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Eplerenone improves endothelial function and arterial stiffness and inhibits Rho-associated kinase activity in patients with idiopathic hyperaldosteronism: a pilot study

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Abstract

Objective: Primary aldosteronism is one of the most common cause of secondary hypertension. It is well known that the incidence of cardiovascular events is higher in patients with primary aldosteronism than in patients with essential hypertension. In a previous study, we showed that aldosterone-producing adenoma is associated with vascular function and structure. The aim of this study was to evaluate the effects of eplerenone on vascular function in the macrovasculature and microvasculature, arterial stiffness and Rho-associated kinase (ROCK) activity in patients with idiopathic hyperaldosteronism (IHA).

Methods: Vascular function, including reactive hyperemia index (RHI), flow-mediated vasodilation (FMD) and nitroglycerine-induced vasodilation (NID), arterial stiffness including

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Conflicts of interest

There are no conflicts of interest.

brachial–ankle pulse wave velocity (baPWV) and brachial intima–media thickness (IMT) and ROCK activity in peripheral leukocytes were measured before and after 12 weeks of treatment with eplerenone in 50 patients with IHA.

Results: After 12 weeks, eplerenone decreased the aldosterone renin ratio but did not alter SBP and DBP. Eplerenone treatment increased log RHI from 0.56 ± 0.25 to 0.69 ± 0.25 ($P < 0.01$) and NID from 12.8 ± 5.8 to $14.9 \pm 6.9\%$ ($P = 0.02$) and it decreased baPWV from 1540 ± 263 to 1505 ± 281 ($P = 0.04$) and ROCK activity from 1.20 ± 0.54 to 0.89 ± 0.42 ($P < 0.01$), whereas there was no significant change in FMD (increase from 4.6 ± 3.4 to $4.6 \pm 3.6\%$, $P = 0.99$) or brachial IMT (decrease from 0.28 ± 0.07 to 0.28 ± 0.04 mm, $P = 0.14$).

Conclusion: Eplerenone improves microvascular endothelial function, vascular smooth muscle function, arterial stiffness and ROCK activity in patients with IHA.

Keywords

aldosterone; endothelial function; eplerenone; idiopathic hyperaldosteronism hypertension; mineralocorticoid receptor antagonist; Rho-associated kinases

INTRODUCTION

Primary aldosteronism is one of the most frequent causes of secondary hypertension. It is well known that the incidence of cardiovascular events is higher in patients with primary aldosteronism than in patients with essential hypertension (EHT) [1,2]. Endothelial dysfunction is the initial step in the development of atherosclerosis [3,4]. Recently, flow-mediated vasodilation (FMD) in the brachial artery and log-transformed reactive hyperemia index (log RHI) measured by reactive hyperemia-peripheral arterial tonometry (RH-PAT) have been widely used as methods for assessment of endothelial function. Several studies have shown that aldosterone impairs endothelial function [5–7]. Aldosterone directly inhibits endothelial NO synthase (eNOS) phosphorylation, leading to a decrease in eNOS activity in human umbilical vein endothelial cells [8,9], and it induces vascular structural change via increases in inflammation and fibrosis in the vasculature, resulting in endothelial dysfunction [10,11]. We demonstrated that macrovascular endothelial function assessed by FMD was impaired in patients with aldosterone-producing adenoma (APA) compared with that in patients with idiopathic hyperaldosteronism (IHA) or patients with EHT. In addition, microvascular endothelial function assessed by log RHI was impaired in patients with IHA compared with that in patients with EHT.

Rho-associated kinases (ROCKs) are one of the first downstream targets of the small guanosine triphosphate-binding protein RhoA. The RhoA/ROCKs pathway plays a physiological role in the pathogenesis of atherosclerosis [12–14]. Elevated ROCK activity plays an important pathophysiological role in the development and maintenance of hypertension [15–20]. An increase in ROCK activity is associated with cardiovascular disease and cardiovascular events [17]. Recently, we reported that ROCK activity was correlated with circulating aldosterone levels [7].

Treatment for primary aldosteronism differs among disease types of primary aldosteronism. Recommended treatment for unilateral APA is adrenalectomy, and recommended treatment

for IHA is medical therapy using mineralocorticoid receptor antagonists. We reported that adrenalectomy improved vascular function and inhibited ROCK activity in patients with APA [7]. However, in patients with IHA, the effects of a mineralocorticoid receptor antagonist on endothelial function, arterial stiffness and ROCK activity is unclear.

The purpose of this study was to evaluate the effects of the mineralocorticoid receptor antagonist eplerenone on vascular function including endothelium-dependent vasodilation assessed by FMD and log RHI and endothelium-independent vasodilation assessed by nitroglycerine-induced vasodilation (NID), on arterial stiffness assessed by brachial–ankle pulse wave velocity (baPWV) and brachial intima–media thickness (IMT) and on leukocyte ROCK activity in patients with IHA.

METHODS

Participants

Fifty patients with IHA (21 men and 29 women; 50 ± 12 years) were enrolled from Hiroshima University Hospital. This was a single-center, observational study. Vascular function, including RHI, FMD and NID, brachial IMT, baPWV and ROCK activity were measured before and after 12 weeks of treatment with eplerenone in all patients. The initial dose of eplerenone was 50 mg once daily. If SBP was greater than 140 mmHg or DBP was greater than 90 mmHg after the first month, the dose of eplerenone was increased to 100 mg. Other antihypertensive drugs were adjusted according to the physician's judgement to reach a target of 140/90 mmHg or less. The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all of the subjects.

Primary aldosteronism, including the classification of primary aldosteronism, was defined according to the report of the guidelines for diagnosis and treatment of primary aldosteronism: the Japan Endocrine Society 2009 [21]. Briefly, a diagnosis of primary aldosteronism was confirmed by the captopril-challenge test, upright furose-mide-loading test, and saline-loading test after screening for an aldosterone-to-renin ratio [ARR; PAC (pg/ml)/PRA (ng/ml per h)] of more than 200. Then, to identify the lateralization of aldosterone secretion, PAC and plasma cortisol concentration were measured in adrenal venous blood using an adrenal vein sampling technique under adrenocorticotrophic hormone stimulation in all patients with primary aldosteronism. No patient had multiple endocrine neoplasias. Patients with familial hyperaldosteronism type 1 or 2 and patients with aldosterone-producing carcinoma were also not included in this study. No antihypertensive agents were taken by the patients for more than 2 weeks before the study.

Study protocol

Participants fasted the previous night for at least 12 h. The study began at 0830 h. The participants were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22–25°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after

maintaining the supine position, log RHI, FMD, and NID were measured. The observers were blind to the form of examination.

Measurement of reactive hyperemia index

Peripheral arterial pulse amplitude was measured using a PAT device that was placed on each first finger (Endo-PAT2000, Itamar Medical, Caesarea, Israel) [22]. The inflation pressure of the PAT device was set to 10 mmHg below DBP or at least 70 mmHg. After baseline pulse amplitude had been recorded from each finger for 5 min, the blood pressure cuff was inflated in the test arm for 5 min to whichever inflated pressure would be higher: 200 or 60mmHg + SBP. Pulse amplitude was recorded simultaneously from both fingers. After the cuff had been deflated, pulse amplitude was recorded for up to 5 min. The ratio of PAT was calculated automatically through a computer algorithm (Itamar Medical). Log RHI was calculated for subsequent analysis. As the raw values of RHI had a heteroscedastic error structure, we used a natural logarithmic transformation. Inter-coefficients and intra-coefficients of variation for the pulse wave amplitude were 5.5 and 6.0%, respectively, in our laboratory.

Measurements of flow-mediated vasodilation and nitroglycerine-induced vasodilation

Vascular response to reactive hyperemia in the brachial artery was used for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter [23].

A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5–10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. Blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 s, and then the blood pressure cuff was inflated to 50 mmHg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and for 10 s immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD [(Peak diameter — baseline diameter)/baseline diameter] was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity

(corrected for the angle) by heart rate and vessel cross-sectional area ($-r^2$). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

The response to nitroglycerine was used for assessment of endothelium-independent vasodilation. NID was measured as described previously [23]. Briefly, after acquiring baseline rest images for 30 s, a sublingual tablet (75 μ g nitroglycerine) was given, and images of the artery was recorded continuously until the dilation reached a plateau after administration of nitroglycerine. Participants who had received nitrate treatment and participants in whom the sublingually administered nitroglycerine tablet was not dissolved during the measurement were excluded from this study. Nitroglycerine-induced vasodilation was automatically calculated as a percent change in peak vessel diameter from the baseline value. Percentage of nitroglycerine-induced vasodilation [(peak diameter — baseline diameter)/baseline diameter] was used for analysis. Intercoefficients and intra-coefficients of variation for the brachial artery diameter were 1.6 and 1.4%, respectively, in our laboratory.

Measurement of Rho-associated kinase activity

ROCK activity was assayed in peripheral blood leukocytes as the amount of phospho-Thr853 in the myosin-binding subunit of myosin light chain phosphatase (MLCPh), because myosin-binding subunit on MLCPh is one of the downstream targets of ROCK. Blood was collected at room temperature in heparinized tubes (20U/ml) [17,24]. After adding an equal volume of 2% dextran, each sample was kept at room temperature for 30 min. The supernatant was spun at 1450 rpm for 10 min. Red blood cells in the resulting cell pellet were lysed with the addition of water and spun at 1450 rpm for 10 min after the addition of Hank's balanced salt solution (Hyclone, Logan, Utah, USA). The resulting leukocyte pellet was resuspended in medium 199 (Sigma Chemical Co., Saint Louis, Missouri, USA) and the number of cells was counted using a hemacytometer. Cells were fixed in 10% trichloroacetic acid and 10 mmol/l dichloro-diphenyltrichloroethane. After centrifugation, the cell pellets were stored at -80°C for western blot analysis. Cells pellets were dissolved in 10 μ l of 1 mol/l Tris base and then mixed with 100 μ l of extraction buffer (8 mol/l urea, 2% sodium dodecyl sulfate, 5% sucrose, and 5% 2-mercapto- ethanol). Equal amounts of cell extracts were subjected to 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. NIH 3T3 cell lysates were used as a positive control and to standardize the results of western blot analyses from several membranes. After serum starvation for 20 h, confluent cells were stimulated with 10 μ mol/l lysophosphatidic acid for 10 min and then subsequently fixed and harvested in 10% trichloroacetic acid and 10 mmol/l dichlorodiphenyltrichloroethane. Following centrifugation at 1450 rpm for 10 min at 4°C , precipitates were dissolved in 10 μ l of 1 mol/l Tris base and mixed with 100 μ l of extraction buffer. An equal volume of positive control cell lysate was used for each gel. Membranes were incubated with rabbit anti-phospho-specific Thr853–myosin-binding subunit polyclonal antibody (Biosource Invitrogen, Carlsbad, California, USA), rabbit anti-myosin-binding subunit polyclonal antibody (Covance Laboratories, Evansville, Indiana, USA), or antiactin monoclonal antibody (Sigma). Bands were visualized using the ECL system (Amersham-Pharmacia Co., London, UK). Images were captured using Adobe Photoshop, and the band intensities were

quantified using National Institutes of Health Image 1.61. Rho-associated kinase activity was expressed as the ratio of phospho myosin-binding subunit in each sample to phospho myosin-binding subunit in each positive control divided by total myosin-binding subunit in each sample per total myosin-binding subunit in each positive control.

Measurement of brachial–ankle pulse wave velocity

Aortic compliance was assessed noninvasively on the basis of Doppler ultrasound measurements of PWV along the descending thoracoabdominal aorta, as previously published and validated [25]. Briefly, baPWV, an index of arterial stiffness, was determined by two pressure sensors, placed on the right ankle and left brachial arteries to record each pulse wave simultaneously, and the time lag (t) between the notches of the two waves, using a pulse wave velocimeter (Form PWV/ABI, model BP-203RPE, Colin Co.). The distance (D) between the two recording sensors was calculated automatically by inputting the value of individual height. The PWV value was calculated as $PWV = D/t$. PWV was measured for five consecutive pulses, and averages were used for analysis.

Measurement of brachial intima–media thickness

Before FMD measurement, baseline longitudinal ultrasonographic images of the brachial artery, obtained at the end of diastole from each of 10 cardiac cycles, were automatically stored on a hard disk for off-line assessment of IMT with a linear, phased-array high-frequency (10-MHz) transducer using an UNEXEF18G ultrasound unit (UNEX Co) [26]. Measurement of IMT was automatically performed on A-mode images of the far wall of the brachial artery. The analysis system automatically chose the measurement point where an image of the posterior intimal interface was clearly obtained. If the measurement point was inappropriate, another clear image site could be manually selected for measurement. A total of 21 points over a 3-mm length of IMT in the 10-mm longitudinal image depicted in the analysis display were measured and the mean value per image was automatically calculated. IMT was measured at the same point in each image. The average of mean values obtained from 10 cardiac cycles was defined as IMT of the brachial artery. When measuring carotid IMT, we had an anatomical landmark, such as the carotid-artery bulb. Unfortunately, it is difficult to measure the same site of the brachial artery attributable to the lack of an anatomical landmark. Measurement of IMT in the brachial artery was performed at the proper site where the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained at 5–10 cm above the elbow. However, there was little influence of inpatient and interpatient variability in the measurement location of the brachial artery at 5–10 cm above the elbow in the present study, because the interface on the intima media of brachial artery is relatively smooth and intima-media thickening is not localized or plaques are not presented, resulting in diffuse intima–media thickening. The coefficients of variation of intra-observer and inter-observer brachial IMT measurements were 3.1 and 4.0%, respectively.

Statistical analysis

Results are presented as means $\pm$ SD for continuous variables and as percentages for categorical variables. Statistical significance was set at a level of $P < 0.05$. Continuous variables were compared by using the paired Student's t -test. Categorical variables were

compared by means of the chi-square test. Relationships between variables were determined by Spearman correlation coefficients analysis. The data were processed using JMP pro version 13 (SAS institute. Cary, North Carolina, USA).

RESULTS

The baseline clinical characteristics of the 50 patients with IHA before and after treatment with eplerenone are summarized in Table 1. Of the 50 patients with IHA, 21 (42.0%) were men, 21 (42.0%) had dyslipidemia, 6 (12.0%) had diabetes mellitus, 8 (16.0%) were smokers, none had coronary artery disease, and 2 (4.0%) had a history of stroke. The mean dose of eplerenone after 12 weeks was 74.5mg/day. Eplerenone treatment decreased aldosterone-renin ratio from 67.5 ± 65.7 to 49.2 ± 34.4 ($P < 0.01$). After treatment with eplerenone, serum potassium increased from 3.8 ± 0.3 to 4.2 ± 0.4 mmol/l ($P < 0.01$), HbA1c increased from 5.4 ± 0.5 to $5.6 \pm 0.6\%$ ($P < 0.01$) and creatinine increased from 62.8 ± 16.8 to 66.3 ± 16.8 mmol/l ($P < 0.01$). Assessment of vascular function including log RHI, FMD and NID before and after 12 weeks of treatment with eplerenone are shown in Fig. 1. Eplerenone treatment increased log RHI from 0.56 ± 0.25 to 0.69 ± 0.25 ($P < 0.01$) and increased NID from 12.8 ± 5.8 to $14.9 \pm 6.9\%$ ($P = 0.02$) but did not significantly alter FMD from 4.6 ± 3.4 to $4.6 \pm 3.6\%$ ($P = 0.99$). Assessment of vascular structure including brachial IMT and baPWV before and after 12 weeks of treatment with eplerenone are shown in Fig. 2. Eplerenone treatment decreased baPWV from 1540 ± 263 to 1505 ± 281 ($P = 0.04$) but did not significantly alter brachial IMT from 0.28 ± 0.07 to 0.28 ± 0.04 mm ($P = 0.14$). ROCK activity before and after 12 weeks of treatment with eplerenone are shown in Fig. 3. Eplerenone treatment decreased ROCK activity from 1.20 ± 0.54 to 0.89 ± 0.42 ($P < 0.01$).

The baseline clinical characteristics before and after treatment with eplerenone of 31 of the 50 patients with IHA who had no change in antihypertensive drugs after additional eplerenone are summarized in Table 2. Of the 31 patients with IHA, 10 (32.2%) were men, 12 (38.7%) had dyslipidemia, 4 (12.9%) had diabetes mellitus, 4 (12.9%) were smokers, none had coronary artery disease, 2 (6.5%) had a history of stroke. Eplerenone treatment decreased aldosterone-renin ratio from 63.7 ± 45.0 to 36.5 ± 29.8 ($P < 0.01$). After treatment with eplerenone, high-density lipoprotein (HDL) cholesterol increased from 1.7 ± 0.6 to 1.8 ± 0.5 mmol/l ($P < 0.01$), serum potassium increased from 3.8 ± 0.3 to 4.2 ± 0.4 mmol/l ($P < 0.01$), HbA1c increased from 5.4 ± 0.4 to $5.6 \pm 0.6\%$ ($P < 0.01$), and creatinine increased from 59.2 ± 15.0 to 61.9 ± 15.9 mmol/l ($P < 0.01$). Eplerenone treatment increased log RHI from 0.56 ± 0.23 to 0.64 ± 0.25 ($P = 0.03$) and NID from 12.8 ± 5 to $15.1 \pm 5.4\%$ ($P = 0.04$), but FMD was not altered from 4.7 ± 2.6 to $4.5 \pm 3.0\%$ ($P = 0.55$; Fig. 4). Eplerenone treatment did not significantly alter brachial IMT from 0.28 ± 0.09 to 0.28 ± 0.05 mm ($P = 0.46$) or baPWV from 1545 ± 211 to 1532 ± 303 ($P = 0.19$; Fig. 5). Eplerenone treatment decreased ROCK activity from 1.21 ± 0.56 to 0.95 ± 0.49 ($P = 0.04$; Fig. 6).

DISCUSSION

In the present study, we demonstrated for the first time that eplerenone improves microvascular endothelial function, vascular smooth muscle function, and arterial stiffness and inhibits ROCK activity in patients with IHA. Treatment with eplerenone in patients with

IHA for 12 weeks improved log RHI, NID, baPWV and ROCK activity, whereas FMD and brachial IMT were not altered.

The present study also showed that 12-week treatment with eplerenone improved log RHI and NID in patients with IHA, whereas FMD was not altered. These findings suggest that microvascular endothelial function and vascular smooth muscle function are reversible and can be restored by treatment with eplerenone in patients with IHA. Therapy for IHA in the form of administration of mineralocorticoid receptor antagonists (spironolactone and eplerenone) is well established [21,27]. A prospective study showed that there was no difference in the incidence of cardiovascular event between groups of EHT patients and primary aldosteronism patients who were treated by adrenalectomy or administration of spironolactone during 7.4 years of follow-up [28]. Spironolactone treatment improved FMD in hypertensive patients with hyperaldosteronism [5]. In addition, we previously reported that the selective mineralocorticoid receptor antagonist eplerenone improved FMD in patients with EHT [18]. Hylton *et al.* showed that eplerenone improved coronary circulatory function in patients with diabetes mellitus but did not alter FMD, NID or RHI. However, there is no information on the effects of eplerenone on vascular function and structure in patients with IHA. In the present study, it was shown that microvascular endothelial function measured by log RHI and vascular smooth muscle function can be restored by treatment with eplerenone in patients with IHA, whereas macrovascular endothelial function measured by FMD was not altered. In a previous study, we showed that microvascular endothelial function was impaired in patients with IHA compared with that in patients with EHT, whereas there was no difference in macrovascular endothelial function measured by FMD between patients with IHA and patients with EHT. Improvement in microvascular endothelial function and vascular smooth muscle function may decrease the incidence of cardiovascular events.

Several studies have shown that arterial stiffness is increased in patients with primary aldosteronism [29–31]. Strauch *et al.* [32] demonstrated that adrenalectomy improved blood pressure and PWV in patients with primary aldosteronism. In contrast, medical treatment with spironolactone did not improve PWV in patients with primary aldosteronism, including IHA and an unknown subtype of primary aldosteronism. Kalizki *et al.* [33] reported that treatment with eplerenone decreased PWV in patients with resistant hypertension. However, the effect of eplerenone on arterial stiffness in patients with IHA is unclear. In the present study, we demonstrated that eplerenone decreased PWV in patients with IHA, whereas brachial IMT was not altered. PWV was correlated with SBP before eplerenone treatment and change in SBP. It is likely that change in SBP with eplerenone treatment contributes to the decrease in PWV. There is a discrepancy between the effect of mineralocorticoid receptor antagonists on arterial stiffness in patients with IHA and the results of our study and that previous study [33]. Some possible reasons for the discrepancy are postulated. In our study, we included patients with primary aldosteronism in whom successful adrenal vein sampling was performed and who had a definite diagnosis of IHA [21], whereas patients with IHA, APA and an unknown subtype of primary aldosteronism were included in the previous study. Holaj *et al.* [31] demonstrated that 6.3 years of treatment with spironolactone in patients with primary aldosteronism, including APA, IHA and an unknown subtype of primary aldosteronism, improved common carotid IMT. However, in the present study, there

was no significant difference between brachial IMT before and that after 12-week treatment with eplerenone. In addition, the high prevalences of overweight, dyslipidemia, smoking habit and diabetes mellitus as confounding factors may influence arterial stiffness in patients with IHA [26,34,35]. Further study is needed to confirm the effect of eplerenone on arterial stiffness in a population free from confounding factors in large clinical trials.

It has been shown that aldosterone stimulates ROCK activity through binding to the mineralocorticoid receptor in vascular smooth muscle cells [36]. We reported that ROCK activity was significantly higher in patients with APA than in patients with IHA and EHT [7]. In addition, adrenalectomy in patients with APA improved endothelial function and ROCK activity. We showed in a randomized, parallel group study that eplerenone decreased ROCK activity in patients with EHT [18]. In the present study, we showed that eplerenone also decreased ROCK activity in patients with IHA. These findings suggest that one of the mechanisms by which eplerenone improves endothelial function and aortic stiffness in patients with IHA is inactivation of the Rho/ROCK pathway. Eplerenone may reduce ROCK activity by blocking the binding of aldosterone to the mineralocorticoid receptor in vascular smooth muscle cells. Endothelial NO synthase (eNOS) expression is up-regulated by reduction of ROCK activity via an increase of eNOS mRNA stability and eNOS phosphorylation in endothelial cells [37].

In this study, the number of patients with IHA was relatively small. However, we observed a marked augmentation of log RHI and NID and reduction in ROCK activity after treatment with eplerenone in patients with IHA. Further study is needed to confirm the beneficial effects on vascular function and ROCK activity in patients with IHA. In 17 of the 50 patients with IHA, doses of some antihypertensive drugs, including calcium channel blockers, angiotensin II receptor blockers and alpha blockers, were decreased after additional eplerenone as patients had blood pressure of less than 125/75 mmHg after administration of the maximum dose of eplerenone. Doses of antihypertensive drugs other than eplerenone were decreased or administration was stopped by the physician's judgement (Table 3). Interestingly, eplerenone similarly improved vascular function, log RHI and NID and showed a tendency, but not with significance, to inhibit ROCK activity in those patients (Fig. 7). These findings suggest that eplerenone may be more effective than other antihypertensive drugs for improving vascular function in patients with IHA. However, this study was not a randomized and parallel group study. The results of this study need to be confirmed by a randomized and parallel group trial.

Eplerenone did not alter ROCK activity and baPWV in patients in whom doses of antihypertensive drugs other than eplerenone were decreased or administration was stopped. Some studies have shown that calcium channel blockers, ARB and alpha antagonists inhibit ROCK activity and improve arterial stiffness [40–42]. In the present study, after starting administration of eplerenone, doses of antihypertensive drugs were decreased or administration was stopped in some patients in whom blood pressure decreased to less than 125/75 mmHg. There were no patients in whom doses of antihypertensive drugs were increased. Decreasing the doses or stopping the administration of antihypertensive drugs might have contributed to no changes in ROCK activity and baPWV in those patients. It has been shown that a shorter duration of hypertension is one of the predictors of complete

resolution of hypertension by adrenalectomy in patients with APA [43,44]. In the present study, the duration of hypertension was longer in patients in whom doses of antihypertensive drugs other than eplerenone were decreased or administration was stopped than in patients in whom there were no changes in antihypertensive drugs other than eplerenone. A longer duration of hypertension may attenuate the beneficial effects of eplerenone on ROCK activity and baPWV.

In previous studies, mineralocorticoid receptor antagonists did not alter metabolic profiles in patients with heart failure and patients with essential hypertension [38,39]. In the present study, HbA1c significantly increased in patients with IHA after treatment with eplerenone. We do not know the precise reasons for the discrepancy in the effects of mineralocorticoid receptor antagonists on levels of HbA1c and glucose between our study and previous studies. The differences in diseases may influence the effect of mineralocorticoid receptor antagonists on metabolic profiles. In addition, we cannot deny the possibility that the small number of subjects enrolled in our study resulted in the increased HbA1c after treatment with eplerenone. Thus, the results of this study need to be confirmed by a multicenter study in large clinical trials.

In conclusion, treatment with eplerenone for 12 weeks improved log RHI, NID, and baPWV and inhibited ROCK activity in patients with IHA. Eplerenone may reduce future cardiovascular events in patients with IHA.

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Abbreviations:

ACEI	angiotensin-converting enzyme inhibitor
APA	aldosterone-producing adenoma
ARB	angiotensin II receptor blocker
ARR	aldosterone-to-renin ratio
baPWV	brachial–ankle pulse wave velocity
BUN	blood urea nitrogen
EHT	essential hypertension
FMD	flow-mediated vasodilation
HDL	high-density lipoprotein
IHA,	idiopathic hyperaldosteronism

IMT	intima-media thickness
LDL	low-density lipoprotein
MLCPh	myosin light chain phosphatase
NID	nitroglycerine-induced vasodilation
NO	nitric oxide
PA	primary aldosteronism
PAC	plasma aldosterone concentration
PRA	plasma renin activity
RHI	reactive hyperemia index
RH-PAT	reactive hyperemia -peripheral arterial tonometry
ROCK	Rho-associated kinase

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FIGURE 1.

Bar graphs show log reactive hyperemia index (a), flow-mediated vasodilation (b), nitroglycerine-induced vasodilation (c) in patients with idiopathic hyperaldosteronism before and after treatment with eplerenone.

FIGURE 2.

Bar graphs show brachial artery intima-media thickness (a), brachial-ankle pulse wave velocity (b) in patients with idiopathic hyperaldosteronism before and after treatment with eplerenone.

FIGURE 3.

Bar graphs show Rho-associated kinase activity in patients with idiopathic hyperaldosteronism before and after treatment with eplerenone.

FIGURE 4.

Bar graphs show log reactive hyperemia index (a), flow-mediated vasodilation (b), nitroglycerine-induced vasodilation (c) in patients with idiopathic hyperaldosteronism who had no change in antihypertensive drugs after additional eplerenone before and after treatment with eplerenone.

FIGURE 5.

Bar graphs show brachial artery intima-media thickness (a), brachial – ankle pulse wave velocity (b) in patients with idiopathic hyperaldosteronism who had no change in antihypertensive drugs after additional eplerenone before and after treatment with eplerenone.

FIGURE 6.

Bar graphs show Rho-associated kinase activity in patients with idiopathic hyperaldosteronism who had no change in antihypertensive drugs after additional eplerenone before and after treatment with eplerenone.

FIGURE 7.

Bar graphs show log reactive hyperemia index (a), flow-mediated vasodilation (b), nitroglycerine-induced vasodilation (c), brachial artery intima-media thickness (d), brachial-ankle pulse wave velocity (e), and Rho-associated kinase activity (f) in patients with idiopathic hyperaldosteronism who had change in antihypertensive drugs after additional eplerenone before and after treatment with eplerenone.

TABLE 1.

Clinical characteristics of the subjects before and after treatment with eplerenone

Variables	Before treatment with eplerenone (<i>n</i> = 50)	After treatment with eplerenone (<i>n</i> = 50)	P value
Age (years)	50 ± 12		
Sex, men/women	21/29		
BMI (kg/m ²)	25.7 ± 5.2	25.5 ± 5.0	0.86
SBP (mmHg)	134 ± 19	132 ± 14	0.16
DBP (mmHg)	76 ± 11	68 ± 8	0.60
Total cholesterol (mmol/l)	5.2 ± 0.9	5.3 ± 0.9	0.69
Triglycerides (mmol/l)	1.4 ± 0.6	1.5 ± 0.5	0.10
HDL cholesterol (mmol/l)	1.6 ± 0.6	1.7 ± 0.5	0.13
LDL cholesterol (mmol/l)	3.1 ± 0.7	3.1 ± 0.8	0.12
Serum potassium (mmol/l)	3.8 ± 0.3	4.2 ± 0.4	<0.01
Glucose (mmol/l)	5.5 ± 0.9	5.6 ± 0.8	0.09
HbA1c (%)	5.4 ± 0.5	5.6 ± 0.6	<0.01
BUN (mmol/l)	4.6 ± 1.2	5.7 ± 1.1	<0.01
Creatinine (mmol/l)	62.8 ± 16.8	66.3 ± 16.8	<0.01
Plasma aldosterone concentration (ng/dl)	21.1 ± 10.8	29.8 ± 14.0	<0.01
Plasma renin activity (ng/ml per h)	0.5 ± 0.4	1.7 ± 2.7	<0.01
Aldosterone–renin ratio	67.5 ± 65.7	49.2 ± 34.4	<0.01
Medical history, <i>n</i> (%)			
Hypertension	50 (100.0)	50 (100.0)	N/A
Dyslipidemia	21 (42.0)	21 (42.0)	N/A
Diabetes mellitus	6 (12.0)	6 (12.0)	N/A
Previous coronary heart disease	0 (0.0)	0 (0.0)	N/A
Previous stroke	2 (4.0)	2 (4.0)	N/A
Smoker, <i>n</i> (%)	8 (16.0)	8 (16.0)	N/A
Medication, <i>n</i> (%)			
Antiplatelets	2 (4.0)	2 (4.0)	N/A
Calcium channel blockers	34 (68.0)	29 (58.0)	0.30

Variables	Before treatment with eplerenone (n = 50)	After treatment with eplerenone (n = 50)	P value
ACEI	0 (0.0)	0 (0.0)	N/A
ARB	9 (18.0)	3 (6.0)	0.06
Mineralocorticoid receptor blockers	0 (0.0)	50 (100.0)	<0.01
Beta blockers	1 (2.0)	1 (2.0)	N/A
Alpha blockers	5 (10.0)	1 (2.0)	0.09
Diuretics	1 (2.0)	1 (2.0)	N/A
Statins	6 (12.0)	6 (12.0)	N/A
Nitrates	0 (0.0)	0 (0.0)	N/A
Medically treated diabetes mellitus			
Any	6 (12.0)	6 (12.0)	N/A
Insulin dependent	1 (2.0)	1 (2.0)	N/A
Duration of hypertension (years)	8.3 ± 7.9		

ACEI, indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N/A, not applicable. Results are presented as means± SD for continuous variables and percentages for categorical variables.

Clinical characteristics of the patients who had no change in antihypertensive drugs after additional eplerenone before and after treatment with eplerenone

TABLE 2.

Variables	Before treatment with eplerenone (<i>n</i> = 31)	After treatment with eplerenone (<i>n</i> = 31)	<i>P</i> value
Age (years)	50 ± 12		
Sex, men/women	10/21		
BMI (kg/m ²)	25.9 ± 5.4	25.5 ± 5.3	0.85
SBP (mmHg)	134 ± 18	133 ± 12	0.38
DBP (mmHg)	83 ± 11	82 ± 9	0.36
Total cholesterol (mmol/l)	5.2 ± 0.9	5.5 ± 1.1	0.46
Triglycerides (mmol/l)	1.4 ± 0.5	1.6 ± 0.8	0.19
HDL cholesterol (mmol/l)	1.7 ± 0.6	1.8 ± 0.5	<0.01
LDL cholesterol (mmol/l)	3.1 ± 0.7	3.0 ± 0.8	0.15
Serum potassium (mmol/l)	3.8 ± 0.3	4.2 ± 0.4	<0.01
Glucose (mmol/l)	5.6 ± 0.8	5.7 ± 0.8	0.33
HbA1c (%)	5.4 ± 0.4	5.6 ± 0.6	<0.01
BUN (mmol/l)	4.3 ± 1.2	5.7 ± 1.1	<0.01
Creatinine (mmol/l)	59.2 ± 15.0	61.9 ± 15.9	<0.01
Plasma aldosterone concentration (ng/dl)	18.5 ± 8.0	27.8 ± 13.1	<0.01
Plasma renin activity (ng/ml per h)	0.4 ± 0.3	1.8 ± 3.0	0.01
Aldosterone-renin ratio	63.7 ± 45.0	36.5 ± 29.8	<0.01
Medical history, <i>n</i> (%)			
Hypertension	31 (100.0)	31 (100.0)	N/A
Dyslipidemia	12 (38.7)	12 (38.7)	N/A
Diabetes mellitus	4 (12.9)	4 (12.9)	N/A
Previous coronary heart disease	0 (0.0)	0 (0.0)	N/A
Previous stroke	2 (6.5)	2 (6.5)	N/A
Smoker, <i>n</i> (%)	4 (12.9)	4 (12.9)	N/A
Medication, <i>n</i> (%)			
Antiplatelets	1 (3.2)	1 (3.2)	N/A
Calcium channel blockers	18 (58.1)	18 (58.1)	N/A

Variables	Before treatment with eplerenone (n = 31)	After treatment with eplerenone (n = 31)	P value
ACEI	0 (0.0)	0 (0.0)	N/A
ARB	2 (6.5)	2 (6.5)	N/A
Mineralocorticoid receptor blockers	0 (0.0)	31 (100.0)	<0.01
Beta blockers	1 (3.2)	1 (3.2)	N/A
Alpha blockers	0 (0.0)	0 (0.0)	N/A
Diuretics	0 (0.0)	0 (0.0)	N/A
Statins	2 (6.5)	2 (6.5)	N/A
Nitrates	0 (0.0)	0 (0.0)	N/A
Medically treated diabetes mellitus			
Any	2 (6.5)	2 (6.5)	N/A
Insulin-dependent	1 (3.2)	1 (3.2)	N/A
Duration of hypertension (years)	7.0 ± 7.7		

Results are presented as means±SD for continuous variables and percentages for categorical variables. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N/A, not applicable.

Clinical characteristics of the patients who had change in antihypertensive drugs after additional eplerenone before and after treatment with eplerenone

TABLE 3.

Variables	Before treatment with eplerenone (n = 17)	After treatment with eplerenone (n = 17)	P value
Age (years)	52 ± 12		
Sex, men/women	10/7		
BMI (kg/m ²)	25.4 ± 5.1	25.4 ± 4.9	0.43
SBP (mmHg)	132 ± 18	130 ± 17	0.23
DBP (mmHg)	78 ± 11	81 ± 12	0.17
Total cholesterol (mmol/l)	5.1 ± 0.8	4.9 ± 0.5	0.42
Triglycerides (mmol/l)	1.38 ± 0.8	1.4 ± 0.8	0.95
HDL cholesterol (mmol/l)	1.5 ± 0.5	1.5 ± 0.3	0.73
LDL cholesterol (mmol/l)	3.2 ± 0.8	3.3 ± 0.9	0.53
Serum potassium (mmol/l)	3.8 ± 0.4	4.1 ± 0.2	<0.01
Glucose (mmol/l)	5.1 ± 0.5	5.3 ± 0.6	0.09
HbA1c (%)	5.3 ± 0.5	5.5 ± 0.5	0.21
BUN (mmol/l)	4.6 ± 1.1	5.7 ± 1.3	0.02
Creatinine (mmol/l)	71.6 ± 18.6	75.1 ± 16.8	0.02
Plasma aldosterone concentration (ng/dl)	26.3 ± 13.9	34.2 ± 15.7	0.08
Plasma renin activity (ng/ml per h)	0.6 ± 0.4	1.6 ± 2.3	0.03
Aldosterone–renin ratio	79.1 ± 95.0	47.3 ± 42.6	0.08
ROCK activity	1.21 ± 0.53	0.79 ± 0.25	0.11
Medical history, n (%)			
Hypertension	17 (100.0)	17 (100.0)	N/A
Dyslipidemia	9 (52.9)	9 (52.9)	N/A
Diabetes mellitus	1 (5.9)	1 (5.9)	N/A
Previous coronary heart disease	0 (0.0)	0 (0.0)	N/A
Previous stroke	0 (0.0)	0 (0.0)	N/A
Smoker, n (%)			
Medication, n (%)			
Antiplatelets	1 (5.9)	1 (5.9)	N/A

Variables	Before treatment with eplerenone (n = 17)	After treatment with eplerenone (n = 17)	P value
Calcium channel blockers	15 (88.2)	9 (57.9)	0.02
ACEI	0 (0.0)	0 (0.0)	N/A
ARB	7 (41.1)	0 (0.0)	<0.01
Mineralocorticoid receptor blockers	0 (0.0)	17 (100.0)	<0.01
Beta blockers	0 (0.0)	0 (0.0)	N/A
Alpha blockers	5 (29.4)	1 (5.9)	0.03
Diuretics	1 (5.9)	1 (5.9)	N/A
Statins	4 (23.5)	4 (23.5)	N/A
Nitrates	0 (0.0)	0 (0.0)	N/A
Medically treated diabetes mellitus			
Any	4 (23.5)	4 (23.5)	N/A
Insulin-dependent	0 (0.0)	0 (0.0)	N/A
Duration of hypertension (years)		11.0 ± 8.0	
Log RHI	0.54 ± 0.25	0.72 ± 0.24	0.02
FMD (%)	4.5 ± 4.6	5.0 ± 4.5	0.27
NID (%)	12.6 ± 7.7	14.4 ± 9.2	0.04
Brachial artery IMT (mm)	0.29 ± 0.05	0.29 ± 0.03	0.71
baPWV (cm/s)	1506 ± 345	1463 ± 260	0.13

Results are presented as means ± SD for continuous variables and percentages for categorical variables. ACEI, indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; baPWV, brachial-ankle pulse wave velocity; BUN, blood urea nitrogen; FMD, flow-mediated vasodilation; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; N/A, not applicable; NID, nitroglycerine-induced vasodilation; RHI, reactive hyperemia index; ROCK, Rho-associated kinase.