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Mineralocorticoid Receptor Blocker Eplerenone Improves Endothelial Function and Inhibits Rho-Associated Kinase Activity in Patients With Hypertension

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Abstract

Hypertension is associated with endothelial dysfunction and activated Rho-associated kinases (ROCKs). The purpose of this study was to evaluate the effects of the selective mineralocorticoid receptor blocker, eplerenone, on endothelial function and ROCK activity in patients with hypertension. The study was carried out over 48 weeks in 60 untreated patients with hypertension who were randomly assigned to eplerenone, nifedipine, and losartan groups. We evaluated the effects of each treatment on flow-mediated vasodilation (FMD) and ROCK activity in peripheral leukocytes. Eplerenone increased FMD and decreased leukocyte ROCK activity. Nifedipine decreased ROCK activity but did not alter FMD. Losartan increased FMD but did not alter ROCK activity. Hypotensive effects were similar in the three groups, as was nitroglycerin-induced vasodilation during the follow-up period. There were no significant differences between the groups with respect to other parameters. The study results show that eplerenone improves endothelial function and inhibits ROCK activity in patients with essential hypertension.

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SUPPLEMENTARY MATERIAL is linked to the online version of the paper at <http://www.nature.com/cpt>

CONFLICT OF INTEREST

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Rho-associated kinases (ROCKs), one of the first downstream targets of the small GTP-binding protein RhoA, mediate various cellular physiologic functions, including vascular cell proliferation, migration, adhesion, apoptosis, and contraction.^{1–4} The Rho/ROCK pathway regulates the contraction of vascular smooth muscle cells through subsequent phosphorylation of myosin light chain, independent of intracellular calcium concentration. Although ROCK is one of the downstream target molecules of RhoA, activation of ROCKs also induces an increase in vascular smooth muscle cell contraction and remodeling of arteries.⁵ Elevation in ROCK activity would be expected to play an important pathophysiological role in the development and maintenance of hypertension. Hypertension is associated with the Rho/ROCK pathway.^{6–9} It is expected that pharmacological interventions, including administration of antihypertensive agents, inhibit ROCK activity. Indeed, we have recently shown that a calcium channel blocker, amlodipine, inhibits leukocyte ROCK activity in patients with essential hypertension.¹⁰

Endothelial dysfunction is established in the initial step of atherosclerosis and plays an important role in the development of this condition.¹¹ In addition, it is well known that endothelial function is an independent predictor of cardiovascular events.¹² Hypertension is associated with endothelial dysfunction.^{13–15} We and several other investigators have shown that renin–angiotensin system (RAS) inhibitors and lifestyle modifications, including aerobic exercise and reduction in body weight, improve endothelial function in patients with hypertension.^{15–17} These findings suggest that endothelial dysfunction is reversible through an appropriate intervention.

Both endothelial dysfunction and elevated ROCK activity play important roles in the development and maintenance of hypertension.^{9,10} An antihypertensive agent that restores endothelial function and ROCK activity may further reduce the mortality and morbidity associated with cardiovascular complications in patients with hypertension. However, there is no information to date on the effects of antihypertensive agents on endothelial function and ROCK activity in patients with hypertension. Interestingly, the mineralocorticoid receptor blocker eplerenone or the drug spironolactone markedly reduced mortality and morbidity in patients with heart failure or acute myocardial infarction who received conventional treatment including RAS inhibitors.^{18–20} In this study, therefore, we evaluated the effects of eplerenone, the calcium channel blocker nifedipine, and the angiotensin type I receptor blocker losartan on endothelial function and ROCK activity during a 48-week active treatment period in patients with essential hypertension.

RESULTS

Clinical characteristics

Eligible patients with essential hypertension were randomly assigned to three groups of 20 patients each to receive eplerenone, nifedipine, or losartan treatments. Table 1 shows the baseline clinical characteristics of all the patients and the effects of each of these three treatments on the baseline parameters. Eplerenone, nifedipine, and losartan significantly reduced blood pressure after 4 weeks of treatment as compared to baseline values (0 weeks). The blood pressure–lowering effects of eplerenone, nifedipine, and losartan were maintained throughout the 48-week treatment period. Hypotensive effects were similar in the three

groups. Serum levels of lipids and glucose were similar in all treatment periods in all three groups.

Endothelial function

The effects of eplerenone, nifedipine, and losartan on flow-mediated vasodilation (FMD) before and after 4, 12, and 48 weeks of treatment in patients with essential hypertension are shown in Figure 1. FMD values were similar at 0 weeks in the eplerenone, nifedipine, and losartan treatment groups.

Eplerenone.—FMD rose significantly from $5.6 \pm 1.4\%$ to $8.7 \pm 1.8\%$ ($P = 0.01$) by 12 weeks of eplerenone treatment. The ability of eplerenone to increase FMD was maintained throughout the 48-week treatment period ($8.5 \pm 1.7\%$ vs. 0 weeks, $P = 0.01$). FMD values after 12 weeks of treatment were similar to those after 48 weeks of treatment.

Nifedipine.—There was no significant difference between FMD values in the nifedipine group before and after the 48-week study period (0 weeks; $5.8 \pm 1.5\%$ vs. 4 weeks; $6.0 \pm 1.7\%$, 12 weeks; $5.6 \pm 2.0\%$, and 48 weeks; $5.3 \pm 1.9\%$).

Losartan.—FMD rose significantly from $5.4 \pm 1.3\%$ to $8.1 \pm 1.6\%$ ($P = 0.02$) by 12 weeks of losartan treatment. The ability of losartan to increase FMD was maintained throughout the 48-week treatment period ($8.0 \pm 1.7\%$, vs. 0 weeks, $P = 0.01$). FMD values after 12 weeks of treatment were similar to those after 48 weeks of treatment.

Nitroglycerine-induced vasodilation was similar at the beginning and end of the 4-, 12-, and 48-week study periods in each group. Nitroglycerine-induced vasodilation was similar in the three groups (Table 2).

Circulating progenitor cells and cell migration

The effects of eplerenone, nifedipine, and losartan on circulating progenitor cells and cell-migration response to vascular endothelial growth factor (VEGF) before and after 4, 12, and 48 weeks of treatment in patients with essential hypertension are shown in Figures 2 and 3. The number of circulating progenitor cells and the cell-migration response to VEGF were similar at 0 weeks in the eplerenone, nifedipine, and losartan treatment groups.

Eplerenone.—Eplerenone treatment for 12 weeks increased the number of circulating progenitor cells from 724 ± 272 to $1,092 \pm 341/\text{ml}$ ($P = 0.01$) and cell-migration response to VEGF from 32.2 ± 21.7 to $58.4 \pm 27.6/\text{high-power field}$ ($P = 0.03$). The ability of eplerenone to increase the number of circulating progenitor cells and the cell-migration response to VEGF was maintained throughout the 48-week treatment period ($1,046 \pm 324/\text{ml}$ and $60.2 \pm 25.8/\text{high-power field}$ vs. 0 weeks, $P = 0.02$ and $P = 0.01$, respectively). The number of circulating progenitor cells and the cell-migration response to VEGF after 12 weeks of treatment were similar to those after 48 weeks of treatment.

Nifedipine.—There were no significant differences between the number of circulating progenitor cells and the cell-migration response to VEGF at 0 weeks and those at 4, 12, and 48 weeks in the nifedipine group.

Losartan.—Losartan treatment for 12 weeks increased the number of circulating progenitor cells from 701 ± 309 to $1,022 \pm 418/\text{ml}$ ($P = 0.01$) and the cell-migration response to VEGF from 33.1 ± 14.9 to $59.3 \pm 22.4/\text{high-power field}$ ($P = 0.02$). The ability of losartan to increase the number of circulating progenitor cells and the cell-migration response to VEGF was maintained throughout the 48-week treatment period ($1,071 \pm 420/\text{ml}$ and $58.2 \pm 23.8/\text{high-power field}$ vs. 0 weeks, $P = 0.02$ and $P = 0.03$, respectively). The number of circulating progenitor cells and the cell-migration response to VEGF after 4, 12, and 48 weeks of treatment were similar.

ROCK activity

The effects of eplerenone, nifedipine, and losartan on ROCK activity before and after 4, 12, and 48 weeks of treatment in patients with essential hypertension are shown in Figure 4. ROCK activities were similar at 0 weeks in the eplerenone, nifedipine, and losartan treatment groups.

Eplerenone.—Eplerenone significantly reduced ROCK activity after 4 weeks of treatment in comparison with the pretreatment value (0.79 ± 0.23 vs. 0.51 ± 0.18 , $P = 0.02$). The ability of eplerenone to decrease ROCK activity was maintained throughout the 48-week treatment period (12 weeks, 0.53 ± 0.17 , and 48 weeks, 0.50 ± 0.20 , vs. 0 weeks, both $P = 0.01$). ROCK activities after 4, 12, and 48 weeks of treatment were similar.

Nifedipine.—Nifedipine significantly reduced ROCK activity after 4 weeks of treatment in comparison with the pretreatment value (0.81 ± 0.32 vs. 0.52 ± 0.21 , $P = 0.02$). The ability of nifedipine to decrease ROCK activity was maintained throughout the 48-week treatment period (12 weeks, 0.51 ± 0.20 , and 48 weeks, 0.49 ± 0.19 , vs. 0 weeks, both $P = 0.01$). ROCK activities after 4, 12, and 48 weeks of treatment were similar.

Losartan.—Losartan did not alter ROCK activity after 4, 12, or 48 weeks of treatment in comparison with the pretreatment value (0 weeks; 0.85 ± 0.28 vs. 4 weeks; 0.80 ± 0.32 , 12 weeks; 0.83 ± 0.30 , and 48 weeks; 0.76 ± 0.33). Protein expression levels of total myosin binding subunit (t-MBS) were similar in all treatment periods in the three groups.

DISCUSSION

In the present study, we demonstrated that, in patients with hypertension, eplerenone increased FMD and decreased ROCK activity, that nifedipine decreased ROCK activity but did not alter FMD, and that losartan increased FMD but did not alter ROCK activity. To our knowledge, this is the first study showing a beneficial effect of eplerenone on endothelial function and ROCK activity in patients with hypertension.

Endothelial function

In a blinded, randomized, parallel-group study, eplerenone and losartan, but not nifedipine, increased FMD in patients with hypertension. Several putative mechanisms have been postulated to explain how eplerenone and losartan improve endothelial function. It is well known that RAS inhibitors augment endothelium-dependent vasodilation through an

increase in nitric oxide (NO) bioavailability (e.g., activation of the PI3/Akt pathway, increase in endothelial nitric oxide synthase mRNA stability, and decrease in oxidative stress and inflammation).^{21,22}

The results of several experimental and clinical studies support our results demonstrating that RAS inhibitors can restore endothelial function.^{16,17,23,24} A balance between angiotensin II and NO is important in the regulation of vascular tone.²⁵ Angiotensin II increases vascular superoxide production through activation of membrane-associated nicotinamide adenine dinucleotide phosphate oxidase, resulting in NO inactivation and toxic peroxynitrite production.²⁵ Consequently, RAS inhibitors may increase NO by inhibiting angiotensin II production. Furthermore, under physiologic conditions, endogenous bradykinin is limited by angiotensin-converting enzyme. Bradykinin binds to B₂ receptors on the endothelial cell surface to release NO.²⁶ RAS inhibitors prevent bradykinin degradation, leading to an increase in NO release.

In addition to angiotensin II, aldosterone plays an important role in the regulation of endothelial function. In Dahl salt-sensitive rats and two-kidney, one-clip rats, eplerenone improved endothelial function through enhancement of expression of the endothelial NO synthase gene,^{27,28} suggesting that eplerenone directly increases NO production by activation of endothelial NO synthase in hypertension. Inhibition of the aldosterone/mineralocorticoid receptor may also contribute to decrease in oxidative stress, resulting in inhibition of NO inactivation.²⁹ There is no information on the effect of a mineralocorticoid receptor blocker on endothelial function in patients with essential hypertension. However, it has been shown that the nonselective mineralocorticoid receptor blocker spironolactone improves FMD and endothelium-dependent vasodilation induced by acetylcholine in patients with hyperaldosteronism and in patients with heart failure.^{30,31} In contrast, spironolactone markedly worsened endothelium-dependent vasodilation in patients with type 2 diabetes mellitus,³² although eplerenone did not alter endothelial function in those patients.³³ It is likely that impairment of glucose profile and increase in plasma angiotensin II levels with spironolactone treatment contribute to impairment of endothelial function in patients with diabetes mellitus. Although eplerenone improved FMD (thereby exerting a beneficial effect on endothelial function) in patients with essential hypertension in our study, this effect may not prove to be beneficial in patients with other atherosclerotic diseases and metabolic disorders. Although eplerenone improves endothelial function, further studies are needed to determine whether this effect actually translates into beneficial effects in cardiovascular disease.

In the present study, the number of circulating progenitor cells and the cell-migration response to VEGF were higher after eplerenone or losartan therapy. Recently, Hill *et al.*³⁴ found, by measuring FMD levels in healthy men, that endothelial function is associated with the number of circulating progenitor cells. One possible mechanism through which eplerenone or losartan improves endothelial function could be by increasing the number and migration of circulating progenitor cells, thereby leading to an increase in NO and re-endothelialization.

Although the antihypertensive drugs used in our study were equally effective in reducing blood pressure, eplerenone and losartan, but not nifedipine, improved FMD. Our findings are consistent with the results of previous studies showing that not every method of achieving reduction in blood pressure directly alters endothelium-dependent vasodilation in brachial, renal, and small arteries in patients with essential hypertension.^{35,36} Consequently, a mere reduction in blood pressure through the use of effective antihypertensive drugs may not always lead to improved endothelial function in patients with essential hypertension.

Previous large clinical trials have clearly shown that eplerenone reduces mortality and morbidity in patients with heart failure and in patients with acute myocardial infarction^{19,20} and that losartan reduces mortality and morbidity from cardiovascular causes in patients with hypertension.³⁷ A meta-analysis clearly showed that endothelial function is an independent predictor of cardiovascular outcomes.¹² Improvement in endothelial function by eplerenone or losartan may contribute to reduction in cardiovascular outcomes in patients with heart failure and patients with hypertension.

ROCK activity

ROCK activity also plays an important role in the development and maintenance of hypertension. In a blinded, randomized, parallel-group study, eplerenone and nifedipine, but not losartan, decreased leukocyte ROCK activity in patients with hypertension. Increased ROCK activity is reversible by appropriate treatment with eplerenone or nifedipine. It has been reported that aldosterone induces ROCK activity by binding to the mineralocorticoid receptor in vascular smooth muscle cells and cardiomyocytes.^{38,39} In rats with aldosterone/salt-induced hypertension and Dahl salt-sensitive rats, ROCK activity is elevated, leading to vascular remodeling and tissue injury in the heart and kidney.^{28,40} Treatment with mineralocorticoid receptor blockers prevented cardiovascular injury through inhibition of ROCK activity in these animal models.⁴⁰ These findings suggest that eplerenone may directly inhibit ROCK activity in patients with essential hypertension. Although treatment with eplerenone was shown to be associated with a decrease in ROCK activity, the limitations of our study prevented us from determining whether eplerenone could directly inhibit ROCK. Future studies are needed to confirm the precise mechanisms through which the aldosterone/mineralocorticoid receptor is associated with induction ROCK activity *in vitro* and *in vivo*.

The precise mechanism involved in the decrease in ROCK activity by the calcium channel blocker nifedipine remains unclear. Recently, in a cross-sectional study, we showed that leukocyte ROCK activity was lower in a calcium channel blocker-treated group than in a group treated with RAS inhibitors, β -blockers, and diuretics.¹⁰ In addition, in a double-blind, randomized, parallel-group study, the calcium channel blocker amlodipine, but not losartan, was shown to decrease leukocyte ROCK activity in patients with hypertension.¹⁰

The hypotensive effects were similar in the eplerenone, nifedipine, and losartan groups. In addition, there was no significant correlation between changes in ROCK activity and changes in blood pressure in the eplerenone and nifedipine groups. Therefore, it is unlikely that decrease in blood pressure by antihypertensive agents is directly involved in the reduction in ROCK activity. Losartan lowered blood pressure to an extent similar to that of

epplerenone without affecting leukocyte ROCK activity; it therefore appears unlikely that the mechanism through which losartan, at the concentrations used, lowers blood pressure involves inhibition of ROCK. Although the effects of losartan on ROCK activity may be inferred by determining RhoA activity, it must be remembered that RhoA has many downstream targets other than ROCK. In addition, the measurement of RhoA-GTP may be unreliable in our leukocyte samples, given the processing of the samples. It is thought that eplerenone or nifedipine reduces vascular resistance, at least in part, through inhibition of the Rho/ROCK pathway. Inhibition of ROCK activity may be an additional potential therapeutic target in patients with essential hypertension.

Study limitations

Currently, in the clinical setting, the measurement of vascular response to intra-arterial or intravenous infusion of ROCK inhibitors is the gold standard for assessing ROCK activity.^{9,41} However, these methods are burdensome and time-consuming for the patients. We, and several other investigators, have shown that ROCK activity in peripheral leukocytes is enhanced in hypertension, metabolic syndrome, and coronary artery disease, and also in smokers.^{10,42–44} In addition, we have recently shown that leukocyte phospho- (p)MBS/t-MBS, which can be measured noninvasively, significantly correlates with the forearm blood flow response to intra-arterial infusion of the ROCK inhibitor fasudil.⁴⁵ Although ROCK activity in peripheral leukocytes may not directly reflect vascular ROCK activity, a noninvasive method for measuring leukocyte ROCK activity would nevertheless be useful for this purpose.

In conclusion, an antihypertensive agent that restores endothelial function and ROCK activity may further reduce the mortality and morbidity associated with cardiovascular complications in patients with hypertension. The selective mineralocorticoid receptor blocker eplerenone has been shown to be one of the agents that can improve endothelial function and inhibit ROCK activity. Large clinical trials have clearly shown that eplerenone decreases mortality and morbidity in patients with heart failure. Eplerenone-induced improvement in endothelial function and decrease in ROCK activity may have contributed to the promising results in these trials. Further studies are needed to understand more clearly the biology of ROCK activity in a clinical setting and to evaluate the long-term effect of eplerenone on cardiovascular events in patients with hypertension.

METHODS

Subjects.

We studied 60 untreated patients with essential hypertension (45 men and 15 women; mean age, 53 ± 9 years). Hypertension was defined as systolic blood pressure of more than 140 mm Hg and/or diastolic blood pressure of more than 90 mm Hg, measured with the subject in a seated position, on at least three occasions. Patients with secondary forms of hypertension were excluded on the basis of complete history, physical examination, radiological and ultrasound examinations, urinalysis, plasma renin activity, plasma aldosterone and norepinephrine concentrations, serum creatinine, potassium, calcium, and free thyroxine concentrations, and 24-h urinary excretion of 17-hydroxycorticosteroids, 17-

ketogenic steroids, and vanillylmandelic acid. None of the patients enrolled in the study had a history of cardiovascular or cerebrovascular disease, diabetes mellitus, liver disease, or renal disease. The study protocol was approved by the ethics committee of Hiroshima University Graduate School of Biomedical Sciences. Written informed consent was obtained from all subjects before participation.

Study protocol.

This was a blinded, randomized study with a parallel-group design. A total of 60 patients with essential hypertension were randomized to blinded treatment with eplerenone (Pfizer Pharmaceutical, Tokyo, Japan; $n = 20$; 15 men and 5 women; mean age, 54 ± 10 years) at a dose of 50 mg, nifedipine (Bayer Pharmaceutical, Osaka, Japan; $n = 20$; 15 men and 5 women; mean age, 55 ± 11 years) at a dose of 40 mg, or losartan potassium (Banyu Pharmaceutical, Tokyo, Japan; $n = 20$; 15 men and 5 women; mean age, 53 ± 9 years) at a dose of 100 mg once daily in the morning, during a 48-week active treatment period. None of the patients had a history of antihypertensive treatment before the study. After a 4-week run-in period, the pretreatment values in the three groups were compared. Active treatment was then carried out for 48 weeks, and the time courses of the effects of eplerenone, nifedipine, and losartan on FMD, an index of endothelial function, and leukocyte ROCK activity were evaluated. During the 48-week treatment period, one patient in the eplerenone group, two patients in the nifedipine group, and one patient in the losartan group dropped out.

All subjects fasted for at least 12 h the night before the study commenced. After they had remained in the supine position for 30 min, basal ROCK activity and fasting serum concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and glucose were measured. Measurements of FMD and leukocyte ROCK activity were performed at the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment.

Measurement of FMD.

FMD was measured using ultrasonography with an automated edge tracking system (UNEXEF18G; UNEX, Nagoya, Japan). See Supplementary Data online for additional details.

Measurement of the number of circulating progenitor cells.

The number of circulating progenitor cells was analyzed using flow cytometry. See Supplementary Data online for additional details.

Characterization of progenitor cells.

Mononuclear cells were isolated using Ficoll density-gradient centrifugation of human blood buffy coats from 50 ml of peripheral blood. See Supplementary Data, including Supplementary Figure S1 online for additional details

Migration assay.

Progenitor cell migration was evaluated using a modified Boyden chamber assay, as previously described.⁴⁶ See Supplementary Data online for additional details.

Measurement of ROCK activity.

ROCK activity was assayed in peripheral blood leukocytes as the amount of phospho-Thr853 in the p-MBS of myosin light-chain phosphatase as previously described.^{10,47} See Supplementary Data online for additional details.

Analytical methods.

Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/ml) and in polystyrene tubes. See Supplementary Data online for additional details.

Statistical analysis.

The results are presented as mean values $\pm$ SD. Values of $P < 0.05$ were considered to indicate statistical significance. The Mann–Whitney U test was used to evaluate differences between groups. Comparisons of time course curves of parameters were analyzed using two-way analysis of variance for repeated measures on one factor, followed by Bonferroni correction for multiple-paired comparisons. Multi-group comparisons of variables were carried out using oneway analysis of variance followed by Bonferroni correction. Values of $P < 0.05/\text{number of comparisons}$ were considered as indicating statistical significance while performing Bonferroni correction. Missing values during follow-up periods were imputed using the last-observation-carried-forward method. The data were processed using the software package StatView IV (SAS Institute, Cary, NC) or Super Analysis of Variance (Abacus Concepts, Berkeley, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Flow-mediated vasodilation (FMD) in the eplerenone, nifedipine, and losartan groups before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment. * $P < 0.05$ vs. 0 weeks in the same group.

Figure 2.

Representative measurements of the number of circulating progenitor cells (CPCs) by flow cytometry in a patient treated with eplerenone, a patient treated with nifedipine, and a patient treated with losartan before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment (top). Comparison of the number of CPCs in patients in the eplerenone, nifedipine, and losartan groups before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment (bottom). * $P < 0.05$ vs. 0 weeks in the same group.

Figure 3.

Representative measurements of the migration of circulating progenitor cells (CPCs) labeled with DAPI by fluorescence in a patient treated with eplerenone, a patient treated with nifedipine, and a patient treated with losartan before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment (top). Comparison of the migration of CPCs in patients in the eplerenone, nifedipine, and losartan groups before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment (bottom). * $P < 0.05$ vs. 0 weeks in the same group. DAPI, 4',6-diamidino-2-phenylindole; hpf, high-power field.

Figure 4.

Representative data from Western blot analysis for phospho-myosin-binding subunit (p-MBS), total-myosin-binding subunit (t-MBS), and β tubulin in a patient treated with eplerenone, a patient treated with nifedipine, and a patient treated with losartan before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment (top). ROCK activity (p-MBS/t-MBS) in patients in the eplerenone, nifedipine, and losartan groups before the beginning of treatment (0 weeks) and after 4, 12, and 48 weeks of treatment (bottom). * $P < 0.05$ vs. 0 weeks in the same group.

Table 1

Clinical characteristics of the eplerenone, nifedipine, and losartan groups

Eplerenone group				
Variable	0 weeks	4 weeks	12 weeks	48 weeks
Body mass index (kg/m ²)	24.4 ± 2.6	24.3 ± 2.5	24.3 ± 2.7	24.1 ± 2.6
Systolic blood pressure (mm Hg)	159.5 ± 14.6	133.1 ± 12.3 [*]	131.8 ± 12.3 [*]	132.7 ± 12.6 [*]
Diastolic blood pressure (mm Hg)	95.3 ± 10.2	83.3 ± 7.2 [*]	82.5 ± 7.1 [*]	82.3 ± 7.5 [*]
Heart rate (bpm)	70.7 ± 8.7	70.3 ± 8.5	70.1 ± 8.8	70.6 ± 9.1
Total cholesterol (mmol/l)	4.98 ± 0.79	4.91 ± 0.82	4.88 ± 0.79	4.92 ± 0.81
Triglycerides (mmol/l)	1.19 ± 0.40	1.17 ± 0.39	1.18 ± 0.36	1.18 ± 0.35
HDL cholesterol (mmol/l)	1.40 ± 0.27	1.41 ± 0.29	1.40 ± 0.31	1.41 ± 0.32
LDL cholesterol (mmol/l)	3.51 ± 0.78	3.42 ± 0.69	3.41 ± 0.74	3.39 ± 0.72
Serum glucose (mmol/l)	4.8 ± 0.3	4.8 ± 0.4	4.8 ± 0.3	4.8 ± 0.3
Smoker (%)	30			
Nifedipine group				
Variable	0 weeks	4 weeks	12 weeks	48 weeks
Body mass index (kg/m ²)	24.2 ± 2.7	24.1 ± 2.8	24.1 ± 2.7	24.1 ± 2.7
Systolic blood pressure (mm Hg)	159.8 ± 14.4	131.9 ± 11.4 [*]	130.3 ± 11.6 [*]	130.1 ± 11.9 [*]
Diastolic blood pressure (mm Hg)	96.1 ± 10.5	81.7 ± 7.7 [*]	81.2 ± 7.5 [*]	80.9 ± 7.4 [*]
Heart rate (bpm)	70.2 ± 9.2	71.2 ± 9.6	72.7 ± 9.8	72.4 ± 9.6
Total cholesterol (mmol/l)	4.88 ± 0.83	4.86 ± 0.91	4.92 ± 0.88	4.95 ± 0.92
Triglycerides (mmol/l)	1.22 ± 0.43	1.21 ± 0.41	1.19 ± 0.37	1.20 ± 0.35
HDL cholesterol (mmol/l)	1.42 ± 0.26	1.43 ± 0.31	1.41 ± 0.33	1.42 ± 0.29
LDL cholesterol (mmol/l)	3.45 ± 0.73	3.44 ± 0.69	3.42 ± 0.71	3.41 ± 0.75
Serum glucose (mmol/l)	4.9 ± 0.4	4.9 ± 0.4	4.9 ± 0.4	4.9 ± 0.3
Smoker (%)	30			
Losartan group				
Variable	0 weeks	4 weeks	12 weeks	48 weeks
Body mass index (kg/m ²)	24.3 ± 2.5	24.3 ± 2.4	24.2 ± 2.3	24.3 ± 2.4

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Variable	Eplerenone group			
	0 weeks	4 weeks	12 weeks	48 weeks
Systolic blood pressure (mm Hg)	158.7 ± 13.9	134.6 ± 11.8 [*]	134.2 ± 11.9 [*]	133.9 ± 12.1 [*]
Diastolic blood pressure (mm Hg)	95.4 ± 9.8	84.5 ± 7.7 [*]	84.1 ± 7.4 [*]	83.5 ± 7.6 [*]
Heart rate (bpm)	69.8 ± 8.6	71.1 ± 8.9	70.2 ± 8.8	70.7 ± 9.1
Total cholesterol (mmol/l)	5.01 ± 0.83	5.03 ± 0.91	4.98 ± 0.86	4.97 ± 0.79
Triglycerides (mmol/l)	1.21 ± 0.42	1.22 ± 0.44	1.19 ± 0.38	1.17 ± 0.36
HDL cholesterol (mmol/l)	1.39 ± 0.24	1.40 ± 0.30	1.41 ± 0.32	1.40 ± 0.31
LDL cholesterol (mmol/l)	3.44 ± 0.72	3.42 ± 0.71	3.40 ± 0.75	3.40 ± 0.76
Serum glucose (mmol/l)	4.9 ± 0.4	4.9 ± 0.3	4.9 ± 0.3	4.8 ± 0.4
Smoker (%)	30			

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^{*} *P* < 0.05 vs. 0 weeks in the same group.

Table 2

Vascular response of the eplerenone, nifedipine, and losartan groups

Eplerenone group				
Variable	0 weeks	4 weeks	12 weeks	48 weeks
Brachial artery diameter, mm	4.14 ± 0.67	4.13 ± 0.64	4.15 ± 0.66	4.12 ± 0.61
Increased hyperemic blood flow, %	431 ± 132	412 ± 140	438 ± 123	441 ± 142
Flow-mediated vasodilation, %	5.6 ± 1.4	6.9 ± 1.6	8.7 ± 1.8 *	8.5 ± 1.7 *
Nitroglycerine-induced vasodilation, %	10.3 ± 3.3	10.2 ± 3.1	10.7 ± 3.7	10.5 ± 3.4
Nifedipine group				
Variable	0 weeks	4 weeks	12 weeks	48 weeks
Brachial artery diameter, mm	4.09 ± 0.57	4.12 ± 0.62	4.13 ± 0.68	4.16 ± 0.71
Increased hyperemic blood flow, %	398 ± 129	420 ± 134	408 ± 145	414 ± 139
Flow-mediated vasodilation, %	5.8 ± 1.5	6.0 ± 1.7	5.6 ± 2.0	5.3 ± 1.9
Nitroglycerine-induced vasodilation, %	10.1 ± 3.7	10.2 ± 3.9	10.4 ± 3.8	10.4 ± 3.9
Losartan group				
Variable	0 weeks	4 weeks	12 weeks	48 weeks
Brachial artery diameter, mm	4.13 ± 0.69	4.14 ± 0.72	4.15 ± 0.71	4.13 ± 0.66
Increased hyperemic blood flow, %	422 ± 146	439 ± 151	428 ± 126	413 ± 137
Flow-mediated vasodilation, %	5.4 ± 1.3	6.2 ± 1.4	8.1 ± 1.6 *	8.0 ± 1.7 *
Nitroglycerine-induced vasodilation, %	10.5 ± 3.5	10.6 ± 4.0	10.6 ± 3.9	10.4 ± 3.6

*P < 0.05 vs. 0 weeks in the same group.