Antihypertensive Effects of Chicken Extract against Deoxycorticosterone Acetate-Salt-Induced Hypertension in Rats

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We investigated the antihypertensive effect of Brand’s Essence of Chicken (BEC), a popular chicken extract used as a traditional remedy, using deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Animals were unilaterally nephrectomized, and then separated into a sham-operated group (sham group) and a DOCA-salt-treated group. The latter was further separated into a normal diet group and a BEC (freeze-dried powder, 0.1 w/w%)-containing diet group. Systolic blood pressure of the normal diet group progressively increased in comparison with that of the sham group. The DOCA-salt-induced hypertension was markedly suppressed by feeding a BEC-containing diet. Systolic blood pressure after 5 weeks was 128±2 mmHg in sham group, 181±4 mmHg in the DOCA-salt-treated normal diet group and 139±5 mmHg in the DOCA-salt-treated BEC diet group, respectively. The treatment with DOCA and salt for 5 weeks significantly increased the weights of heart and left ventricle, but these increases were significantly suppressed in the BEC group. When the degree of vascular hypertrophy of the aorta was histochromically evaluated, DOCA-salt-induced increases in wall thickness and wall area of the vessels were significantly decreased by the BEC-feeding. Histopathological renal damage of fibrinoid-like necrosis in glomeruli, thickening of small arteries and tubular dilatation were observed in the DOCA-salt-treated normal diet group, but this damage was efficiently reduced by the BEC-feeding. In addition, BEC-feeding decreased urinary excretion of protein, which was elevated by the treatment with DOCA and salt. Thus, BEC seems to be useful as a prophylactic treatment in the development of hypertension and related tissue injuries.

Key words essence of chicken; deoxycorticosterone-salt; hypertension; vascular hypertrophy; renal injury

In some Asian regions, particularly in Chinese communities in Southeast Asia, Brand’s Essence of Chicken (BEC) is used as a traditional health food for various purposes, including recovery from postpartum sickness, the physical development of athletes, recovery from mental stress and enhancement of the mental efficiency of students.1) However, little is known as to whether the supplement of BEC has a beneficial effect against the development and progression of cardiovascular diseases such as hypertension. The present study was designed to evaluate whether a dietary supplement of BEC has a preventive effect in the development of experimental hypertension induced by deoxycorticosterone acetate (DOCA) and salt, which has been used extensively as a useful model of human hypertension. We report here that dietary BEC can markedly suppress the elevation of blood pressure, cardiovascular hypertrophy and renal damage in DOCA-salt hypertensive rats.

MATERIALS AND METHODS

Materials BEC (70 ml/bottle, Cerebos Pacific, Ltd., Singapore) was freeze-dried, and the resultant powder (about 6 g from 1 bottle of BEC) was mixed with normal commercial diet (NMF, Oriental Yeast Co., Ltd.) at 0.1 w/w% (BEC-containing diet). A feeding of this diet (15—30 g/rat/d) is equal to a dosage of about 0.175—0.35 ml of BEC/rat/d, and corresponds to approximately half to one bottle of BEC/human/day.

Animal Experiments Male Sprague-Dawley rats (6 weeks old) were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the right kidney was removed via a right flank incision. After a 1-week postsurgical recovery period, rats were separated into a sham-operated group and a DOCA-salt group. The latter was further separated into a normal diet group and a BEC-containing diet group. The sham group was fed a normal diet and tap water ad libitum. Rats of the DOCA-salt group were treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg), and 1% NaCl was added to their tap water for drinking. Systolic blood pressure (SBP) was monitored weekly by a tail cuff and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan). After 5 weeks, the animals were placed in metabolic cages, urine was collected overnight, and then all rats were bled from the abdominal aorta to obtain blood samples. The heart, left kidney and aorta were excised and weighed. The thoracic aorta and left kidney were used for morphometric analysis.

Histological Studies The thoracic aorta and the left kidney of each rat was preserved in phosphate-buffered 10% formalin, after which the tissues were chopped into small pieces, embedded in paraffin, cut at 4 μm and stained with hematoxylin and eosin. Three different cross sections of each vessel placed under a microscope were photographed, and the vessel wall area and thickness were determined using an image analyzer (AE-6905C, ATTO, Tokyo).

Statistical Analysis All values were expressed as mean±S.E.M. For statistical analysis, we used one-way ANOVA followed by Tukey-Kramer multiple comparison tests. For all comparisons, differences were considered significant at p<0.05.
RESULTS

Effects of BEC Feeding on Body and Heart Weights of DOCA-Salt Hypertensive Rats  At the end of the experimental period (5 weeks), a gain in body weight of DOCA-salt rats fed a normal diet was significantly less than that in sham-operated rats, while BEC feeding led to the recovery of body weight loss. The heart weight-to-body weight ratio and the left ventricular weight-to-body weight ratio were markedly increased by the treatment with DOCA and salt for 5 weeks, but BEC feeding produced significant lowering effects on these alterations (Table 1).

Effects of BEC Feeding on the Blood Pressure of DOCA-Salt Hypertensive Rats  At the beginning of the experiment, the SBP of the sham, DOCA-salt-treated normal-diet and DOCA-salt-treated BEC-diet groups were 113±2, 113±3 and 112±2 mmHg, respectively. As shown in Fig. 1, SBP in the normal-diet group was progressively elevated by the treatment of DOCA and salt. After 1 week of treatment, there was a significant increase in SBP of the normal-diet group compared with that of the sham group, and thereafter this hypertensive effect was gradually accelerated. The elevation of SBP in rats fed the BEC-containing diet was much less than that of the normal-diet group, and a significant lowering effect on DOCA-salt-induced hypertension was observed throughout 1 to 5 weeks. SBPs after 5 weeks were 128±2 mmHg in the sham group, 181±4 mmHg in the DOCA-salt-treated normal-diet group and 139±5 mmHg in the DOCA-salt-treated BEC-diet group, respectively (Fig. 1).

In preliminary experiments, when the BEC-containing diet was given to sham rats (n=3) for 5 weeks, changes in SBP of these animals (126±3 mmHg at 5 weeks) were similar to those of the normal-diet group.

Effects of BEC Feeding on the Vascular Hypertrophy of DOCA-Salt Hypertensive Rats  Figure 2 shows typical examples of light micrographs of representative cross sections of the aorta of the 3 groups of animals. The wall thickness of the aorta from the DOCA-salt rat fed normal diet was much greater than that seen in the sham-operated animal. BEC feeding clearly ameliorated the above structural change induced by DOCA-salt treatment. The data on morphometric

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n=6)</th>
<th>Normal diet (n=6)</th>
<th>BEC diet (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>393±7</td>
<td>339±14*</td>
<td>385±12†</td>
</tr>
<tr>
<td>HW/BW (g/kg)</td>
<td>2.76±0.05</td>
<td>3.71±0.16**</td>
<td>3.12±0.10††</td>
</tr>
<tr>
<td>LVW/BW (g/kg)</td>
<td>1.82±0.04</td>
<td>2.69±0.14**</td>
<td>2.20±0.08††</td>
</tr>
</tbody>
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Values are mean±S.E. *p<0.05; **p<0.01, compared with sham group. †p<0.05; ††p<0.01, compared with DOCA-salt-treated normal diet group. BW, body weight; HW, heart weight; LVW, left ventricular weight; DOCA, deoxycorticosterone acetate.
analysis are summarized in Table 2. There were great increases in the wall thickness and wall area, both of which represent a degree of vascular hypertrophy, in the DOCA-salt-treated normal-diet group compared with the sham-operated group. BEC feeding efficiently suppressed the DOCA-salt-induced vascular hypertrophy.

Effects of BEC Feeding on the Urinary Protein Excretion of DOCA-Salt Hypertensive Rats  The level of urinary excretion of protein in the DOCA-salt-treated normal-diet group was markedly elevated compared with that in sham group, and the elevation was significantly suppressed by the BEC feeding (Fig. 3).

Effects of BEC Feeding on the Histological Renal Damage of DOCA-Salt Hypertensive Rats  Figure 4 shows typical examples in renal tissues of sham rat and DOCA-salt rats fed normal or BEC diet. Histological examination of the kidney of normal diet-fed DOCA-salt rats revealed tissue injury characterized by tubular dilatation containing proteinaceous casts, thickening of small arteries and fibrinoid-like necrosis in glomeruli. Such damage was effectively ameliorated by the feeding of BEC diet.

DISCUSSION

In the present study, we demonstrated that BEC feeding efficiently suppressed the development of hypertension induced by DOCA and salt. Cardiovascular hypertrophy, renal tissue injury and elevation of urinary protein excretion, all of which are well known markers of hypertensive diseases, were also significantly ameliorated by the BEC feeding. Since BEC feeding was started at the prehypertensive stage, our results suggest that treatment with BEC may be a prophylactic regimen against the development of hypertension.

DOCA-salt hypertensive rats have been used extensively

<table>
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<tr>
<th>Group</th>
<th>Wall thickness (μm)</th>
<th>Wall area (mm²)</th>
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</thead>
<tbody>
<tr>
<td>Sham (6)</td>
<td>101±3</td>
<td>0.451±0.021</td>
</tr>
<tr>
<td>DOCA-salt (normal diet) (6)</td>
<td>139±7**</td>
<td>0.724±0.034**</td>
</tr>
<tr>
<td>DOCA-salt (BEC diet) (5)</td>
<td>119±4†</td>
<td>0.600±0.027†*</td>
</tr>
</tbody>
</table>

Values are mean±S.E. *p<0.05; **p<0.01, compared with sham group. †p<0.05, compared with DOCA-salt-treated normal diet group. DOCA, deoxycorticosterone acetate.
as a useful model of human hypertension. It is known that increased sympathetic nerve activity and various humoral factors, including vasopressin, play an important role in the pathogenesis of DOCA-salt hypertension.\textsuperscript{2} Recent studies reported that the synthesis or release of endothelin-derived relaxing factor (EDRF) and responses to EDRF are impaired in this model of hypertension.\textsuperscript{4,5} In addition, we found the hypotensive effect of FR 139317 or ABT-627, both of which are selective endothelin ET\textsubscript{A} receptor antagonists, in DOCA-salt hypertensive rats, thereby suggesting that endothelin and ET\textsubscript{A} receptors are closely involved in the maintenance of DOCA-salt hypertension.\textsuperscript{6,7} The mechanisms by which BEC prevents the development of DOCA-salt hypertension are unclear, but feeding with BEC for 5 weeks may affect the activities of the above neural and/or humoral factors.

Hypertension, in humans and in experimental animals, is often accompanied by vascular hypertrophy.\textsuperscript{7} In our study, morphological analysis of aorta in DOCA-salt rats showed arteriosclerotic changes, with significant increases in wall thickness and wall area. There was also a thickening of small arteries in histological studies of the kidney. Since hypertension itself is a main causal factor of hypertrophy, it is possible that the blunting of the rise in blood pressure of BEC-fed rats is associated with the absence of vascular hypertrophy. On the other hand, a previous study\textsuperscript{8} using experimental hypertensive rats demonstrated that the attenuation of vascular hypertrophy was observed after captopril treatment at a dose which did not lower blood pressure effectively. Moreover, vasorelaxing agents such as hydralazine failed to suppress vascular hypertrophy, even at a hypotensive dose.\textsuperscript{9} These observations suggest that factors other than blood pressure per se are involved in the vascular hypertrophy. In order to clarify whether BEC selectively prevents vascular hypertrophy, the effects of a lower dose or non-hypotensive dose should be examined.

The cardiac hypertrophy in DOCA-salt hypertensive rats has been well documented.\textsuperscript{10} In the present study, both the heart weight-to-body weight ratio and the left ventricular weight-to-body weight ratio were markedly increased by the treatment with DOCA and salt, but BEC feeding produced significant lowering effects on these alterations. The view stated above also may be applicable to the relationship between the changes in blood pressure and the cardiac hypertrophy.

BEC is an extract of chicken muscle which is processed with water under high temperature conditions. It contains mainly protein, amino acids and peptides such as carnosine. Carnosine is a dipeptide composed of \(\beta\)-alanine and \(\L\)-histidine, and is found in high concentrations in BEC. A recent study demonstrated endothelin-independent vasodilatory actions of this peptide in rat aortic rings.\textsuperscript{11} This peptide possesses antioxidant and free radical scavenging functions, which may contribute to its hypotensive action.\textsuperscript{12} Based on findings that a production of oxygen free radicals such as superoxide anion is enhanced in vascular tissues from hypertensive animals, and that oxidative stress is closely related to the development of hypertension.\textsuperscript{13—15} Most recently, we found the hypotensive effect of carnosine in DOCA-salt-treated animals (Y. Matsumura et al., unpublished data, 2001). Taken together, carnosine may be a possible candidate as an active component for the antihypertensive activity of BEC.

In conclusion, we clearly demonstrated the antihypertensive action of BEC. Further studies to identify the mechanism underlying this antihypertensive activity are in progress in our laboratory.

REFERENCES