Original Article

Effects of Eplerenone and Salt Intake on Left Ventricular Remodeling after Myocardial Infarction in Rats

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Eplerenone, a selective aldosterone blocker, has been shown to attenuate cardiac fibrosis and decrease cardiovascular events in both experimental and clinical studies. We examined the cardioprotective effect of eplerenone in myocardial infarction (MI) rats receiving different levels of salt in their diet. The MI rats were randomly divided into five groups: Group CL, animals received a low-salt diet (0.015%); Group EpL, a low-salt diet with eplerenone (100 mg/kg/day in food); Group CH, a high-salt diet (0.9%); Group EpH, a high-salt diet with eplerenone; and Group C, a normal salt diet (0.3%). These diets were continued for 4 weeks. Echocardiographic and histomorphological examinations revealed that the administration of eplerenone significantly improved the cardiac function, significantly suppressed compensatory cardiac hypertrophy and significantly reduced cardiac fibrosis in both the interstitial and the perivascular areas in the high-salt diet group (Group EpH). However, eplerenone had no observable effects in the low-salt diet group (Group EpL). Also, these examinations demonstrated that the left ventricular remodeling after MI was suppressed and the cardiac function was improved in the group receiving a low-salt diet without eplerenone (Group CL), even though there was a significant increase of aldosterone level in blood, in comparison to the group receiving a high-salt diet without eplerenone (Group CH). These results indicate that the cardioprotective effect of eplerenone varies depending on the salt intake. (*Hypertens Res* 2006; 29: 627–634)

Key Words: selective aldosterone blocker, salt intake, left ventricular remodeling, myocardial infarction

Introduction

The Randomized Aldactone Evaluation Study (RALES) demonstrated that the non-selective aldosterone blocker spironolactone clinically decreased morbidity and mortality in patients with chronic heart failure (1). The Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) further demonstrated that the aldosterone selective blocker eplerenone reduced the risk of cardiovascular events in patients with heart failure after myocardial infarction (MI) (2). Eplerenone has been reported to show very high selectivity for the mineralocorticoid recep-

tors, and has fewer anti-androgenic- and progesterone-related side effects than spironolactone (3, 4). In some experimental studies, eplerenone has been reported to have a variety of actions, such as an anti-inflammatory effect, the prevention of cardiac fibrosis (5, 6), the suppression of nicotinamide adenine dinucleotide (phosphate) NAD(P)H oxidase activity, and the prevention of endothelial dysfunction (7). Recently, various reports have noted the relationship between salt intake and the impact of aldosterone on the cardiovascular system (8–11), and aldosterone blockers have been reported to prevent salt-induced cardiac injury (12–14). Also, in an experiment using stroke-prone spontaneously hypertensive rats (SHRSPs) given different levels of salt intake, eplerenone

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Table 1. Physiological and Hemodynamic Change

Parameters	Group C	Group CL	Group EpL	Group CH	Group EpH
BW (g)	329±17	269±15* [#]	264±15* [#]	317±10	322±7.2
HW (mg)	1,159±61	940±62*	833±35* [#]	999±43	1,063±47
HW/BW (mg/g)	3.63±0.38	3.62±0.47	3.2±0.13	3.23±0.2	3.33±0.16
SBP (mmHg)	114±3.6	108±9.1	113±11	105±2.2	110±2.9
DBP (mmHg)	76±5.2	81±7.6	84±8.0	76±1.0	79±2.8
HR (bpm)	393±21	425±10	400±14	401±12	409±10

The values are the mean±SEM. * $p<0.05$ vs. Group C. ** $p<0.01$ vs. Group C. [#] $p<0.05$ vs. Group EpH. BW, body weight; HW, heart weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

was reported to be less effective in the low-salt-loaded state (15). This raises the question of whether there is a difference in the cardioprotective effect of eplerenone under different levels of salt intake. In order to answer this question, we administered eplerenone to MI rats given high- and low-salt diets, and also examined the effect of eplerenone and salt intake on left ventricular remodeling after MI.

Methods

Myocardial Infarction Model Rats

All experimental procedures were approved by the institutional guidance committee of the Jikei University School of Medicine. Male Wistar rats (Sankyo Lab Service, Tokyo, Japan) weighing 200–250 g were used in this study. We produced MI rats as previously described (16). First, the rats were anesthetized with an intramuscular injection of ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (10 mg/kg). Next, each rat was intubated with a polyethylene tube and connected to a respirator (Shinano Co., Tokyo, Japan) with the tidal volume adjusted for the rat's weight and a breathing rate of 80 times per min. During subsequent operative procedures, each rat was anesthetized with 0.5–1.0% isoflurane. After a 10-min equilibration period, a left thoracotomy was performed through the cavity between the fourth and fifth ribs. The pericardial sac was resected and the left anterior descending artery (LAD) of the rat was ligated with 5-0 silk surgical sutures under microscopy. The ligation was confirmed by changes in the color tone of the ischemic area. 5-0 silk surgical sutures were then used to suture the thoracic cavity and to close the thorax. The rats were then weaned from the respirator, and the intratracheal tube was removed once they were breathing spontaneously and had begun to move. Four weeks after the operation, the heart was excised and the heart weight (HW) and body weight (BW) were measured.

Experimental Protocol

After the operation, the MI model rats were divided into the following five groups: Group CL animals received a low-salt

diet; Group EpL, a low-salt diet with eplerenone (100 mg/kg per day in food); Group CH, a high-salt diet; Group EpH, a high-salt diet with eplerenone (100 mg/kg per day in food); and Group C, a normal salt diet.

The rats in the low-salt diet group were given rat chow containing 0.015% NaCl. The rats in the normal salt diet group were given rat chow containing 0.3% NaCl. The rats in the high-salt diet group were given water containing 0.9% NaCl. These NaCl loading regimens were conducted for 4 weeks.

Echocardiographic Examination and Physiological Analysis

Four weeks after the operation, echocardiographic examinations were performed with an SSD-5000 echocardiographic system using a 15-MHz transducer (ALOKA Co., Ltd., Tokyo, Japan). The left ventricular posterior wall thickness (LVPWT) and interventricular septum thickness (IVST) were measured. The degree of compensatory cardiac hypertrophy in the left ventricle of each rat was estimated using the LVPWT/IVST ratio. In order to evaluate the cardiac function, the ejection fraction (EF) and fractional shortening (FS) were measured. In order to examine the hemodynamic changes in all of the groups, the systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured using a photoelectric tail-cuff pulse detection system (BP 98A; Softron, Tokyo, Japan) 4 weeks after the operations.

Histological Examination

We assessed cardiac fibrosis in each rat as previously described (17). Four weeks after the procedure, the heart was removed and excised into three pieces horizontally along the short-axis. The pieces were fixed in a solution containing 4% paraformaldehyde, and then were embedded in paraffin, and sliced into sections measuring 4 µm in thickness. The sections to be used for the histological examinations were stained with Sirius red in order to reveal the collagen deposition rate. Digital photomicrographic images were taken using a Nikon digital image system (Nikon, Tokyo, Japan), and then the digital images were analyzed using NIH image software (NIH, Bethesda, USA). The myocardial fibrosis rates both in the

Fig. 1. *M-mode echocardiogram records for each group. A: Sham-operated rats; B: Group EpL; C: Group CL; D: Group EpH; E: Group C; and F: Group CH.*

interstitial area and in the perivascular area were calculated as (fibrotic area/myocardial cross-sectional area) $\times 100$. Two researchers performed all assessments in a blinded manner.

Measurement of Plasma Aldosterone Levels

A blood sample was promptly collected from each rat under ether anesthesia. Immediately after the sample was taken, it was centrifuged in order to separate the plasma portion, which was preserved at -20°C . The aldosterone concentration in these serum samples was measured by means of a radioimmunoassay using an ALDOSTERONE-RIAKIT II (SRL, Tokyo, Japan).

Statistical Analysis

A statistical analysis was performed using the StatView 5 software package (SAS Institute Inc., Cary, USA). All data were expressed as the mean $\pm$ SEM. When comparing parameters, an analysis of co-variance was performed using

Fisher's test, and values of $p < 0.05$ were considered to indicate statistical significance.

Results

Physiological and Hemodynamic Changes

Table 1 shows the physiological and hemodynamic data at 4 weeks after MI was produced. In Groups CL, EpL, CH and EpH, the SBP, DBP and HR showed no significant differences in comparison to those in Group C. These results suggest that the dosages of eplerenone and the salt-loading levels did not affect the hemodynamics of the MI model rats in this experiment. The BW and HW were recorded for all groups and then the HW/BW ratio was calculated. The BW and HW were significantly lower in Group CL than in Group C. On the other hand, the BW and HW showed no significant difference between Group CH and Group C. In addition, no significant differences were observed in the HW/BW ratios among the five groups.

Fig. 2. The results of echocardiographic findings in A: the ejection fraction (EF) and B: the fractional shortening (FS) at 4 weeks after MI in each group. * $p < 0.05$ vs. Group CH.

Fig. 3. The results of echocardiographic findings in LVPWT/IVST at 4 weeks after MI in each group. LVPWT, left ventricular posterior wall thickness; IVST, intraventricular septum thickness. * $p < 0.05$ vs. Group CH, ** $p < 0.01$ vs. Group CH, # $p < 0.01$ vs. Group C.

Cardiac Function

In order to assess the cardiac function, echocardiographic examinations were performed 4 weeks after MI. M-mode echocardiograms from each group are shown in Fig. 1. The EF and FS data are shown in Fig. 2. The administration of eplerenone significantly improved the EF and FS in the high-salt diet groups (Group CH vs. Group EpH), but it had no significant effect in the low-salt diet groups (Group CL vs. Group EpL).

Compensatory Cardiac Hypertrophy

Figure 3 shows the LVPWT/IVST ratio 4 weeks after MI. The LVPWT/IVST ratio was significantly lower in Group CL than in Group C. This result showed that a low-salt diet suppressed compensatory cardiac hypertrophy. The administration of eplerenone significantly reduced the ratio in the groups on high-salt diets (Groups CH and EpH), but it did not affect the groups on low-salt diets (Groups CL and EpL).

Cardiac Fibrosis

Figure 4 shows typical photomicrographic images of the non-infarcted area in the left ventricular myocardium (A–E: interstitial area in the myocardium; F–J: perivascular area). Depositions of collagen fiber (stained red) in the myocardium were detected using Sirius red staining. The degrees of cardiac fibrosis in the myocardial interstitial and perivascular areas are shown in Fig. 5A and B, respectively. In the myocardial interstitial area (Fig. 5A), Group CL showed significantly less cardiac fibrosis than Group C. Furthermore, in the high-salt diet groups (Groups CH and EpH), cardiac fibrosis in the myocardial interstitial area was significantly suppressed by the administration of eplerenone. Eplerenone had no observable effect in the low-salt diet groups (Groups CL and EpL). A similar effect was observed in the perivascular area (Fig. 5B). Group CL had a significantly lower degree of cardiac fibrosis than Group C. Among the high-salt diet groups (Groups CH and EpH), cardiac fibrosis in the perivascular area was significantly suppressed by the administration of eplerenone. However, among the low-salt diet groups (Groups CL and EpL), eplerenone had no observable effect. These results showed that eplerenone significantly suppressed cardiac fibrosis after MI in the high-salt diet groups,

Fig. 4. Photomicrographs of cardiac sections stained with Sirius red to visualize collagen deposition. A and F: Group C; B and G: Group CH; C and H: Group EpH; D and I: Group CL; E and J: Group EpL. The collagen depositions in A–E were assessed in the interstitial area, and those in F–J were assessed in the perivascular area (magnification: $\times 100$ objective; bar: 100 μm).

Fig. 5. The quantitation of collagen deposition is normalized by a cross-sectional area of the hearts in the interstitial area (A), or the perivascular area (B). # $p < 0.05$ vs. Group C, * $p < 0.05$ vs. Group CH, ** $p < 0.01$ vs. Group CH.

Fig. 6. The plasma aldosterone levels at 4 weeks after MI in each group. * $p < 0.05$ vs. Group C, † $p < 0.05$ vs. Group CH, # $p < 0.01$ vs. Group EpH, † $p < 0.01$ vs. Group CL.

but it did not suppress cardiac fibrosis in the low-salt diet groups, and also showed that a low-salt diet significantly suppressed cardiac fibrosis.

Plasma Aldosterone Levels

In order to assess the effects of eplerenone and salt intake, we measured the plasma aldosterone levels (Fig. 6). The plasma aldosterone levels were significantly higher in Group CL than in Group C, and significantly higher in Group CL than in Group CH. The treatment with eplerenone tended to increase the plasma aldosterone levels in the high-salt diet groups, and it also significantly increased the plasma aldosterone levels, particularly in the low-salt diet groups.

Discussion

Eplerenone has been reported to suppress left ventricular remodeling by reducing cardiac fibrosis in MI rats given a normal salt diet (18, 19). Spironolactone has been reported to prevent cardiac fibrosis and cardiac hypertrophy induced by a

high-salt diet (13). In our study using MI rats, we demonstrated for the first time that eplerenone significantly suppresses cardiac fibrosis as a major factor of the left ventricular remodeling after MI in animals given a high-salt diet, but not in those given a low-salt diet.

It has been recognized that aldosterone causes cardiac fibrosis as one type of end-organ damage, and the effect does not depend on blood pressure (20–23). Aldosterone has been shown to contribute to cardiac fibrosis under salt loading. Regarding the mechanism of this effect, it has been reported that prior to the occurrence of cardiac fibrosis, the infiltration of inflammatory cells such as monocytes and macrophages, and the expression of proinflammatory molecules such as cyclooxygenase-2 (COX-2), monocyte chemoattractant protein-1 (MCP-1), and osteopontin all increased in rats given aldosterone and salt (24). Moreover, eplerenone has been reported to suppress the infiltration of inflammatory cells and the expression of these proinflammatory molecules (6). These reports supported our present results that the progression of cardiac fibrosis induced by salt loading is significantly suppressed by eplerenone.

It is known that low-salt intake activates the renin-angiotensin system, enhances aldosterone synthesis from the adrenal glands, and increases the aldosterone level in blood (25). In our study, the aldosterone level in blood in rats given a low-salt diet was significantly higher than that in rats given a high-salt diet. Nevertheless, rats given a low-salt diet showed significantly less cardiac fibrosis than rats given a high-salt diet, and significantly improved cardiac function. Brilla and Weber reported that rats receiving aldosterone with a low-salt diet had significantly higher blood aldosterone levels than those receiving aldosterone with a high-salt diet, but none of the rats in their study developed cardiac fibrosis (20). This report also supports our present results, which show that a low-salt diet positively contributed to cardioprotection, even though it increased the renin-angiotensin-aldosterone system (RAAS) in blood. Our present results also suggest that a low-salt diet alone is nearly as effective at suppressing cardiac

remodeling and improving cardiac function as administration of eplerenone in a high-salt diet.

Both cardiac fibrosis and compensatory cardiac hypertrophy are important factors in the process of left ventricular remodeling after MI. It has been reported that aldosterone synthesis in local cardiac tissues and its effects are related with the process of left ventricular remodeling (26). Aldosterone blockers have been reported to suppress cardiac hypertrophy and cardiac fibrosis (27, 28). Takeda *et al.* reported that a high-salt intake accelerates the expression of the aldosterone synthase gene, CYP11B2 mRNA, in the cardiac tissues while also increasing cardiac aldosterone synthesis locally, which may affect cardiac hypertrophy independently of the RAAS in blood (29). Recently, Ye *et al.* reported that the CYP11B2 and 11 β -hydroxylase (CYP11B1) genes are not expressed in the rat heart (30). In the present study, however, eplerenone prevented left ventricular remodeling after MI in animals receiving a high-salt diet, and we therefore suggest that eplerenone blocks mineralocorticoid receptors in the heart.

In our experiment using MI rats, eplerenone significantly suppressed compensatory cardiac hypertrophy, particularly in the animals receiving a high-salt diet, but did not do so in animals receiving a low-salt diet. Our results suggest that eplerenone suppresses the progression of cardiac hypertrophy, possibly *via* the cardiac aldosterone synthesis induced by salt loading.

It has been reported that the cardiac fibrosis and cardiac hypertrophy induced by deoxycorticosterone acetate (DOCA) and salt occur through the activation of Na⁺/H⁺ exchanger 1 (NHE1) (31). Furthermore, eplerenone has been reported to block the nongenomic effects of aldosterone on NHE1 in mesenteric resistance vessels (32). These results suggest that the pathway through the nongenomic effect of aldosterone on NHE1 may play an important role in the cardioprotective effect of eplerenone under salt loading.

In conclusion, in the present study eplerenone prevented the progression of left ventricular remodeling after MI in animals fed a high-salt diet, but did not exert such cardioprotective effects in animals fed a low-salt diet. Also, a low-salt diet without eplerenone treatment resulted in equal prevention of the progression of left ventricular remodeling compared to a high-salt diet with eplerenone. These results indicate that the cardioprotective effects of eplerenone on left ventricular remodeling after MI are dependent on the salt intake.

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