Cardiac Damage Prevention by Eplerenone: Comparison With Low Sodium Diet or Potassium Loading

Diego V. Martinez, Ricardo Rocha, Mamiko Matsumura, Eveline Oestreicher, Margarita Ochoa-Maya, Weranuj Roubsanthisuk, Gordon H. Williams, Gail K. Adler

Abstract—To determine the extent to which dietary sodium modulates aldosterone-induced cardiovascular damage, and to determine whether increased dietary potassium can prevent this damage, we used the Nω-nitro-L-arginine methyl ester (L-NAME)/angiotensin II (Ang II) rat model of cardiac injury. This model is dependent on the presence of aldosterone for the occurrence of myocardial damage. Two sets of experiments were performed. In the first set, the following groups were studied: (1) 1% NaCl to drink (control group); (2) L-NAME/Ang II with water to drink (low salt group); (3) L-NAME/Ang II/1% NaCl (high salt group); (4) L-NAME/Ang II/1% NaCl/eplerenone (eplerenone group). Systolic blood pressure increased similarly in all groups compared with controls. Compared with the controls, the high salt group, but not the low salt or eplerenone groups, developed significant myocardial damage. In the second set of experiments three groups of animals were studied: (1) L-NAME/Ang II/1%NaCl (high salt group) (2) L-NAME/Ang II/1%NaCl/eplerenone (eplerenone group), and (3) L-NAME/Ang II/1%NaCl with an extra 1% KCl in food (high dietary potassium group). Eplerenone, but not dietary potassium supplementation, prevented the development of cardiac damage.

Thus, mineralocorticoid receptor antagonist treatment and low sodium diet were effective in preventing cardiac damage, which suggests that a minimal level of aldosterone and a moderately high sodium diet are both required for the development of the cardiovascular damage in the L-NAME/Ang II model. The inability of potassium supplementation to reduce myocardial damage suggests that eplerenone’s protective effect is not due to its potassium-sparing ability, but is rather related to some other feature of its selective aldosterone antagonism. (Hypertension. 2002;39(part 2):614-618.)

Key Words: sodium • potassium • cardiovascular diseases • aldosterone • rats

Seven decades have passed since Simpson and Tait first described a new adrenal salt retaining hormone: aldosterone. Studies rapidly established the role of the renin-angiotensin-system in the control of aldosterone secretion, and it became clear that the renin-angiotensin-aldosterone system (RAAS) was a major regulator of sodium and potassium balance, blood volume, and arterial blood pressure. In recent years, attention has focused on the role of the RAAS in mediating cardiac and renal damage and stroke. Increased activity of the RAAS is associated with increased end-organ damage in hypertensive subjects. Blocking angiotensin II’s (Ang II) actions or reducing Ang II production reduces end-organ damage in individuals with congestive heart failure or hypertension. While most of the attention was focused on the role of Ang II in producing these effects, recent data support that aldosterone also is a causative agent. Clinical studies report a positive correlation between aldosterone levels and the severity of end-organ damage. In patients with severe heart failure on optimum therapy, including diuretics, digitalis, and ACE inhibitors, the addition of the mineralocorticoid receptor antagonist spironolactone reduced cardiac morbidity and mortality by 30% over a two-year period.

Animal models have also been used to demonstrate the role of aldosterone in the development of end-organ damage. Dorrow and Miller and Selye et al produced cardiac lesions by repeated injections of deoxycorticosterone acetate (DOCA). In the DOCA-salt model, administration of a mineralocorticoid to uninephrectomized rats on a high sodium diet induced hypertension and cardiac fibrosis. Cardiac fibrosis was reduced by administration of mineralocorticoid receptor antagonists, spironolactone, or canrenone. In stroke-prone spontaneously hypertensive rats (SHRSP), the administration of aldosterone antagonists or adrenalectomy markedly reduced the incidence of stroke and renal microvascular lesions. In both the DOCA-salt and SHRSP models, end-organ damage was dependent on the presence of a very high sodium intake.

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We have shown that administration of L-NAME and Ang II to rats on a moderately high sodium diet similarly causes myocardial damage that can be prevented by adrenalectomy or administration of the aldosterone receptor antagonist eplerenone. Administration of aldosterone to adrenalectomized, L-NAME/Ang II treated rats on a high sodium diet again induces the damage. In these animal models of cardiovascular damage, the role of aldosterone has been firmly established. However, the role of the two ions (sodium and potassium) has not been established. Using the L-NAME/Ang II model, the purpose of the present study was to determine the extent to which dietary sodium modulates aldosterone-induced cardiovascular damage and to determine whether increased dietary potassium could prevent cardiovascular damage similar to the effect of aldosterone receptor blockade.

Methods

Animals

The present studies, conducted in accordance with institutional guidelines for the humane treatment of animals, used male Wistar rats (n=72), weighing 225 g to 250 g, obtained from Charles River Laboratories, Inc (Wilmington, Mass). All animals were housed in a room lighted 12 hours/day at an ambient temperature of 22±1°C. Animals were allowed 1 week to recover after arrival and had free access to Purina Rat Chow 5001 (Ralston Purina Co) and tap water until the initiation of the experiment.

The L-NAME/Ang II/NaCl model was used to induce cardiovascular damage. 1% NaCl was administered in drinking water ad libitum starting 3 days before L-NAME and continuing until the end of the experiment. L-NAME (40 mg/kg per day) from Sigma was administered in the drinking water for 14 days. Ang II dose was 112 or 225 μg/kg per day as indicated below. The minipumps were implanted subcutaneously in the back of the animal neck after rats were anesthetized with isoflurane. On day 14, animals were anesthetized with intraperitoneal pentobarbital. Blood samples were taken for hormone measurements using the abdominal aorta catheterization. Animals were euthanized, and the hearts were removed, weighed, and stored in 10% phosphate-buffered formalin. The tissue was later processed for light microscopy.

To assess the influence of dietary sodium restriction, rats were randomized to one of four treatment groups. The control NaCl group (n=7) received 1% NaCl in drinking water. The L-NAME/Ang II/1% NaCl group (n=8) received the standard treatment. The L-NAME/Ang II/1% NaCl/eplerenone group (n=8) received standard treatment plus eplerenone (100 mg/kg per day PO, day 0 to 14). Eplerenone was dissolved in 0.5% methylcellulose and administered twice a day by gavage. The L-NAME/Ang II/low salt group (n=9) received distilled water to drink plus L-NAME and Ang II. The dose of Ang II was 112 μg/kg per day in the above groups. From day 0 to 14, rats received Rat Chow (Cat No 7286/5881 M) from Purina Test Diet containing 0.02% sodium and 1.10% potassium.

To assess the influence of dietary potassium loading, rats were randomized to one of these treatment groups: L-NAME/Ang II/1%NaCl (n=16), L-NAME/Ang II/1% NaCl/eplerenone (n=16), L-NAME/Ang II/1%NaCl/high K+ (n=8). The dose of Ang II was 225 μg/kg per day. From day 0 to 14, rats received Rat Chow from Purina Test Diet containing 0.4% sodium and 1.10% potassium except that the high potassium group had 1% KCl added to the rat chow.

Assays and Analyses

Animals in all groups were handled and weighed daily and maintained in separate metabolic cages. Twenty-four-hour food intake, fluid intake, and urine output were measured daily. Systolic blood pressure (SBP) was measured on day 0 and 13 in awake animals by tail-cuff plethysmography using a Natsume KN-210 manometer and tachometer (Peninsula Laboratories, Inc). Rats were allowed to rest quietly for 5 minutes in a Lucite chamber (warmed previously to 30°C) before measurement of blood pressure. Blood pressure was measured ten times over approximately 10 minutes, and rats were returned to their cages. The Lucite chambers are specially designed to limit the movements of the animal without harming it. Rats were properly trained to the restrainers for periods of 20 minutes every day for one week; blood pressure was not measured during this time.

Plasma aldosterone concentration was measured with a standard RIA kit from Diagnostic Products; sodium and potassium were analyzed with the AVL 987 electrolyte analyzer; and creatinine was analyzed with the Beckman creatinine II analyzer (Model 6642).

Histology

Hearts were stained with hematoxylin and eosin for light microscopy. Two or three sections of the heart were analyzed for each animal. Sections were taken from different parts of the heart and contained both right and left ventricles. A scale from 0 to 4 was used to score the level of myocardial injury in each section, and an average score for each animal was obtained: 0 represented no damage; 1 represented the presence of myocytes demonstrating early damage changes such as nuclear pyknosis or karyolysis, noncontracting marginal wavy fibers, and eosinophilic staining of the cytoplasm associated with the presence of scattered neutrophilic infiltrates; 2 indicated one clear area of damage (loss of myocardial cell with heavy neutrophilic infiltrates); 3 indicated two or more separate areas of damage comprising less than 50% of the myocardium; 4 was assigned to hearts in which areas of damage comprising more than 50% of the myocardium.

Statistical Analysis

Data were analyzed by one-way analysis of variance, ANOVA (Prism 3.0). A significant difference between group data were subjected to the Bonferroni test. P value less than 0.05 was accepted as statistically significant.

Results

Effect of Dietary Sodium on L-NAME/Ang II-Induced Cardiac Damage

The 24-hour urinary sodium to creatinine ratio measured in urine collected from day 13 to 14 was significantly (P<0.001) lower in animals on a low sodium diet compared with all groups on a high sodium diet (which did not differ from each other), consistent with the differences in dietary sodium intake. Likewise, plasma aldosterone levels measured on day 14 were ten times higher in the low salt/L-NAME/Ang II group than in animals on a high-sodium diet (P<0.001) (Table 1).

Baseline SBP was similar in all treatment groups (P>0.7). By day 13, there was an increase in SBP in all groups (P<0.05). However, animals receiving L-NAME/Ang II had significantly higher SBP than did 1%NaCl-drinking controls (P<0.05) (Table 1). Day 13 SBP did not differ between L-NAME/Ang II-treated groups. The heart weight to total body weight ratio was similar in all treatment groups and not different from control animals.

Histological examination of the hearts from L-NAME/Ang II/1% NaCl-treated animals revealed biventricular myocardial injury characterized by loss of cross-striation of myofibers, homogenization of cytoplasm, loss of cellular membranes, and a severe influx of inflammatory cells. This damage was significantly more than that observed in control
animals receiving a high sodium diet alone (HDS = 1.5 ± 0.8 versus 0 in controls; \( P < 0.05 \)) (Figure 1). A representative photomicrograph of these lesions is shown in Figures 2A and 2B. In contrast, the extent of cardiac damage induced by L-NAME/Ang II was significantly less if animals consumed a low sodium diet (HDS = 0.4 ± 0.4) or were treated with eplerenone (HDS = 0.3 ± 0.3) (Figures 2C and 2D). The L-NAME/Ang II/low sodium group and L-NAME/Ang II/high-sodium/eplerenone group demonstrated levels of myocardial damage similar to those in the 1% NaCl-drinking controls.

**Effect of Dietary Potassium on L-NAME/Ang II-Induced Cardiac Damage**

Animals on a high sodium diet received L-NAME/Ang II combined with high (2.1%) dietary potassium, normal (1.1%) dietary potassium, or normal dietary potassium plus eplerenone. Rats fed a high potassium diet had a significantly higher 24-hour urinary potassium to creatinine ratio (Table 2) than did animals on a normal potassium diet, which was consistent with the differences in potassium intake. Plasma aldosterone, SBP, and heart weight to body weight ratio did not differ between groups (Table 2). The aldosterone levels were higher in these dietary potassium studies than in the dietary sodium studies because the Ang II infused was twice as large (225 versus 112 \( \mu \)g/kg per day).

Histological examination of the hearts from L-NAME/Ang II-treated animals on a high sodium/normal potassium diet showed cardiac damage (HDS = 2.3 ± 0.5). The extent of damage was significantly reduced in animals that also received eplerenone (HDS = 1.2 ± 0.6, \( P < 0.01 \)). In contrast, dietary potassium supplementation did not prevent the development of cardiac damage in L-NAME/Ang II/high sodium-treated rats. (HDS = 2.7 ± 0.4, \( P > 0.05 \) versus high sodium group) (Figure 3).

**Discussion**

Induction of cardiac damage by combined L-NAME and Ang II administration appears to be dependent on both a moderately high sodium diet and aldosterone activation of mineralocorticoid receptors. Furthermore, the protective effects of a low sodium diet or a mineralocorticoid receptor antagonist did not appear to be related to their effects on blood pressure or potassium homeostasis.

The observation that low dietary sodium intake provides cardiac protection in L-NAME/Ang II-treated animals, despite a 10-fold increase in plasma aldosterone levels, is consistent with other models of aldosterone-induced cardiovascular injury. In both the DOCA/salt and SHRSP models, cardiovascular injury is dependent on animals consuming a high sodium diet.18,24 The mechanisms by which high sodium intake promotes aldosterone-mediated cardiovascular injury are unclear, but they do not appear to be related to changes in blood pressure or volume homeostasis.

In the present study, both a low sodium diet and eplerenone prevented cardiac damage without reducing SBP. A similar dissociation of blood pressure and cardiovascular protection was seen in SHRSP when blockade of the RAAS reduced nephrosclerosis and stroke without lowering SBP.22,23,26,27 Another indication of the independence of mineralocorticoid-induced cardiac damage and blood pressure is the DOCA/salt model in which reducing SBP by central nervous system administration of a mineralocorticoid receptor antagonist did

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**Figure 1.** Histopathological scores (mean ± SEM) for myocardial necrosis in hearts obtained from studies on the effect of dietary sodium on L-NAME/Ang II-induced cardiac damage. Two or three sections from each heart were examined under light microscopy and scored according to the extent of myocardial damage on a semiquantitative scale from 0 to 4, with 0 representing no damage and 4 representing damage to more than 50% of the myocardium.

**TABLE 1.** Effect of Dietary Sodium and Eplerenone on L-NAME/AngII-Treated Animals

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>SBP Day 0* (mm Hg)</th>
<th>SBP Day 13* (mm Hg)</th>
<th>Plasma Aldosterone† (ng/dL)</th>
<th>HW/BW‡ (mg/g)</th>
<th>Urinary Na/Cr§ (mEq/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% NaCl</td>
<td>7</td>
<td>113 ± 14</td>
<td>134 ± 14**</td>
<td>4 ± 2#</td>
<td>3.1 ± 0.1</td>
<td>55.7 ± 5.9#</td>
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<tr>
<td>1% NaCl L-NAME/AngII</td>
<td>8</td>
<td>112 ± 12</td>
<td>168 ± 25†††</td>
<td>10 ± 11#</td>
<td>3.4 ± 0.4</td>
<td>41.8 ± 5.0#</td>
</tr>
<tr>
<td>1% NaCl L-NAME/AngII/Epl</td>
<td>9</td>
<td>117 ± 11</td>
<td>163 ± 13†††</td>
<td>12 ± 11#</td>
<td>3.2 ± 0.3</td>
<td>51.3 ± 6.6#</td>
</tr>
<tr>
<td>Low sodium L-NAME/AngII</td>
<td>9</td>
<td>110 ± 10</td>
<td>154 ± 13†††</td>
<td>105 ± 27</td>
<td>3.2 ± 0.3</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

Values are the mean ± SE.

*Tail-cuff measurements of SBP obtained on day 0 and on day 13 of L-NAME.
†Plasma aldosterone determined on day 14.
‡Heart weight (HW) to body weight (BW) ratio determined on day 14.
§Sodium (Na) to creatinine (Cr) ratio in 24-hour urine collected day 13–14.
¶P < 0.001 vs LN/Ang II/Low salt.
#P < 0.05 vs day 0.
not reduce cardiac damage. Finally, in the RALES study, spironolactone reduced cardiac morbidity and mortality in humans with severe heart failure without altering blood pressure. The average dose of spironolactone was only 25 mg/d—a dose thought to have little effect on volume homeostasis. These findings suggest that low sodium intake provides cardiac protection, even though plasma aldosterone levels and blood pressure are high. The results in the present experiments support the hypothesis that aldosterone-induced cardiac damage is dependent on the relationships between the mineralocorticoid receptor, sodium intake, and intracellular messengers, and not on plasma aldosterone levels per se.

Treatment with mineralocorticoid receptor antagonists reduce urinary K⁺ excretion, raising the possibility that increased body potassium could be a mediator of the beneficial cardiovascular effects of eplerenone in the current studies. In support of this are a number of studies suggesting that a high-potassium diet reduces hypertension and protects against vascular injury. However, increasing dietary potassium from a normal to an elevated level did not reduce L-NAME/Ang II/1% NaCl-induced cardiac damage, suggesting that positive potassium balance was not cardioprotective. A similar lack of cardiovascular protection with dietary potassium supplementation was observed in the DOCA-salt model. One fundamental difference in the L-NAME/Ang II model versus previous ones documenting potassium’s protective role is the normal potassium in these animals. Taken together, the present and previous data suggest that in the presence of high sodium intake, low potassium per se can induce damage. This damage can be reduced by administration of potassium. However, aldosterone blockade has a beneficial effect on cardiac damage, independent of a potential effect on potassium homeostasis, that is most easily appreciated when potassium intake is normal. This latter scenario may be more representative of clinical conditions when aldosterone receptor blockade may prove to be particularly beneficial.

In summary, the combined administration of Ang II and L-NAME to rats on a moderately high sodium diet is an effective method of inducing hypertension and myocardial damage. Mineralocorticoid receptor antagonist treatment (eplerenone) and low sodium diet were effective in preventing cardiac damage, thus suggesting that a minimal level of aldosterone and a moderately high sodium diet are both required for the development of the cardiovascular damage seen in the L-NAME/Ang II animal model. The inability of

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<th>Urinary K/Cr§ (mEq/mg)</th>
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</thead>
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<tr>
<td>1% NaCl L-NAME/AngII</td>
<td>16</td>
<td>121±0.1</td>
<td>186±0.1#</td>
<td>30±6.4</td>
<td>4±0.1</td>
<td>5.0±1.2‡</td>
</tr>
<tr>
<td>1% NaCl L-NAME/AngII/Epl</td>
<td>16</td>
<td>122±0.1</td>
<td>186±0.1</td>
<td>31.8±4.7</td>
<td>3.5±0.2</td>
<td>6.2±1.5‡</td>
</tr>
<tr>
<td>1% NaCl/2.1%K L-NAME/AngII</td>
<td>8</td>
<td>116±0.1</td>
<td>173±0.1#</td>
<td>21±4.8</td>
<td>3±0.2</td>
<td>24.4±3.7</td>
</tr>
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</table>

Values are the mean±SE.

*Tail-cuff measurements of SBP obtained on day 0 and on day 13 of L-NAME.
†Plasma aldosterone determined on day 14.
‡Heart weight (HW) to body weight (BW) ratio determined on day 14.
§Sodium (Na) to creatinine (Cr) ratio in 24 hour urine collected day 13–14.
¶P<0.001 vs high K⁺ diet.
#P<0.05 vs day 0.
Figure 3. Histopathological scores (mean±SEM) for myocardial necrosis in hearts obtained from studies on the effect of dietary potassium on L-NAME/Ang II-induced cardiac damage. Two or three sections from each heart were examined under light microscopy and scored according to the extent of myocardial damage on a semiquantitative scale from 0 to 4, with 0 representing no damage and 4 representing damage in more than 50% of the myocardium.

potassium supplementation to reduce myocardial damage in this model suggests that eplerenone’s protective effect is not due to its potassium-sparing ability but rather to some other features of its selective aldosterone antagonism.

Acknowledgments
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References
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