (Table 2). The number of patients who required dose doubling was significantly higher in the ACE inhibitor and AT1-receptor antagonist groups, whereas the percentage of patients receiving mono-
treatment was higher in the calcium antagonist groups, whereas the percentage of patients receiving mono-
treatment was higher in the ACE inhibitor and AT1-receptor antagonist groups. Lipid profile and glucose plasma levels were unchanged throughout the treatment period (Table 3).

FMD was significantly increased (P<0.01) (from 0.25±0.10 mm to 0.32±0.12 mm, MPI from 5.1±2 to 6.4±2.4%, AUC from 308±139 to 501±204 U) after perindopril (Figure 2). In contrast, FMD was unchanged as compared with baseline values after telmisartan (from 0.27±0.10 mm to 0.27±0.10 mm, MPI from 5.5±2.1% to 5.6±1.9%), nifedipine (from 0.26±0.11 mm to 0.24±0.10 mm, MPI from 5.2±2.1% to 4.8±1.9%), amlodipine (from 0.27±0.10 mm to 0.25±0.09 mm, MPI from 5.4±2.0% to 5.1±1.8%), atenolol (from 0.27±0.10 mm to 0.28±0.09 mm, MPI from 5.5±2.1% to 5.7±1.9%), or nebivolol (from 0.26±0.10 mm to 0.28±0.12 mm MPI from 5.3±2.2% to 5.6±2.4%) administration (Figure 2). The degree of changes in FMD after perindopril (+1.5±2.1%) was significantly (P<0.01) different from that obtained with other treatments (telmisartan, +0.3±2.9%; nifedipine, −0.5±2.4%; amlodipine, −0.3±2.5%; atenolol, +0.4±2.1%; nebivolol, +0.5±2.2%). It is important to observe that none of the different compounds modified the response to GTN (Figure 2) or resting BA diameter and RH (Table 2).

Oxidative stress was significantly reduced after perindopril, telmisartan, nifedipine, and amlodipine administration but remained unchanged after β-blocker–based therapy (Figure 3).

### Table 2. Blood Pressure Control Among Differently Treated Essential Hypertensive Patients During the Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perindopril Group (n=28)</th>
<th>Telmisartan Group (n=29)</th>
<th>Nifedipine Group (n=28)</th>
<th>Amlodipine Group (n=28)</th>
<th>Atenolol Group (n=29)</th>
<th>Nebivolol Group (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F</td>
<td>18/10</td>
<td>18/11</td>
<td>17/11</td>
<td>17/11</td>
<td>18/11</td>
<td>17/11</td>
</tr>
<tr>
<td>Age, y</td>
<td>51±11</td>
<td>50±9</td>
<td>52±11</td>
<td>53±8</td>
<td>53±9</td>
<td>53±8</td>
</tr>
<tr>
<td>Higher drug dose (%)</td>
<td>28 (100)</td>
<td>28 (100)</td>
<td>18 (64)</td>
<td>19 (68)</td>
<td>22 (76)</td>
<td>22 (79)</td>
</tr>
<tr>
<td>Diuretic added (%)</td>
<td>7 (25)</td>
<td>6 (21)</td>
<td>2 (7)</td>
<td>3 (11)</td>
<td>10 (34)</td>
<td>7 (25)</td>
</tr>
<tr>
<td>Patients with BP &lt;140/90 mm Hg (%)</td>
<td>22 (79)</td>
<td>22 (76)</td>
<td>22 (79)</td>
<td>23 (82)</td>
<td>24 (83)</td>
<td>21 (75)</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. BP indicates blood pressure.

*P<0.001 vs baseline.
administered at a dose causing a degree of vasodilation comparable with FMD as well as in RH, the stimulation evoked to activate endothelial relaxation, our results confirm the presence of impaired endothelium-dependent vasodilation in the conduit arteries of patients with essential hypertension.17,21,22

The original and interesting finding of this study is that administration of the ACE inhibitor perindopril was the only treatment able to improve FMD (both in terms of absolute and percent diameter increments). Since RH and response to GTN did not change after treatment, this suggests that perindopril can improve endothelium-dependent vasodilation in the BA of patients with essential hypertension. Such a finding is in agreement with previous evidence demonstrating that acute intravenous administration of perindoprilat can reverse impaired FMD in the epicardial coronary artery without overt atherosclerosis of patients with essential hypertension.23 In contrast, available evidence indicates that ACE inhibitors are not effective on impaired endothelium-dependent vasodilation to metacholine or acetylcholine in the forearm microcirculation of patients with essential hypertension.24–26 The discrepancy might be explained by the difference in vascular beds explored or stimulation used to activate endothelial function. It is worth noting that the effectiveness of ACE inhibitors in improving endothelial function in conduit arteries is confirmed in epicardial and brachial arteries of patients with coronary artery disease.27–29

It is intriguing that administration of the AT1-receptor antagonist telmisartan did not improve endothelial dysfunction in this population of hypertensive patients. Although no other results concerning the effect of treatment with AT1-receptor antagonists on FMD of conduit arteries are available in patients with essential hypertension, these compounds have been shown to be capable of reversing endothelial dysfunction in patients with coronary artery disease.29,30 and their effect appears to be similar to that exerted by ACE inhibitors.29

Another relevant finding of the present study is that calcium antagonist treatment, both with nifedipine and amlodipine, induced no FMD modifications in the BA of patients with essential hypertension. This result is at variance with previous evidence demonstrating that nifedipine administration improved FMD in the BA of hypertensive patients.13 An explanation for
this divergence may lie in the different sample size. In particular, the small number of patients in the latter study population could have led to a type 1 mistake, given the low reproducibility of FMD.15

However, it should be kept in mind that treatment with amlodipine did not improve FMD in the BA of patients with coronary artery disease.28 It also needs to be underlined that if the microcirculatory district is considered, then the results appear completely different, since both in the forearm and subcutaneous microvasculature calcium antagonists are highly effective in reversing endothelial dysfunction in hypertensive patients,12,31

Finally, the present results also indicate that β-blocker treatment did not modify FMD in the BA of patients with essential hypertension. Although previous evidence obtained in the subcutaneous and muscle microcirculation has demonstrated the lack of efficacy of atenolol,2,33 in contrast, nebivolol, a selective β1-adrenoceptor antagonist with vasodilating properties mediated by NO,34,35 was shown to be capable of improving the response to acetylcholine in the forearm of hypertensive patients.36 On the other hand, as pointed out above, the efficacy of various compounds can may differ according to the vascular district considered.

Taken together, these results demonstrate that the ACE inhibitor perindopril was the only compound that improved endothelium-dependent vasodilation in the peripheral conduit arteries of patients with essential hypertension. The effect of the ACE inhibitor is very likely to be specific and not related to methodological problems. First, important clinical characteristics of the different study subgroups that could independently affect endothelial function, such as lipid and glucidic profile or smoking history, were similar at baseline and did not prove to be significantly modified during the 6-month treatment period. Moreover, the antihypertensive effect of the various treatments was superimposable, thus excluding FMD modification attributable to different blood pressure reduction. Finally, since noninvasive assessment of endothelium-dependent vasodilation by FMD requires an accurate methodology to ensure adequate reproducibility,14 a methodological bias could affect the results. In our laboratory, the adequate experimental setup including online computerized analysis of BA diameter ensures good reproducibility, as previously tested16,17 and confirmed in the present study.

A crucial issue to be addressed is the possible mechanism responsible for the beneficial effect of ACE inhibition on BA endothelium-dependent vasodilation. In essential hypertension, one of the most important mechanisms determining endothelial dysfunction is the production of oxidative stress, at least in the peripheral microcirculation1 or in the coronary epicardial vessels.37 In the present study, this possibility was indirectly explored by measuring plasma parameters of oxidative stress. Interestingly, administration of perindopril, telmisartan, nifedipine, or amlodipine was able to reduce plasma MDA and lipoperoxides and increase FRAP, a marker of total antioxidant capability, confirming previous evidence that ACE inhibitors, AT-1 antagonists, and calcium antagonists can interfere with oxidative stress.29,31,32 However, in our study, the beneficial effect of treatment on oxidative stress was not universally associated with improvement in endothelial function. Two possible explanations could be put forward to account for this discrepant effect. First, the drug activity on systemic markers of oxidative stress may not reflect the effect on intracellular oxidative mechanisms. Alternatively, the increase in endothelium-dependent vasodilation observed after perindopril treatment may not be determined by antioxidant activity. One peculiar effect of ACE inhibitors is the accumulation of bradykinin, which can increase endothelium-dependent vasodilation by a pathway involving hyperpolarization.5,38 Thus, it could be hypothesized that the beneficial effect of perindopril on conduit artery endothelium-dependent vasodilation may be related to bradykinin-dependent mechanisms. This would be in line with the lack of effect observed for AT-1 antagonists or calcium antagonists, compounds, which, at least in humans, act on endothelial function by mechanisms possibly independent from bradykinin.29,31,32

Finally, the present results further support the concept that endothelium acts as an autocrine-paracrine system,1 and therefore that conclusions regarding mechanisms and therapeutic measures should be limited to the experimental model explored.
Perspectives
This study indicates a specific beneficial effect of ACE inhibition not shared by the other main antihypertensive drug classes on conduit artery endothelium-dependent vasodilation in patients with essential hypertension. Although endothelial dysfunction is an independent promoter of cardiovascular events, no clinical data demonstrate that the reversal of endothelial dysfunction can improve the prognosis of patients with hypertension or other cardiovascular risk factors. Thus, in the near future, large-scale clinical trials are required to demonstrate that treatment of endothelial dysfunction in patients with essential hypertension can exert a beneficial effect on cardiovascular events, adjunctive to that achieved by BP control.

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References
Different Effect of Antihypertensive Drugs on Conduit Artery Endothelial Function
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