Selective Mineralocorticoid Receptor Blocker Eplerenone Reduces Resistance Artery Stiffness in Hypertensive Patients

Carmine Savoia, Rhian M. Touyz, Farhad Amiri, Ernesto L. Schiffrin

Abstract—Some antihypertensive agents may improve resistance artery remodeling in hypertensive patients whereas other agents may not, for similar blood pressure reduction. We questioned whether the selective mineralocorticoid receptor blocker eplerenone improves resistance artery remodeling in hypertensive patients versus the β-blocker atenolol. Sixteen hypertensive patients were randomly assigned to double-blind daily treatment with eplerenone or atenolol. Resistance arteries from gluteal subcutaneous tissue were assessed on a pressurized myograph. After 1 year of treatment, systolic and diastolic blood pressures were similarly well controlled in both groups. Endothelial function did not change with treatment in either group. Media/lumen ratio and cross-sectional area were unchanged in either the atenolol or the eplerenone group. In atenolol-treated patients, the arterial wall became stiffer, whereas in the eplerenone-treated patients, it became less stiff and similar to that of a normotensive control group. The media collagen/elastin ratio was reduced only after eplerenone treatment. Circulating concentrations of osteopontin, monocyte chemoattractant protein-1, basic fibroblast growth factor, interleukin-8, and interleukin-10 were significantly reduced only by eplerenone. However, plasma interleukin-1 receptor antagonist concentration was significantly reduced by both drugs. In conclusion, in hypertensive patients, blood pressure control for 1 year with atenolol was associated with increased wall stiffness of resistance arteries, whereas eplerenone treatment was associated with reduced stiffness, decreased collagen/elastin ratio, and a reduction in circulating inflammatory mediators. These data raise the possibility that eplerenone treatment of hypertensive patients when normalizing blood pressure could potentially be associated with better vascular protection and outcomes than the β-blocker atenolol, which remains to be demonstrated. (Hypertension. 2008;51[part 2]:432-439.)

Key Words: aldosterone • vascular remodeling • extracellular matrix • collagen • elastin • inflammatory biomarkers

Normalization of elevated blood pressure (BP) in hypertensive individuals is associated with reduced target-organ damage and incident cardiovascular morbidity and mortality. Small artery structural alterations may precede many clinical manifestations of end-organ damage in hypertensive patients and predict the occurrence of future cardiovascular events. Activation of the renin-angiotensin-aldosterone system has been associated with increased cardiovascular morbidity and mortality in hypertensive patients. We and others have shown that antihypertensive drugs that interfere with the renin-angiotensin-aldosterone system exert potentially beneficial effects on vascular structure beyond BP control in hypertensive patients at variable risk for cardiovascular disease by improving the functional and anatomic alterations of resistance arteries typically found in experimental and human hypertension and which participate in increased peripheral resistance.

Aldosterone is a mineralocorticoid synthesized not only in the adrenal cortex but potentially also in blood vessels. Aldosterone may increase BP by actions on blood vessels independent of its effects on the kidney that influence salt and water balance. Activation of mineralocorticoid receptors may contribute to cardiovascular dysfunction, inflammation, fibrosis, and cardiovascular damage. Preclinical studies have shown that aldosterone exerts important pathophysiological effects on the cardiovascular system and causes vascular injury in the brain, heart, and kidneys. However, in most patients with essential hypertension, plasma aldosterone levels are within the reference range. Yet, clinical trials in patients with hypertension and/or left ventricular hypertrophy/dysfunction or congestive heart failure have shown a beneficial effect of treatment with mineralocorticoid receptor antagonists on cardiac performance and cardiovascular mortality. Mineralocorticoid antagonism attenuates cardiovascular damage by mechanisms that seem to be in part independent of changes in systolic BP and that involve direct blockade of aldosterone’s cardiovascular proinflammatory and profibrotic effects.
In the present study we tested the hypothesis that remodeling of resistance arteries from mild-to-moderate hypertensive patients could be improved by the selective mineralocorticoid receptor blocker eplerenone compared with the β-blocker atenolol at equal levels of BP control.

Methods

Patients
The study protocol was approved by the ethics committee of the Clinical Research Institute of Montreal, where the study was carried out. Normotensive and stage 1 (mild-to-moderate) hypertensive patients (aged 30 to 70 years) provided written informed consent to participate in the study. Clinic sitting BP was measured after 15 minutes of rest; diastolic BP was read as phase V Korotkoff sounds. Control subjects had systolic and diastolic BPs <135 and <85 mm Hg, respectively. Recumbent systolic and diastolic BPs of hypertensive patients were >140 and/or >90 mm Hg, respectively, on ≥3 occasions. None of the patients had previously received antihypertensive medication. The absence of secondary forms of hypertension was confirmed by usual diagnostic techniques. Exclusion criteria included smoking >10 cigarettes per day, serum creatinine concentration >200 μmol/L, symptomatic ischemic heart disease or myocardial infarction within the previous 6 months, congestive heart failure, or systemic disease.

Trial Design
Gluteal subcutaneous biopsies were obtained under local anesthesia during a run-in period of 4 weeks on placebo, and a second biopsy was obtained after 1 year of treatment. These were performed as already reported by us for previous studies.4–6 Biopsies were performed only once on normotensive subjects. Patients were randomly, double-blindly assigned to treatment with 50 mg of eplerenone or atenolol daily. If systolic and diastolic BP remained >140 and/or >90 mm Hg, respectively, after 2 weeks, the dosage of eplerenone or atenolol was raised to 100 mg daily. Four weeks later, open-label hydrochlorothiazide (12.5 mg, raised later to 25 mg if needed) was added to achieve goal BP <140 and <90 mm Hg.

Vascular Studies
The study of resistance arteries was performed by individuals unaware of the groups that samples belonged to. Small arteries (lumen diameter 150 to 300 μm) were isolated from subcutaneous tissue immediately after the biopsy and mounted on a pressurized myograph. Experiments and calculations of vascular morphology and mechanical properties were carried out as described in previous publications.4–6

Collagen and Elastin Determination
Pressurized (60-mm Hg) resistance arteries were fixed in Russell fixative and embedded in paraffin. For collagen type I, 5-μm sections were stained with Sirius red F3BA (0.1% [pH 2.0] in saturated picric acid solution, Sigma). Elastin was identified with Verhoeff-van Giesson stain.20 Microscopic images were analyzed by Northern Eclipse 7.0 (EMPIX Imaging Inc).

Measurement of Inflammatory Biomarkers
Blood was collected in EDTA tubes by antecubital vein puncture during a run-in placebo period of 4 weeks. Another blood sample was obtained after 1 year of treatment. Blood was collected only once in normotensive control subjects. Blood was centrifuged immediately and plasma stored at −80°C. Assays were done simultaneously to reduce interassay variability by an operator unaware of their provenance. Plasma levels of monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-1β and IL-6, IL-8, IL-1 receptor a (IL-1Ra), IL-10, basic fibroblast growth factor (bFGF), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM-1) were measured by Bioplex assay with microbeads (Bio-Rad Laborato-

Results
Subject demographics are presented in the table. Body weight and body mass index were similar in patients and the control group and were unchanged in the former during the study. Systolic and diastolic BPs were equally well controlled by atenolol and eplerenone. *P<0.05 after random assignment to atenolol or eplerenone vs before treatment.

Data Analysis
Results are presented as means±SEMs. Comparisons were performed by 2-tailed Student’s t test, 1-way ANOVA followed by the Newman-Keuls test, or 2-way or repeated-measures ANOVA, as appropriate. Regression analysis was done by the least-squares method. P<0.05 was considered statistically significant.
the control group and did not change significantly under treatment (Figure 2a). N^G^-nitro-L-arginine methyl ester significantly reduced acetylcholine-induced dilatation equally in all of the vessels (Figure 2b), suggesting preserved endothelial function and NO bioavailability.

Resistance arteries from hypertensive patients exhibited similar lumen diameter but significantly greater media thickness (125%; \( P<0.05 \)) and media/lumen ratio (46%; \( P<0.05 \)) than normotensive subjects (Figure 3a through 3c). Media cross-sectional area was similar in vessels from hypertensive patients compared with control subjects (16.51 ± 1.16 \( \times 10^3 \) \( \mu \text{m}^2 \) versus 14.56 ± 1.7 \( \times 10^3 \) \( \mu \text{m}^2 \), respectively; +13%; \( P \) value not significant [NS]; Figure 3d). Remodeling and growth indexes were 81% and 13%, respectively, supporting the concept that vessels in the hypertensive subjects exhibited eutrophic remodeling.

Data are mean ± SEM, unless otherwise specified. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

\( *P<0.05 \) posttreatment vs pretreatment.

\( \dagger P<0.05 \) vs control.

**Table. Clinical Parameters of Subjects Studied**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Atenolol, Basal</th>
<th>Atenolol, 1 y</th>
<th>Epilrenone, Basal</th>
<th>Epilrenone, 1 y</th>
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<tr>
<td>No.</td>
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<td>8</td>
<td>8</td>
<td>8</td>
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<td>Age, y</td>
<td>51.2±1.7</td>
<td>44.3±4.0</td>
<td>+1</td>
<td>53.7±3.2</td>
<td>+1</td>
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<td>Body weight, kg</td>
<td>75.1±4.3</td>
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<td>81.5±4</td>
<td>88.9±4.9</td>
<td>89.6±4.7</td>
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<td>BMI, kg/m²</td>
<td>26.8±1</td>
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<td>28.8±1</td>
<td>32.2±2</td>
<td>32.4±1.9</td>
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<td>SBP, mm Hg</td>
<td>110.5±3.3</td>
<td>143.3±3.7</td>
<td>121.4±3.1†</td>
<td>141.8±3.5†</td>
<td>129.1±1.9†</td>
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<td>DBP, mm Hg</td>
<td>72.8±2.3</td>
<td>95.3±1.8†</td>
<td>79.2±2.6†</td>
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<td>84.5±1.3†</td>
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<td>Pulse pressure, mm Hg</td>
<td>37.7±2.2</td>
<td>48.8±4.7</td>
<td>42.1±2.7*</td>
<td>51.7±3.9</td>
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<td>MAP, mm Hg</td>
<td>85.3±2.5</td>
<td>111.7±1.3†</td>
<td>93.2±2.5†</td>
<td>107.3±1.2†</td>
<td>99.3±1.07†</td>
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<td>Heart rate, bpm</td>
<td>70.1±3.2</td>
<td>69.8±2.9</td>
<td>64.3±2.2*</td>
<td>62.8±2.4</td>
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<td>Urea, mmol/L</td>
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<td>5.7±0.4</td>
<td>5.5±0.2</td>
<td>5.2±0.6</td>
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<td>Creatinine, μmol/L</td>
<td>85.3±6.2</td>
<td>81.1±5.1</td>
<td>83.1±5.0</td>
<td>81.2±6.4</td>
<td>95.5±6*</td>
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<td>Na⁺, mEq/L</td>
<td>141.0±0.5</td>
<td>141.9±0.8</td>
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<td>140.8±0.5</td>
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<td>K⁺, mEq/L</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4±0.1</td>
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<td>1.6±0.1</td>
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<td>LDL cholesterol, mmol/L</td>
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<td>3.2±0.4</td>
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<tr>
<td>Triglycerides, mmol/L</td>
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<td>1.3±0.3</td>
<td>1.3±0.2</td>
<td>1.6±0.2</td>
</tr>
</tbody>
</table>

Figure 2. a. Endothelium-dependent (acetylcholine [Ach]) and -independent (sodium nitroprusside [SNP]) relaxation of resistance arteries from normotensive individuals and hypertensive patients. Vessels were preconstricted with norepinephrine (1 μmol/L). b. Effect of the NO synthase (NOS) inhibitor N^G^-nitro-L-arginine methyl ester (L-NAME) on acetylcholine-induced relaxation. \( *P<0.05 \) Ach + L-NAME vs Ach in all groups.
treatment, lumen diameter was unchanged by atenolol (−1.8% versus before treatment; P value NS) or eplerenone treatment (−4% versus before treatment; P value NS; Figure 3a). Media thickness tended to be reduced albeit not significantly in the atenolol treated group (−17%; P value NS). However, in the eplerenone group, media thickness was significantly reduced (−22%; P<0.05; Figure 3b). There was no significant change in the media/lumen ratio or the cross-sectional area at the end of the study with either treatment (media/lumen ratio −16% versus before treatment in both groups; P value NS; Figure 3c; media cross-sectional area: 13.93±2.1×10^3 μm^2, −16%, under atenolol, and 12.1±147×10^3 μm^2, −27%, under eplerenone treatment; P value NS; Figure 3d).

Stress-strain curves of vessels from the hypertensive patients were shifted to the left (increased stiffness) compared with vessels from normotensive control subjects (Figure 4). A further leftward shift occurred under atenolol, whereas the stress-strain curve of vessels from eplerenone-treated patients shifted to the right, becoming equal to that of the control subjects.

Vascular content of type I collagen and elastin was similar in both groups of hypertensive patients before treatment. Only after eplerenone treatment was vascular collagen content significantly reduced and elastin significantly increased compared with vessels from patients before the treatment (Figure 5a through 5c; P<0.05). The collagen-to-elastin ratio (Figure 5d) was significantly reduced after eplerenone treatment compared with other groups, whereas changes under atenolol treatment did not achieve statistical significance.

Plasma levels of osteopontin, MCP-1, bFGF, the inflammatory cytokine IL-8, the anti-inflammatory cytokine IL-10, and IL-1Ra were similar in patients before random assignment (Figure 6a through 6f). Eplerenone significantly reduced osteopontin, MCP-1, bFGF, and IL-8 (Figure 6a through 6d), as well as IL-1Ra and IL-10 (Figure 6e and 6f). Atenolol-induced changes of these inflammatory markers did not achieve statistical significance except for IL-1Ra. Levels of IL-1β, IL-6, and adhesion molecules remained unchanged under both treatments (data not shown).

### Discussion

Major findings from this double-blind, parallel-design, 1-year trial on a small group of previously untreated subjects with mild-to-moderate hypertension who accepted to have gluteal subcutaneous biopsies for sampling of resistance vessels demonstrate the following: (1) treatment with the selective mineralocorticoid antagonist eplerenone with good BP control reduced stiffness and the collagen/elastin ratio in resistance arteries; (2) resistance vessels from atenolol-treated patients exhibited increased stiffness and unchanged collagen/elastin ratio, despite similar effective BP control; and (3)
there was improved inflammatory status in eplerenone-treated patients.

Hypertensive subjects in the present study demonstrated eutrophic small artery remodeling as expected in patients with newly diagnosed uncomplicated hypertension. We have reported previously that structural remodeling (increased media/lumen ratio) of resistance arteries occurs as an early stage in the progression of the target-organ damage in mild-to-moderate (stage 1) essential hypertension. Structural abnormalities of resistance arteries may amplify vasoconstrictor responses, thereby contributing to elevated vascular tone, which may participate in the mechanisms that increase BP. The vasoconstricted state seems to become embedded in the newly secreted extracellular matrix, which may participate in the mechanisms that increase BP. The vasoconstricted state seems to become embedded in the newly secreted extracellular matrix, which may represent a mechanism leading to eutrophic remodeling of resistance arteries. Vasodilatation under antihypertensive treatment has been implicated to play a role in the correction of vascular remodeling rather than just the lowering of BP. Vascular remodeling was not significantly improved in the present study either by eplerenone or atenolol after 1 year of treatment, despite good BP control with both agents. This might, therefore, be explained in part by the fact that neither atenolol nor eplerenone act as a vasodilator agent. However, stiffness of small arteries from hypertensive patients was reduced only by eplerenone, whereas in patients treated with atenolol, arterial wall stiffness was enhanced. Aldosterone induces fibrosis in the heart, blood vessels, and kidney, particularly in the presence of high salt. In the clinical setting, selective antagonism of mineralocorticoid receptors has been associated with reduced fibrosis in human myocardium and improved survival in patients with ventricular dysfunction and heart failure. Here we demonstrate for the first time that the collagen/elastin ratio of small arteries from hypertensive patients was reduced only after eplerenone treatment, suggesting a direct and BP-independent effect of mineralocorticoid receptor blockade on components of the vascular extracellular matrix, in agreement with previous preclinical data and effects demonstrated on the heart in recent multicenter trials in humans.

Inflammatory processes are important participants in the pathophysiology of hypertension and vascular remodeling,
and small artery damage may be associated with increased arterial stiffness and low-grade vascular inflammation. BP control with eplerenone was associated with reduced circulating systemic inflammatory markers in hypertensive patients. We showed recently that, in high-risk hypertensive patients, BP control obtained mainly with drugs that antagonize components of the renin-angiotensin-aldosterone system, specifically using angiotensin receptor blockers, improves vascular structure and ameliorates systemic inflammation. Aldosterone may in part mediate actions that are usually attributed to the direct effect of angiotensin II, such as vascular remodeling, increased oxidative stress, and inflammation of the vascular wall and the heart. Mineralocorticoids and, in particular, aldosterone may activate inflammatory pathways in the cardiovascular system. In humans, intravenous infusion of angiotensin II increased circulating concentrations of inflammatory cytokines through a mineralocorticoid receptor–dependent mechanism. Aldosterone induced the synthesis of the inflammatory cytokines MCP-1 and osteopontin directly, promoting vascular inflammation and remodeling, as well as development of atherosclerosis.

bFGF has long been recognized to stimulate proliferation of cultured fibroblasts, endothelial cells, smooth muscle cells, and skeletal myoblasts and is also involved in regulation of cell survival, migration, and extracellular matrix production or degradation. Animal studies have demonstrated that mineralocorticoid receptor antagonists decrease the expression of inflammatory markers. Here we show that eplerenone was associated with a reduction of proinflammatory mediators, including MCP-1, osteopontin, bFGF, and IL-8, the latter recently described as an emerging inflammatory biomarker associated with incident coronary artery disease in apparently healthy individuals. The anti-inflammatory cytokines IL-10 and IL-1Ra were also reduced by eplerenone, possibly as a consequence of countervailing mechanisms in response to the reduction of inflammatory markers. Atenolol treatment failed to significantly reduce most of the biomarkers of inflammation examined, which may be related to the fact that most β-blockers lack anti-inflammatory properties, with the exception of carvedilol.

In this study, patients exhibited preserved endothelial function and NO availability. Small artery structural alterations may precede the development of hypertension and may occur earlier than endothelial dysfunction. In the remod-

Figure 6. Plasma levels of proinflammatory cytokines (a) osteopontin, (b) MCP-1, (c) bFGF, and (d) IL-8 and of anti-inflammatory (e) IL-10 and (f) cytokine receptor IL-1Ra. Ate indicates atenolol; Epl, eplerenone. *P<0.05, †P<0.05 vs other groups.
eled vessel, the chronically altered local pressure-flow relationship may result in adaptive resetting of the endothelium, resulting in blunting of the reduction in NO generation, as well as of the production of other endothelium-derived relaxants, and, therefore, relative preservation of endothelium-dependent relaxation. This may also counteract the deterioration of the structure of proximal resistance arteries from young subjects with moderate hypertension. The blood lipid profile of the patients was normal before and after the end of the study, which could contribute to the preserved endothelial function of this group of hypertensive patients. Indeed, a substantial degree of endothelial dysfunction present in hypertension has been attributed to an abnormal lipid profile.\textsuperscript{39,40}

Conclusion, Limitations, and Perspectives

Normalization of elevated BP is the mainstay of treatment to decrease cardiovascular morbidity and mortality in hypertension.\textsuperscript{3} Target-organ damage at early stages of hypertension, such as vascular remodeling and stiffening, endothelial dysfunction, and low-grade inflammation of the wall of resistance arteries represents a therapeutic target beyond BP control to reduce cardiovascular risk.\textsuperscript{3} Our results suggest that strategies intended to lower BP in stage 1 (mild-to-moderate) hypertensive patients using selective mineralocorticoid receptor antagonists may reduce vascular stiffness and collagen deposition and may exert vascular anti-inflammatory effects beyond BP reduction. These conclusions should be taken in the context of the limitation introduced by the small number of subjects studied here because of difficulty in recruiting previously untreated hypertensive individuals for the invasive, albeit well-tolerated, procedure needed for these studies. The present investigation, by providing the first evidence in hypertensive humans that selective mineralocorticoid receptor blockade reduces collagen deposition and stiffness of small arteries, suggests that outcomes in hypertensive subjects may be improved by treatment with these agents, which remains to be demonstrated.

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Disclosures

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References


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