Preventive effects of low molecular mass potassium alginate extracted from brown algae on DOCA salt-induced hypertension in rats

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ABSTRACT

Available evidence indicates that brown algae may be beneficial for the treatment of high blood pressure. Our recent study demonstrated that low molecular mass potassium alginate (L-PA), one of the major polysaccharides extracted from brown algae, decreased systolic blood pressure (SBP) in spontaneous hypertensive rats. The present study investigated the effects of L-PA on deoxycorticosterone acetate (DOCA) salt-induced hypertension in rats. Hypertension was induced by biweekly subcutaneous injections of 50 mg/kg DOCA plus 1% NaCl in drinking water. The control group received saline injections. L-PA (250 or 500 mg/kg), KCl (239 mg/kg), or volume-matched solvent was administered orally once daily for 30 days. DOCA salt administration significantly increased SBP, sodium excretion, serum sodium content, circulating plasma volume (CPV), plasma atrial natriuretic peptide (ANP) content, heart and renal weight indices, and mortality and decreased plasma aldosterone (ALD) and serum potassium levels in the vehicle-treated DOCA salt group compared with the control group. However, L-PA dose-dependently normalized the above changes induced by DOCA salt, with the exception of further increasing sodium excretion, while KCl did not affect the changes caused by DOCA salt, with the exception of slightly ameliorating hypokalemia and mortality. These findings suggest that L-PA may offer a novel form of potassium supplementation with greater antihypertensive and sodium excretion actions than KCl and may likely be beneficial for the primary prevention and treatment of hypertension and its cardiovascular sequelae.

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1. Introduction

Hypertension affects approximately 25% of the adult population worldwide, and its prevalence is predicted to increase by 60% by 2025 [1]. It is the major risk factor for cardiovascular disease and is responsible for the most deaths worldwide. Various genetic and environmental factors are known to be involved in the pathogenesis of primary hypertension, among which excess sodium intake or hypokalemia has long been regarded as the pivotal environmental factor for this disorder [2]. Weinberger et al. estimated that approximately 25% of the normotensive population is “salt-sensitive”, and their arterial pressures are abnormally responsive to excess dietary NaCl, which may be a more accurate predictor of subsequent cardiovascular diseases as well as morbidity than normotension itself [3,4]. Additionally, Weinberger et al. found that this condition is more prevalent in primary hypertension patients, of whom 50–70% showed obvious sensitivity of arterial pressure to NaCl. In contrast, potassium depletion has been shown to contribute to the pathogenesis of hypertension and subsequent cardiovascular and renal injury [2]. However, potassium supplementation appears to decrease blood pressure and prevent strokes in high blood pressure patients [5]. Moreover, forms of potassium that do not contain chloride, such as those found naturally in fruits, vegetables, and other foods, might offer greater cellular entry in exchange for sodium and increased antihypertensive effects [6]. Therefore, ameliorating sodium retention and potassium deficits in the body, rather than KCl treatment itself, may provide an important strategy for the primary prevention and treatment of hypertension and its cardiovascular sequelae.

The available evidence indicates that brown algae may be used as a seasoning in dried flake form and as a table salt replacement.
for high blood pressure patients [7]. Studies have shown that algicin acid, one of the major polysaccharides present in the extracellular matrix of brown algae, has antiviral properties and stimulates human keratinocyte growth, VEGF-mediated growth, and migration of human endothelial cells [8–10]. Our recent study indicated that low molecular mass potassium alginate (L-PA) could decrease blood pressure in spontaneous hypertensive rats [11]. L-PA is a polysaccharide extracted from brown algae, *Laminaria japonica*, cultured in Qingdao sea water in China by specific means of fractionation and neutralization with potassium hydroxide. The average molecular weight of L-PA was determined to be 1800 Dalton by high performance steric exclusion chromatography analysis. Its potassium content was 25%, and its polysaccharide chemical composition was determined as a linear glycuronoglycan consisting of β-1,4 linked D-mannuronic acid and L-guluronic acid units in the pyranose ring form [12]. The present study thus investigated the preventive effects of L-PA on experimental hypertension induced by deoxycorticosterone acetate plus 1% sodium chloride (DOCA salt) in rats. Moreover, we compared the effects of L-PA and KCl on DOCA salt-induced hypertension.

2. Materials and methods

2.1. Chemicals and reagents

The test compound, L-PA, was provided by Dalian Yaweite Bioengineering Co., Ltd., China (Lot number: 060806). Its potassium content was 25% (w/w). L-PA was dissolved in distilled water for animal treatment. Deoxycorticosterone acetate (DOCA, purity > 99.9%) was provided by Pharmaceutical Co., Ltd., Shenzhen, Zhejiang, China (Lot number: 200708-1). The plasma atrial natriuretic peptide (ANP) and aldosterone (ALD) assay kits were purchased from North Biological Technology Research Institute, Beijing, China. The other reagents were the highest grades available.

2.2. Animals

SPF male Sprague Dawley rats (five to six weeks old, 200–220 g) were purchased from the Institute of Experimental Animal, Sichuan Medical Science Academy, China. The rats were housed in a temperature- and humidity-controlled room (25 °C) with a 12 h light/dark cycle for one week to be acclimated to this environment before the experiment. During this period, rats were fed standard chow diet containing 3 mg Na+/g pellet and 5 mg K+/g pellet (GB 14924.3-2001, China) and were provided water ad libitum.

2.3. Pharmacological treatment and blood pressure measurement

After one week acclimation to the environment, systolic blood pressure (SBP) was measured using the tail-cuff method on a rat blood pressure monitoring system (RBP-1B, Beijing, China). The rats then were randomly assigned to the following five groups based on SBP: control (n = 10), DOCA salt (n = 20), 250 or 500 mg/kg L-PA (n = 10 per group), and 239 mg/kg KCl (n = 10). Hypertension was induced by DOCA salt as reported previously [13]. Briefly, rats received subcutaneous (s.c.) injections of 50 mg/kg DOCA dissolved in olive oil twice per week and were provided with drinking water containing 1% NaCl for 30 days. Animals in the control group received s.c. volume-matched saline injections and were provided with drinking water without 1% NaCl. Meanwhile, L-PA, KCl, or volume-matched vehicle (distilled water) was administered orally once daily (9:00–10:00 a.m.) for 30 days.

2.4. Biochemical analysis

To measure the excretion of urinary and fecal Na+ and K+ during the experimental period, the animals were individually housed in metabolic cages for 24 h for urine or feces collection on Days 0, 20, and 30. Urinary and fecal Na+ and K+ values were measured by M6 atomic absorption spectrophotometry (Thermo Fisher Scientific, Waltham, MA, USA). After urine and feces collection on Day 30, blood samples were taken from the arterial catheter in chloral hydrate-anesthetized rats (300 mg/kg, i.p.). The heparinized plasma was obtained by centrifuging the blood samples using 1% heparin solution as anticoagulant (9/1, v/v) at 3500 rpm for 10 min at 4 °C. The plasma ANP and ALD values were determined by radioimmunoassay on an LS 6500 Multi-Purpose Scintillation Counter (Beckman Coulter, Fullerton, CA, USA) with ANP and ALD kits, respectively (North Biological Technology Research Institute, Beijing, China). Meanwhile, serum was prepared by centrifuging the blood samples at 4000 rpm for 10 min after bathing for 30 min in 37 °C water. Serum sodium and potassium levels then were detected using a clinical electrolyte analyzer (HC 9883, Hang Chuang Medical Treatment Facility Co., Ltd., Shenzhen, China).

2.5. Determination of circulating plasma volume and organ weight indices

One day after blood samples were taken, all animals were intravenously injected with 0.2% Evans Blue (EB, 1 ml/kg). Five minutes following EB administration, heparinized rat blood samples were collected, and plasma was separated as above to determine absorbance at 620 nm using a UV-2102C spectrophotometer (Unico, Shanghai, China). The circulating plasma volume (CPV) was calculated from the following equation [14]:

\[
CPV\left(\frac{ml}{100gBW}\right) = \frac{EB(ml) \times OD_{\text{standard}}/OD_{\text{sample}}}{BW(g)} \times 100\%
\]

Animals then were decapitated. Hearts and kidneys were excised, washed with cold phosphate-buffered saline, dried with soft facial tissue, and weighed. The heart and kidney weight indices were recorded as organ weight (g)/100 g body weight.

2.6. Statistical analysis

All data are expressed as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Values of P < 0.05 were considered significantly significant.

3. Results

3.1. Effects of L-PA on mortality and electrolyte metabolism

Table 1 shows that DOCA salt induced a significant increase in death rate in rats on Day 30 (45%) compared with the control group (0%). Death rate was dose-dependently prevented by oral administration of L-PA at doses of 250 mg/kg (20%) and 500 mg/kg (0%) or KCl at a dose of 239 mg/kg (20%).

On Day 0, all groups showed no differences in the excretion of both urinary and fecal sodium and potassium (P > 0.05), and the control group had constant sodium and potassium excretion over the 30-day experimental period (P > 0.05). DOCA salt administration greatly augmented sodium excretion through urine (P < 0.01, Days 20 and 30) and feces (P < 0.05, Day 30) and significantly decreased potassium excretion through urine (P < 0.05, Day 20) and feces (P < 0.01, Day 20) compared with the control group.

Pretreatment with L-PA dose-dependently increased sodium excretion through urine (P < 0.01 at 500 mg/kg on Day 20) and
Effects of low molecular mass potassium alginate (L-PA) and potassium chloride (KCl) on mortality and electrolyte metabolism in DOCA salt hypertensive rats.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Vehicle-treated DOCA sal</th>
<th>L-PA (250 mg/kg) + DOCA sal</th>
<th>L-PA (500 mg/kg) + DOCA sal</th>
<th>KCl (239 mg/kg) + DOCA sal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Day 20</td>
<td>10</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Day 30</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Urinary Na⁺ (mEq/kg/24h)</td>
<td>3.10 ± 0.41</td>
<td>3.21 ± 1.13</td>
<td>3.06 ± 1.09</td>
<td>3.20 ± 1.26</td>
<td>3.30 ± 0.67</td>
</tr>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 20</td>
<td>2.99 ± 0.86*</td>
<td>24.00 ± 11.21</td>
<td>28.17 ± 13.38</td>
<td>36.50 ± 9.69</td>
<td>20.70 ± 11.74</td>
</tr>
<tr>
<td>Day 30</td>
<td>3.11 ± 1.26*</td>
<td>22.84 ± 11.10</td>
<td>17.61 ± 3.50</td>
<td>12.46 ± 5.33**</td>
<td>23.23 ± 15.85</td>
</tr>
<tr>
<td>Urinary K⁺ (mEq/kg/24h)</td>
<td>6.94 ± 1.68</td>
<td>7.01 ± 1.68</td>
<td>6.42 ± 1.68</td>
<td>6.78 ± 2.76</td>
<td>6.91 ± 0.92</td>
</tr>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 20</td>
<td>6.17 ± 0.59*</td>
<td>5.13 ± 0.84</td>
<td>5.98 ± 1.23</td>
<td>7.29 ± 0.78**</td>
<td>9.95 ± 1.50**</td>
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<tr>
<td>Day 30</td>
<td>7.04 ± 1.30</td>
<td>6.64 ± 2.26</td>
<td>8.77 ± 1.41**</td>
<td>10.48 ± 1.16*</td>
<td>10.26 ± 2.72**</td>
</tr>
<tr>
<td>Fecal Na⁺ (mEq/kg/24h)</td>
<td>1.35 ± 0.27</td>
<td>1.19 ± 0.43</td>
<td>0.93 ± 0.17</td>
<td>0.98 ± 0.23</td>
<td>1.39 ± 0.17</td>
</tr>
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<td>Day 0</td>
<td></td>
<td></td>
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<tr>
<td>Day 20</td>
<td>1.41 ± 0.32</td>
<td>1.36 ± 0.45</td>
<td>1.64 ± 0.31</td>
<td>1.85 ± 1.14</td>
<td>1.67 ± 0.90</td>
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<td>Day 30</td>
<td>1.41 ± 0.19</td>
<td>2.21 ± 0.93</td>
<td>2.68 ± 1.45</td>
<td>3.35 ± 0.29*</td>
<td>2.01 ± 0.94</td>
</tr>
<tr>
<td>Fecal K⁺ (mEq/kg/24h)</td>
<td>2.27 ± 0.40</td>
<td>2.12 ± 0.28</td>
<td>1.98 ± 0.27</td>
<td>2.11 ± 0.47</td>
<td>2.24 ± 0.13</td>
</tr>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 20</td>
<td>2.17 ± 0.17*</td>
<td>1.20 ± 0.33</td>
<td>1.27 ± 0.27</td>
<td>1.26 ± 0.59</td>
<td>1.41 ± 0.58</td>
</tr>
<tr>
<td>Day 30</td>
<td>1.97 ± 0.66</td>
<td>1.56 ± 0.42</td>
<td>2.63 ± 0.66</td>
<td>2.65 ± 0.51</td>
<td>1.75 ± 0.67</td>
</tr>
<tr>
<td>Serum Na⁺ (mEq/L)</td>
<td>134.2 ± 1.4</td>
<td>139.2 ± 1.9</td>
<td>138.4 ± 1.7</td>
<td>138.3 ± 2.5</td>
<td>138.8 ± 2.4</td>
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<tr>
<td>Day 0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 20</td>
<td>6.0 ± 0.7*</td>
<td>4.5 ± 0.6</td>
<td>4.7 ± 0.6</td>
<td>5.1 ± 0.7</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>Day 30</td>
<td>0.17 ± 0.02*</td>
<td>0.22 ± 0.04</td>
<td>0.20 ± 0.02</td>
<td>0.17 ± 0.03*</td>
<td>0.23 ± 0.05</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01, compared with vehicle-treated DOCA salt group at the same time.

a The alive number of rats on Days 0, 20, and 30 are indicated in all measured parameters. Serum Na⁺ and K⁺ values and CPV were measured on Day 30.

3.2. Effects of L-PA and KCl on SBP

Fig. 1 shows that administration of DOCA salt significantly increased SBP on Days 20 and 30 (P < 0.01 vs. control group), which was effectively and dose-dependently prevented by oral administration of L-PA at doses of 250 and 500 mg/kg (P < 0.05 and P < 0.01 vs. vehicle-treated DOCA salt group, respectively). However, 239 mg/kg KCl, which contains the same amount of potassium as 500 mg/kg L-PA, did not affect DOCA salt-induced hypertension in rats.

3.3. Plasma concentrations of ANP and ALD

Fig. 2 shows that DOCA salt administration significantly increased plasma ANP content and decreased plasma ALD content compared with the control group, an effect that was significantly and dose-dependently prevented by 250 or 500 mg/kg L-PA treatment once daily for 30 days (P < 0.05, P < 0.01 vs. vehicle-treated DOCA salt group, respectively). KCl treatment at a dose of 239 mg/kg had no significant effect on plasma concentrations of ANP and ALD in DOCA salt-induced hypertensive rats compared with vehicle-treated DOCA salt controls (P > 0.05).

3.4. Organ weight indices

Fig. 3 shows that DOCA salt treatment significantly increased heart and kidney weight indices (P < 0.01, P < 0.05 vs. control group, respectively), effects that were effectively prevented by 250 mg/kg L-PA.
and 500 mg/kg L-PA once daily for 30 days \((P < 0.01, P < 0.05, \text{respectively})\). However, 239 mg/kg KCl did not affect heart and kidney weight indices in DOCA salt-induced hypertensive rats.

4. Discussion

Previous studies have shown that administration of mineralocorticoid together with salt results in sodium retention, potassium depletion, hypertension, extensive tissue damage, and even death, while activating natriuretic systems and suppressing sodium- and water-retaining systems to increase sodium excretion \([2,15]\). Consistent with previous reports, the results of the present study show that administration of DOCA plus 1% NaCl to drinking water increased SBP, CPV, and plasma ANP values, cardiac and renal hypertrophy, and mortality and decreased plasma ALD in rats, effects that were accompanied by excess excretion of urinary and fecal sodium and hypokalemia.

The presence of hypokalemia in hypermineralocorticoid states has been shown repeatedly to affect cardiac structure, leading to myocardial necrosis or fibrosis, and myocardial function, resulting in impaired cardiac contractility and ventricular arrhythmias. \([16]\). Additionally, hypokalemia induces renal glomerular, tubular, and vascular lesions in hypertensive rats \([17]\). Population studies have shown an inverse relationship between potassium intake and the prevalence of hypertension and the risk of stroke or mortality \([18]\). Experimentally, potassium supplementation has been shown to decrease high blood pressure, prevent stroke, and reduce mortality in stroke-prone spontaneously hypertensive, Dahl salt-sensitive, renal hypertensive, or NaCl-poisoned rats \([17,19–21]\). The anti-hypertensive effect of dietary potassium has been attributed to a number of actions \([2]\), including natriuresis and diuresis \([22]\), vasodilation due to stimulation of vascular Na-K-ATPase activity \([23]\), and increased uptake of norepinephrine into postganglionic sympathetic nerve terminals \([24]\), actions on the renin-angiotensin-aldosterone system, kallikrein-kinin system, and central neural regulation \([25–27]\). In contrast to previous reports, although KC1 supplementation in the present study significantly reversed hypokalemia and decreased death rate in DOCA salt-induced hypertensive rats, KC1 treatment did not affect other changes induced by DOCA salt, including increased sodium excretion, SBP, CPV, and plasma ANP values, and cardiac and kidney hypertrophy, and decreased plasma ALD content. A possible explanation for this difference may be attributable to a lower dose of KCl used in the present study. Dietary potassium has been reported to protect against powerful, dose-dependent protection in both normotensive and hypertensive subjects as well as in rats \([22,28]\). Manger et al. observed that a dietary concentration of 2.6% KCl, but not a low (0.7%) dietary concentration of KCl, maintained essentially normal hemodynamics and plasma volume in Dahl salt-sensitive rats fed a high salt diet \([22]\). In our study, 239 mg/kg KCl containing 125 mg/kg potassium was orally administered once daily, which was even lower than the 0.7% dietary concentration of KCl in Manger’s study. With KC1 treatment at a dose of 239 mg/kg, the decrease in death rate induced by DOCA salt may be attributable to reversal of hypokalemia in DOCA salt-induced hypertensive rats. Generally, groups with the higher average pressures also suffered the highest mortality. Therefore, the mechanisms by which KC1 moderated mortality in DOCA salt-induced hypertensive rats independent of BP as well as cardiac and kidney hypertrophy remain unknown.

Over 30,000 varieties of algae plants are known worldwide, most of which live in sea water and some of which have long been used as human foods. The extensive sources of edible marine algae provide the potential for exploring and developing healthy products and medicines. The present study demonstrated for the first time that L-PA, a polysaccharide extracted from the edible brown algae Laminaria japonica cultured in Qingdao sea water in China, dose-dependently prevented the development of DOCA salt-induced hypertension in rats. Interestingly, 500 mg/kg L-PA and 239 mg/kg KCl, both containing equal potassium loading (125 mg K/kg), had similar effects on hypokalemia and the increase in urinary potassium excretion in DOCA salt rats. However, these two forms of potassium supplementation exerted differential effects on sodium excretion, electrolyte metabolism-regulating hormones, hypertension, cardiac and kidney hypertrophy, and mortality in DOCA salt rats. Pretreatment with L-PA at a dose of 500 mg/kg completely prevented the changes induced by DOCA salt, with the exception of further increasing excess sodium excretion through early urinary and late fecal pathways. KCl at a dose of 239 mg/kg did not affect DOCA salt-induced hypertension, with the exception of having inhibitory effects on hypokalemia and death rate. Moreover, L-PA at a dose of 250 mg/kg exerted a reduction in mortality in DOCA salt rats that was similar to 239 mg/kg KCl, an effect that was accompanied by an apparent correction of the imbalance of ANP and ALD, hypertension, and cardiac and kidney indices, as well as a mild increase in urinary and fecal sodium excretion. Our present observations suggest that both
hypokalemia and high blood pressure might play important roles in the remodeling of cardiovascular and renal systems and mortality under conditions of mineralocorticoid and salt excess. L-PA effectively prevented cardiovascular and renal hypertrophy and mortality by reversing both hypokalemia and high blood pressure in DOCA salt-treated rats, whereas KCl augmented the survival of DOCA salt-treated rats likely by ameliorating hypokalemia. The present study, however, did not directly provide an answer to how L-PA induces greater sodium excretion and antihypertensive, cardioprotective, and nephroprotective actions than KCl, but a few explanations may be proposed. Potassium supplementation with L-PA might offer larger cellular entry in exchange for sodium and thus greater antihypertensive effects than KCl [6]. Furthermore, the possibility that L-PA might exert direct regulation on vasoconstriction and sodium resorption in renal tubules cannot be completely excluded. One limitation of the present study is the rather short duration of L-PA treatment. Thus, we cannot exclude the possibility that more prolonged L-PA might have resulted in greater benefit or that the beneficial effects of L-PA might be overcome by the effects of high salt intake and mineralocorticoid excess.

In conclusion, the present study demonstrated for the first time that oral administration of L-PA could effectively prevent the development of hypertension and its subsequent cardiac and renal hypertrophy as well as mortality under conditions of mineralocorticoid and salt excess, effects that are likely associated with reversal of sodium retention and subsequent blood volume expansion. Moreover, the preventive effect of L-PA on DOCA salt-induced hypertension was more effective than equal potassium loading via KCl administration. These data support the hypothesis that L-PA may be a form of potassium supplementation with greater antihypertensive and sodium excretion effects than KCl, with likely benefits for the primary prevention and treatment of hypertension and its cardiovascular sequelae.

References
