Correction of Arterial Structure and Endothelial Dysfunction in Human Essential Hypertension by the Angiotensin Receptor Antagonist Losartan

Ernesto L. Schiffrin, MD, PhD, FRCPC; Jeong Bae Park, MD; Hope D. Intengan, PhD; Rhian M. Touyz, MD, PhD

Background—Structural and functional alterations of the vasculature may contribute to complications of hypertension. Because angiotensin II may be pivotal in some of these vascular abnormalities, we tested the hypothesis that the angiotensin type 1 (AT₁) receptor antagonist losartan, in contrast to the β-blocker atenolol, would correct resistance artery abnormalities in patients with essential hypertension.

Methods and Results—Nineteen untreated patients with mild essential hypertension (47 ± 2 years, range 30 to 65 years; 57% male) were randomly assigned in double-blind fashion to losartan or atenolol treatment for 1 year. Nine age/sex-matched normotensive subjects were also studied. Both treatments reduced blood pressure to a comparable degree (losartan, from 149 ± 4.1/101 ± 1.6 to 128 ± 3.6/86 ± 2.2 mm Hg, \( P < 0.01 \); atenolol, from 150 ± 4.0/99 ± 1.2 to 130 ± 3.2/84 ± 1.4 mm Hg, \( P < 0.01 \)). Resistance arteries (luminal diameter 150 to 350 mm) dissected from gluteal subcutaneous biopsies were studied on a pressurized myograph. After 1 year of treatment, the ratio of the media width to lumen diameter of arteries from losartan-treated patients was significantly reduced (from 8.4 ± 0.4% to 6.7 ± 0.3%, \( P < 0.01 \)). Arteries from atenolol-treated patients exhibited no significant change (from 8.3 ± 0.3% to 8.8 ± 0.5% after treatment). Endothelium-dependent relaxation (acetylcholine-induced) was normalized by losartan (from 82.1 ± 4.9% to 94.7 ± 1.1%, \( P < 0.01 \)) but not by atenolol (from 80.4 ± 2.7% to 81.7 ± 4.6%). Endothelium-independent relaxation (by sodium nitroprusside) was unchanged after treatment.

Conclusions—The AT₁ antagonist losartan corrected the altered structure and endothelial dysfunction of resistance arteries from patients with essential hypertension, whereas the β-blocker atenolol had no effect. (Circulation. 2000;101:1653-1659.)

Key Words: arteries ■ blood pressure ■ endothelium ■ hypertrophy ■ receptors ■ remodeling

Lowering the blood pressure (BP) of hypertensive individuals in multicenter randomized clinical trials decreases the incidence of stroke, heart and renal failure, and mortality. In contrast, most clinical trials failed to show the beneficial effects on cardiac ischemia expected from population studies for the level of BP reduction achieved (5 to 6 mm Hg). This may imply that BP lowering alone does not normalize altered vessels and that, consequently, vascular events may continue to occur. In hypertensive patients, the extent and consequences of ischemia may be influenced by small-vessel disease. Endothelial dysfunction of small arteries has been implicated in the pathogenesis of myocardial ischemia. To improve the outcome in hypertensive patients, it may be necessary to correct vascular remodeling and endothelial dysfunction, in addition to lowering BP.

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Increased peripheral resistance in hypertension results predominantly from altered structure (narrowed lumen diameter and/or a thickened media, with increased ratio of media width to lumen diameter [M/L]) and function of resistance arteries <350 μm in diameter. Increased M/L results from hypertrophic remodeling (thicker media encroaches on the lumen) or eutrophic remodeling (reduced outer vessel diameter narrows the lumen without net growth) and amplifies vascular responses to vasoconstrictors. These changes may predispose the patient to cardiac ischemia, nephroangiosclerosis, and stroke. Endothelial dysfunction in small vessels in hypertension (eg, impaired acetylcholine-induced endothelium-dependent relaxation) can also contribute to myocardial ischemia and other manifestations of hypertensive target organ damage.
Angiotensin I–converting enzyme inhibitors (ACEIs)\(^{20–23}\)
and angiotensin type 1 (AT\(_1\)) receptor antagonists\(^{22–25}\) have a regressive effect on vascular changes in spontaneously hypertensive rats; these vascular changes involve small coronary, renal, and cerebral arteries. In hypertensive humans, a favorable effect of the ACEIs cilazapril, perindopril, and lisinopril was demonstrated on subcutaneous small artery structure,\(^{26–29}\) whereas the β-blocker atenolol was without effect.\(^{26–31}\) In the present study, we tested the hypothesis that in patients with essential hypertension, treatment for 1 year with the AT\(_1\) antagonist losartan would correct altered resistance arterial structure and endothelial function, whereas treatment with atenolol would not.

**Methods**

**Patients**

The protocol was approved by the Ethics Committee of the Clinical Research Institute of Montreal. Normotensive subjects and patients with essential hypertension (aged 30 to 65 years) provided written informed consent to participate in the study. Control subjects had systolic and diastolic BP <140 and <85 mm Hg, respectively. Recumbent diastolic BP of hypertensive patients was >90 mm Hg on at least 3 occasions. Ten patients had never received antihypertensive drugs, and 9 had not received antihypertensive drugs for >6 months. Diagnosis of essential hypertension was established by the absence of clinical evidence of secondary hypertension and by normal serum electrolytes, creatinine, urinalysis, abdominal echocardiogram, and, when indicated, renal scintiscan, renal arteriogram, or computed abdominal tomography. Exclusion criteria included smoking >5 cigarettes per day, abnormal fasting blood glucose, serum creatinine concentration >150 \(\mu\)mol/L, and systemic diseases. Clinic sitting BP was measured after 15 minutes of rest; diastolic BP was read as phase V of Korotkoff sounds. Twenty-four-hour ambulatory BP monitoring was recorded hourly (8 AM to 10 PM) with a model 90207 SpaceLab recorder. Left ventricular mass was calculated echocardiographically by the Penn Convention.\(^{32}\)

**Trial Design**

Gluteal subcutaneous biopsies measuring 1.0 \(\times\) 0.5 \(\times\) 0.5 cm\(^3\) were obtained under local anesthesia (2% lidocaine) with patients on placebo and after 1 year of treatment; biopsies were performed once in normotensive subjects. Patients were randomly assigned to treatment with 50 mg losartan or atenolol in a double-blind fashion. If diastolic BP was >90 mm Hg after 2 weeks, dosage of the drug was raised to 100 mg, and 4 weeks later, open-label hydrochlorothiazide (12.5 to 25 mg) was added if needed.

**Vascular Studies**

The study of arteries was performed by individuals blinded to the groups to which the vessels belonged. Small arteries (lumen diameter 150 to 350 \(\mu\)m) were isolated from subcutaneous tissue immediately after the biopsy and mounted on a pressurized myograph.\(^{30}\) Vessel segments (2 to 3 mm long) were slipped onto 2 glass microcannulas, one of which was positioned until vessel walls were parallel, and equilibrated in physiological salt solution (mmol/L: NaCl 120, NaHCO\(_3\) 25, KCl 4.7, KH\(_2\)PO\(_4\) 1.18, MgSO\(_4\) 1.18, CaCl\(_2\) 2.5, EDTA 0.026, and glucose 5.5), continuously bubbled with 95% O\(_2\) and 5% CO\(_2\) to achieve a pH of 7.40 at 37°C, pressurized to 60 mm Hg. Endothelium-dependent and -independent relaxations were assessed by measuring dilatory responses to acetylcholine (1 mmol/L to 100 \(\mu\)mol/L) and sodium nitroprusside (10 mmol/L to 1 mmol/L), respectively, in vessels precontracted with norepinephrine (1 \(\mu\)mol/L). Thereafter, vessels were deactivated with physiological salt solution plus 10 mmol/L EGTA to eliminate myogenic tone before measuring structure.

**Data Analysis**

The remodeling index (percentage of difference between internal diameters of hypertensive and normotensive vessels not attributable to growth) was calculated as previously described\(^{40}\):

\[
\frac{[(D_i)_h−(D_i)_n]/[(D_e)_h−(D_e)_n]]}{[(D_i)_n−(D_i)_h]/[(D_e)_n−(D_e)_h]],
\]

where \((D_i)_h\) and \((D_i)_n\) are the internal diameters of normotensive and hypertensive vessels, respectively, and \((D_i)_n\) and \((D_i)_h\) is the remodeled internal diameter, \((D_{remodel})=[(D_i)_h^2−4CSA/\pi]/2\), where \((D_i)_h\) is the external diameter of hypertensive vessels, and CSA\(_h\) is the media cross-sectional area of normotensive vessels. Growth index was calculated as (CSA\(_n−CSA_h)/CSA_h\), where CSA\(_h\) and CSA\(_n\) are cross-sectional areas of normotensive and hypertensive vessels, respectively.

Results are presented as mean±SEM. Comparisons were performed by 2-tailed Student \(t\) test, 1-way ANOVA followed by Newman-Keuls test, and repeated-measures ANOVA, as appropriate. A value of \(P<0.05\) was considered statistically significant.

**Results**

Demographics of subjects appear in Table 1. Only 5 of the 19 hypertensive patients had echocardiographically demonstrable left ventricular hypertrophy (left ventricular mass index [LVMI] >134 g/m\(^2\) in males and 110 g/m\(^2\) in females). Serum electrolytes, creatinine, lipids, and supine plasma renin activity were similar in all groups. Atenolol (average daily dose 67.5 mg) or losartan (average daily dose 88.9 mg) produced similar control of clinic BP in both groups (Figure 1). Ambulatory BP monitoring was similarly well controlled. Addition of 12.5 mg hydrochlorothiazide in 1 atenolol-treated and 4 losartan-treated patients was needed to achieve goal BP. Echocardiographic LVMI did not change significantly. However, ECG voltage criteria of Sokolow and Lyon\(^{33}\) (SV\(_1+RV_1\) or V\(_6\)) showed a decrease of −0.31 mV (95% CI −0.00099 to 0.6232, \(P=0.05\)) in losartan-treated patients versus a decrease of −0.07 mV in atenolol-treated patients. Supine plasma renin activity increased under losartan treatment (\(P<0.01\)).

Resistance vessels exhibited significantly greater media thickness and M/L in hypertensive patients than in normotensive subjects (Table 2). Remodeling and growth indexes were 79.9% and 12.8%, respectively, suggesting eutrophic remodeling.\(^{9,11}\) After 1 year, media thickness and M/L of resistance arteries were significantly smaller in patients after losartan treatment than before treatment, whereas in atenolol-treated patients, these values remained abnormal (Figure 2, Table 2). The 4 patients treated with losartan who required hydrochlorothiazide (8.3±0.5% before and 6.6±0.5% after treatment) had an M/L similar to that of other patients in their group.

Endothelial function tested with acetylcholine-induced relaxation demonstrated that the maximal response to acetylcholine was diminished in untreated hypertensive patients compared with normotensive subjects (Figure 3). Maximal acetylcholine response from untreated hypertensive patients correlated inversely with clinic systolic BP \((r=−0.56, P<0.01)\). Maximal acetylcholine relaxation was similar in the losartan and atenolol groups before treatment (82.1±4.9% and 80.4±2.7%, respectively). It was significantly improved in vessels after losartan treatment (94.7±1.1%, \(P<0.01\)) but not after atenolol treatment (81.7±4.6%). Sodium nitroprusside–induced relaxation was similar in normotensive subjects (98.0±1.0%) and hypertensive subjects (93.7±1.2%)
before and after treatment). In losartan+diuretic–treated patients, relaxation changed from 82.6±9.5% to 94.0±0.7%, similar to patients not receiving diuretic. Before antihypertensive therapy, total serum cholesterol and maximal acetylcholine response were inversely correlated ($r=-0.47$, $P<0.05$). Changes in HDL cholesterol were correlated with improvement in endothelium-dependent relaxation ($r=0.49$, $P<0.05$).

### Discussion

This randomized, double-blind, parallel-design, 1-year trial demonstrates for the first time that AT1 receptor antagonism corrects structural and functional changes in subcutaneous resistance arteries, a model of resistance artery representative of other systemic vessels, in patients with mild to moderate essential hypertension. Equally effective BP control with the β-blocker atenolol failed to influence arterial structure or function.

### Table 1. Subject Demographics and Laboratory Characteristics

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<th>Hypertensives</th>
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<th>Atenolol Treatment</th>
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</tbody>
</table>

Values are mean±SEM. SBP indicates clinic systolic BP; DBP, clinic diastolic BP; ASBP, ambulatory SBP; ADBP, ambulatory DBP; and ND, not done.

* $P<0.01$ vs normotensives; † $P<0.01$ vs before treatment.

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### Figure 1

Systolic and diastolic BP of patients treated with losartan or atenolol for 1 year. Treatment was started at time 0. BP was not significantly different between groups. * $P<0.01$ for time 0 vs each subsequent visit after treatment started (repeated-measures ANOVA).
function, as previously reported in other groups of patients.\textsuperscript{26,28,30}

Structural abnormalities of small arteries in the present study are analogous to those previously reported in untreated hypertensive patients,\textsuperscript{10–14,26–29,34} with remodeling (79.9%) and growth indexes (12.8%) suggesting eutrophic remodeling. Increased M/L was found in small arteries of patients, among whom only 26% had left ventricular hypertrophy and none had proteinuria. An increased M/L in subcutaneous resistance arteries is thus the first detectable manifestation of target organ damage in middle-aged hypertensive subjects. In the present study, echocardiographic LVMI did not change significantly, but ECG voltage criteria (less subject to variability) showed a reduction of left ventricular mass in only the losartan-treated group. In 69 hypertensive patients with left ventricular hypertrophy, another AT\textsubscript{1} antagonist, valsartan, corrected LVMI, whereas atenolol was less effective,\textsuperscript{35} in agreement with our ECG findings.

Angiotensin II via AT\textsubscript{1} receptors may induce remodeling of the arterial wall through smooth muscle growth\textsuperscript{36–38} and collagen deposition,\textsuperscript{39} as found in small arteries in hypertension.\textsuperscript{34,40} AT\textsubscript{1} receptor stimulation is accompanied secondarily by an increased apoptotic rate in the arterial wall\textsuperscript{41} and myocardium,\textsuperscript{42} which could counterbalance cell proliferation, leading to eutrophic remodeling. The regression of M/L in losartan- but not atenolol-treated patients suggests that interference with the actions of angiotensin II, as demonstrated earlier in humans with the use of ACEI\textsuperscript{26–29} and in the present study for the first time with AT\textsubscript{1} antagonists, corrects small artery remodeling in hypertensive patients. AT\textsubscript{1} receptor blockade may be particularly effective in light of reports that dual pathways for angiotensin II generation by angiotensin-converting enzyme and a chymostatin-sensitive enzyme, presumably chymase, exist in human resistance arteries.\textsuperscript{43} AT\textsubscript{1} antagonist–elicited reflex elevation of plasma angiotensin II may stimulate unblocked angiotensin type 2 (AT\textsubscript{2}) receptors, which could modulate vascular remodeling. However, the presence of AT\textsubscript{2} receptors in blood vessels from adults is disputed. Because AT\textsubscript{1} antagonists are vasodilators but atenolol has vasoconstrictor effects,\textsuperscript{44} hemodynamic ef-

<table>
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<th>Losartan Treatment</th>
<th>Atenolol Treatment</th>
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<tr>
<td>Before</td>
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<td>External diameter, $\mu$m</td>
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<td>Internal diameter, $\mu$m</td>
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<td>MCSA, $\mu$m$^2$</td>
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Values are mean±SEM. MCSA indicates media cross-sectional area.

* $P<0.05$ vs normotensives (1-way ANOVA/Student-Newman-Keuls); † $P<0.05$ vs before treatment (repeated-measures ANOVA/Student-Newman-Keuls); ‡ $P<0.05$ vs 1-year losartan treatment (repeated-measures ANOVA/Student-Newman-Keuls).
factors could contribute to the disparate impact of these drugs on small artery structure. BP was equally controlled by atenolol and losartan, without improved vascular structure with the former, suggesting that BP lowering alone did not play a role in our findings. Although we cannot conclude from the present study whether hydrochlorothiazide affected small arteries, 2 studies\textsuperscript{28,45} did not find that diuretics influenced small artery structure in hypertensive patients.

Endothelial dysfunction associated with hypertension, hyperlipidemia, and other risk factors of cardiovascular disease may contribute to complications, including myocardial ischemia.\textsuperscript{5} Acetylcholine-induced relaxation allows evaluation of endothelial function and is impaired in vitro in hypertensive animals\textsuperscript{46,47} and in vivo in the forearm of hypertensive humans in some\textsuperscript{18,49} but not all\textsuperscript{50} studies. In vitro acetylcholine responses of subcutaneous small arteries from hypertensive patients were moderately impaired in response to the highest concentration in some studies\textsuperscript{19,29,30} but were normal in another study.\textsuperscript{50} In the present study, the endothelium-dependent response (maximal dose of acetylcholine) was normalized in losartan-treated patients, whereas it remained impaired in atenolol-treated patients, with the latter finding resembling previous reports in atenolol-treated subjects.\textsuperscript{26,30,31} Blockade of AT\textsubscript{1} receptors with attenuated angiotensin II–induced oxidative stress\textsuperscript{51,52} could decrease the degradation of nitric oxide and result in improved endothelial function. Reflex elevation of plasma angiotensin II elicited by AT\textsubscript{1} antagonists and stimulation of unblocked AT\textsubscript{2} receptors could favorably affect the endothelium, as in the kidney, where AT\textsubscript{2} receptor stimulation elicits the generation of nitric oxide.\textsuperscript{53} Hypercholesterolemia is associated with blunted endothelium-dependent relaxation of small subcutaneous resistance arteries, and its correction improves this abnormality.\textsuperscript{54} In the present study, total cholesterol was correlated inversely with the magnitude of acetylcholine-induced relaxation before treatment, and the change of HDL cholesterol in both hypertensive groups was correlated positively with the change in acetylcholine response after treatment. This could indicate a role of blood lipids in the improvement of endothelial dysfunction in losartan-treated patients.

An important consideration is whether these results imply clinically relevant vascular protection for patients, which will translate into improved outcomes, reduced morbidity, and mortality in hypertension. The present study provides some insight into this question. Subcutaneous small arteries behave structurally and functionally like small arteries from the coronary, renal cortical, and mesenteric vascular beds of hypertensive rats, including the response of these arteries to specific antihypertensive therapy (eg, ACEI, angiotensin receptor antagonists, and calcium channel blockers).\textsuperscript{20–22,24} Structural changes in gluteal subcutaneous vessels parallel those occurring in vessels in the human forearm.\textsuperscript{55} Motz and Strauer\textsuperscript{56} showed that 1 year of enalapril treatment improved coronary microcirculatory reserve in hypertensive patients, in agreement with the improvement in subcutaneous small artery structure and function demonstrated by methodology similar to that used in the present study in ACEI-treated hypertensive patients.\textsuperscript{26–28} Moreover, endothelium-dependent relaxation of subcutaneous resistance arteries\textsuperscript{19} correlates closely with flow-mediated dilatation of the brachial artery (J.B. Park, F. Charbonneau, E.L. Schiffrin, unpublished data, 1999). The latter exhibits a close correlation with epicardial coronary artery endothelium-dependent vasomotor responses to acetylcholine.\textsuperscript{57} Similar to improvement of endothelial function in resistance arteries from hypertensive patients under ACEI therapy,\textsuperscript{29,31} ACEI treatment improves endothelial dysfunction of epicardial coronary arteries in patients with coronary artery disease.\textsuperscript{58} Taken together, these data suggest that vascular protection in subcutaneous resistance arteries under losartan treatment may reflect changes occurring in the coronary, renal, and cerebral circulation.

A limitation of the present study is that vessels were investigated in vitro, where they are devoid of perivascular tethering and support, which may modify their behavior relative to the in vivo situation. Currently, resistance-sized arteries cannot be investigated in humans in vivo. In the present study, we report results obtained with a pressurized myograph, but vessels were also studied on a wire myograph (not reported). Both methods provided closely correlated M/L ($r=0.723$, $P<0.001$, n=44) and functional results, as demonstrated previously\textsuperscript{30} and reviewed recently.\textsuperscript{59} This allows confidence in the reliability of the data and comparison with results of studies performed on human vessels in the past with wire myography.\textsuperscript{10,11,14,19,26–30,34}

In conclusion, treatment with the AT\textsubscript{1} receptor antagonist losartan improved structural abnormalities and normalized endothelial function of small arteries from patients with mild to moderate essential hypertension. None of these effects was found in a parallel group of hypertensive patients treated with the β-blocker atenolol, despite similar BP lowering. Whether the putatively beneficial vascular-protective effects of losartan will translate into improved outcome in hypertension beyond the effect of blood pressure lowering itself, with reduced morbidity (cardiac events, stroke, and progression of hypertensive nephropathy) or mortality, remains to be demonstrated.
Acknowledgments
This study was supported by a group grant from the Medical Research Council of Canada (MRCC) to the Multidisciplinary Research Group on Hypertension and a grant from Merck-Frosst Canada. Dr. Intengan is the recipient of a postdoctoral fellowship from MRCC, and Dr. Touyz is the recipient of a Canadian Hypertension Society-MRCC Scholarship. The authors thank Mireille Kirouac, RN, and Michele Lapointe, RN, for invaluable help in recruitment of patients, carrying out this protocol, and blood sampling and Li Yuan Deng and Jin-S. Li for expert technical help.

References


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Circulation. 2000;101:1653-1659
doi: 10.1161/01.CIR.101.14.1653

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