[Original]

Eplerenone Improves Vascular Endothelial Function in Patients with Primary Aldosteronism: A Pilot Study

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Abstract : Primary aldosteronism (PA) is associated with a high risk of cardiovascular complications. Large-scale clinical studies have demonstrated that mineralocorticoid receptor antagonists (MRA) exhibit organ-protective effects and improve the prognosis of patients with heart failure and myocardial infarction, and daily clinical practice suggests that MRA seem to improve vascular endothelial dysfunction. In this pilot study, we treated 10 PA patients with eplerenone for 3 months. We used Endo-PAT to evaluate the effects of MRA on vascular endothelial function and analyzed the data for correlative factors. The primary outcome measure, the reactive hyperemia index (RHI), was 1.71 before therapy and increased significantly to 2.21. Univariate analysis showed a significant correlation between the rate of change in RHI and that in plasma renin activity (PRA). Since plasma aldosterone concentration increases during MRA therapy, PRA may be the best marker for selecting the most appropriate dose of MRA. PRA can potentially be used for adjusting the dose of MRA, in addition to adjusting blood pressure and serum potassium level.

Keywords : primary aldosteronism, endothelial dysfunction, reactive hyperemia index, eplerenone.

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Introduction

Primary aldosteronism (PA) is a typical form of secondary hypertension and is the underlying etiology of hypertension in approximately 4–19% of cases [1–3]. PA is reported to be associated with a high risk of cerebrovascular and cardiovascular diseases compared to essential hypertension (EH) in patients of similar age and blood pressure [4, 5]. According to the 2019 Japanese Society of Hypertension guidelines for the management of hypertension, drug therapy with mineralocorticoid receptor (MR) antagonists (MRA) as the first choice of treatment should, in principle, be administered continuously throughout life to patients

who have bilateral PA, do not wish to undergo surgery, are inoperable, or do not wish to take any tests after screening [6].

MR is expressed in various organs in addition to the distal renal tubules. The high incidence of cardiovascular complications due to aldosterone may be directly related to inflammation or increased oxidative stress in the vascular endothelium [7]. Since aldosterone itself has a damaging effect on the cardiovascular system, large-scale clinical studies have demonstrated that MRA exhibits an organ-protective effect and improves the prognosis of patients with heart failure or myocardial infarction [8, 9]. Few studies, however, have reported that MRA improves vascular endothelial func-

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tion, i.e., the early stage of arteriosclerosis, in clinical practice. Endo-PAT (Endo-PAT2000, Itamar Medical, Caesarea, Israel) using peripheral arterial tonometry (PAT) has been used as a noninvasive method for the assessment of vascular endothelial function [10], and the usefulness of reactive hyperemia index (RHI) in predicting vascular diseases has been reported [11]. We previously assessed the relation of endothelial function in clinical studies [12–17], but clinical trials using RHI in PA are limited compared to those using flow-mediated dilation (FMD). In this study we treated 10 patients with PA by using eplerenone for 3 months, and used Endo-PAT to evaluate the effects of MRA on the status of vascular endothelial function and explored the factors that correlate with the improvement of this function.

Materials and Methods

Participants

The study participants were 10 patients with PA, who visited the Outpatient Clinics of the Department of Endocrinology, Metabolism and Diabetes, University of Occupational and Environmental Health, between October 2017 and December 2018. They were not using drugs that affect the aldosterone-renin ratio (ARR), such as angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARB) at the time of endocrinological examination, but they were being treated with calcium channel blockers and/or α -blockers. PA was suspected based on screening data of $\text{ARR} \geq 200$. Each participant underwent a captopril load test, a physiological saline load test, and a furosemide standing test, and the diagnosis of PA was confirmed when two or more of the above three tests, which are listed in the Japanese Endocrine Society-PA Diagnostic and Treatment Guideline 2009, proved positive. We excluded patients with diabetes and cardiac arrhythmia on electrocardiogram. For localized diagnosis, adrenal computed tomography (CT) was performed in all 10 patients. None had adrenal tumors on either side. Selective adrenal venous sampling (AVS) was performed in 6 patients and confirmed the presence of bilateral PA. This study was approved by the Ethics Committee of the University of Occupational and Environmental Health, Japan (H27–186).

Study Design

This retrospective study included 10 patients with PA who were treated with 50 mg/day eplerenone. Each patient underwent the Endo-PAT before and 3 months after treatment with eplerenone to measure their RHI. No changes were made to other medications except for anti-hypertensive drugs (calcium channel blockers were reduced or discontinued in 4 cases, and α -blocker was discontinued in 1 case). Blood pressure was evaluated by blood pressure in the examination room, not by home blood pressure. The primary endpoint was changes in RHI after treatment with eplerenone, and the secondary endpoint was the correlation between changes in RHI and changes in plasma aldosterone concentration (PAC), plasma renin activity (PRA) and ARR.

Assessment of endothelial function with Endo-PAT

We assessed vascular function in 10 patients by Endo-PAT. The method used for digital assessment of vascular function using Endo-PAT has been described elsewhere [18].

Statistical analysis

Data were expressed as mean $\pm$ SD. The Wilcoxon test was used for comparison of pre-treatment with the 3 months of treatment (Figure 1, Table 1). The Spearman correlation test was used for analysis of the data listed in Table 2. The level of significance was set at $P < 0.05$. All statistical analyses were conducted using the Statistical Package for Social Association version 21.0 (SPSS Inc., Chicago, IL).

Results

The baseline characteristics of the patients are shown in Table 3. The study participants were 10 patients with PA (3 males; 7 females). The mean age of the participants was 60.4 years. The participants were mildly obese, with mean body mass index of 25.5 kg/m^2 . Renal function was normal, with eGFR of $77.3 \text{ mL/min/1.73 m}^2$. Laboratory tests showed almost normal serum potassium (only 1 patient had hypokalemia).

As shown in Figure 1, the primary outcome measure, the RHI value was 1.71 ± 0.41 before therapy, but increased significantly to 2.21 ± 0.62 at 3 months after

Figure 1. Vascular endothelial function before and after 3-month treatment with eplerenone. RHI: reactive hyperemia index.

Table 1. Effects of 3-month treatment with eplerenone on various parameters

	Before therapy	After therapy	<i>P</i> value
Clinical parameters			
SBP (mmHg)	149.7±19.8	136.1±19.0	0.221
DBP (mmHg)	93.4±12.4	81.8±13.8	0.083
Biochemical parameters			
PAC (pg/ml)	125.7±54.7	192.0±82.4	0.037
PRA (ng/ml/hr)	0.32±0.20	0.94±0.51	0.011
ARR	452±245	326±345	0.203
K (mEq/l)	3.9±0.3	4.3±0.5	0.028

Data are mean ± SD. *P* values for comparison of before and after therapy, by Wilcoxon test. SBP: systolic blood pressure, DBP: diastolic blood pressure, PAC: plasma aldosterone concentration, PRA: plasma renin activity, ARR: aldosterone-renin ratio.

Table 2. Correlation between Δ change in RHI and Δ change in various parameters

Variable	Univariate Analysis	
	<i>r</i>	<i>P</i> value
SBP	-0.479	0.162
DBP	-0.261	0.467
K	0.164	0.651
PAC	-0.370	0.293
PRA	0.939	<0.001
ARR	-0.915	<0.001

Data are *P* value by Spearman rank correlation. RHI: reactive hyperemia index, SBP: systolic blood pressure, DBP: diastolic blood pressure, PAC: plasma aldosterone concentration, PRA: plasma renin activity, ARR: aldosterone-renin ratio.

treatment with eplerenone ($P=0.022$). There were no significant changes in blood pressure and ARR (Table 1), but PAC, PRA and serum potassium significantly increased after the 3-month treatment. With regard to the secondary outcome measures, univariate analysis of factors affecting the Δ change in RHI identified significant correlations with the Δ change in PRA and ARR (Table 2).

Discussion

The main findings of the present study based on the

use of Endo-PAT were that 3-month continuous treatment with MRA improved vascular endothelial function in patients with PA, and that the changes in this function correlated with changes in PRA.

The American Endocrine Society revised the clinical practice guidelines for the diagnosis and treatment of PA in 2016, for the first time in 8 years [19]. The revised guidelines recommended treatment with MRA for patients with positive results in the first screening test who do not wish to or cannot be further tested. Treatment with MRA is also recommended for patients with suspected PA, thus confirming the importance of

Table 3. Baseline characteristics of patients

Variable	mean $\pm$ SD or number
sex (male:female)	3:7
age (years)	60.4 $\pm$ 12.8 (41-76)
body mass index (kg/m ²)	25.5 $\pm$ 3.6 (21.8-33.1)
Systolic blood pressure (mmHg)	146.2 $\pm$ 17.4 (112-168)
Diastolic blood pressure (mmHg)	90.5 $\pm$ 8.6 (76-107)
LDL-C (mg/dl)	125.3 $\pm$ 29.6 (77-182)
HDL-C (mg/dl)	67.5 $\pm$ 18.5 (38-91)
Triglyceride (mg/dl)	115 $\pm$ 70 (42-282)
Aspartate aminotransferase (IU/l)	23.8 $\pm$ 9.1 (13-41)
Alanine aminotransferase (IU/l)	23.1 $\pm$ 17.2 (8-67)
γ -GTP (IU/l)	34.1 $\pm$ 18.8 (15-80)
Cre (mg/dl)	0.70 $\pm$ 0.22 (0.44-1.15)
eGFR (ml/min/1.73 m ²)	77.3 $\pm$ 16.7 (57.4-106.2)
FPG (mg/dl)	95.7 $\pm$ 7.1 (90-109)
HbA1c (%)	5.6 $\pm$ 0.4 (4.9-6.1)
K (mEq/l)	3.9 $\pm$ 0.3 (3.2-4.2)
PAC (pg/ml)	136 $\pm$ 54 (53-213.0)
PRA (ng/ml/hr)	0.33 $\pm$ 0.22 (0.2-0.9)
ARR	480.8 $\pm$ 259.8 (230.0-1065.0)
24hr urine-aldosterone (μ g/day) (n=8)	9.1 $\pm$ 3.1 (4.3-14.3)
Smoking (n)	3
Alcohol (n)	6
Statin (n)	1
CCB (n)	9
α -blocker (n)	1
ARB (n)	0

Data are mean $\pm$ SD or number (range: minimum-maximum). LDL: low-density lipoprotein, HDL: high-density lipoprotein, γ -GTP: gamma-glutamyl transpeptidase, Cre: Creatinine, eGFR: estimated glomerular filtration rate, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, PAC: plasma aldosterone concentration, PRA: plasma renin activity, ARR: aldosterone-renin ratio, CCB: calcium channel blocker, ARB: angiotensin receptor blocker.

medical treatment. Eplerenone, a second-generation MRA with enhanced selectivity for MR, became available in Japan in 2007. It is highly selective for MR and has only a few adverse endocrine reactions, such as gynecomastia, which is observed in patients treated with spironolactone.

Previous studies using FMD reported that PA was associated with poorer vascular endothelial function compared with EH [20-22], and that the percentage of FMD (%FMD) correlated negatively with PAC, ARR [19, 20], and 24-hour urinary aldosterone excretion

[21, 22]. It was also reported that MRA therapy and adrenalectomy improved %FMD in patients with PA [20-22]. In vitro, eplerenone improved endothelial function through the enhancement of the expression of the endothelial nitric oxide (NO) synthase gene [23, 24] suggesting that eplerenone directly increases NO production in hypertension by activation of endothelial NO synthase. Inhibition of the aldosterone/mineralocorticoid receptor may also contribute to a decrease in oxidative stress, resulting in inhibition of NO inactivation [25]. In vivo, spironolactone improved %FMD

and endothelium-dependent vasodilation induced by acetylcholine in patients with hyperaldosteronism and in patients with heart failure [26, 27]. These reports did not examine the factors that contribute to the improvement in %FMD in patients with PA [20–22]. Although our study was retrospective in nature, it is the first to report on the factors that correlate with MRA-related improvement in vascular endothelial function.

Hundemer *et al* reported that 602 patients with PA showed 1.91 times higher incidence of cardiovascular events, 1.34 times higher mortality, 1.26 times higher incidence of diabetes mellitus, and 1.93 times higher incidence of atrial fibrillation than age-matched patients with EH [28]. They also reported that treatment with MRA reduced the incidence of cardiovascular events in PA patients with high PRA (≥ 1.0 ng/ml/hr) to levels comparable to those in EH. In other words, since MRA therapy increases PAC, PRA may be the best marker for selecting the appropriate dose of MRA, as confirmed by our results. The adjustment of the dose of MRA is generally based on serum potassium levels. Based on the results of our study and those of Hundemer *et al*, PRA can potentially be used for adjusting the dose of MRA, in addition to blood pressure and serum potassium level.

The limitations of the present study are: (1) the small sample size of 10 patients, (2) the retrospective study design, and (3) the lack of a control against eplerenone, such as a placebo. We plan to conduct a prospective study in the near future that will include a control group and a larger sample size.

Conflict of Interest

The authors declare no conflict of interest.

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