Effects of Chlorthalidone on Ventricular Hypertrophy in Deoxycorticosterone Acetate–Salt Hypertensive Rats

Antonio M. Cabral, Francine B. Carvalhinho, Elisardo C. Vasquez, Maria A. Cicilini

Abstract  Diuretics have been the mainstay of long-term treatment of hypertension, but there is no evidence suggesting that diuretics may be effective in reducing cardiac hypertrophy associated with hypertension. Thus, the present study was carried out to elucidate if long-term treatment with chlorthalidone (8 mg per animal per day added to food) affects the development of and reverses the ventricular hypertrophy in deoxycorticosterone acetate (DOCA) (8 mg/kg SC twice a week)–salt hypertensive rats. Chlorthalidone was given to one group during all 20 days of DOCA administration (preventive regimen) and to another group 20 days after DOCA treatment was initiated until the 40th day (therapeutic regimen). Chlorthalidone was found to reduce or prevent the development of ventricular hypertrophy, as assessed by a reduction in ventricular mass and cardiac protein as well as arterial hypertension. Both chlorthalidone regimens prevented the increase or induced a significant decrease in the plasma concentration of sodium and in cardiac sympathetic tone, which were both increased in DOCA-salt–treated rats. These data provide evidence that long-term chlorthalidone treatment is effective in preventing or reducing ventricular hypertrophy along with arterial hypertension. However, whether this is due to a reduction in plasma sodium or other additional mechanisms, such as a reduction in cardiac sympathetic tone, remains to be determined. (Hypertension. 1994;23[suppl I]:I-180-I-184.)

Key Words  • chlorthalidone • diuretics • heart hypertrophy • hypertrophy, left ventricular • hypertension, mineralocorticoid • cathepsin B

Although cardiac hypertrophy has been associated with arterial hypertension, in recent years many investigators have demonstrated that this associated ventricular hypertrophy is a phenomenon of multifactorial origin whose development is not solely dependent on an increased pressure load but also on local growth factors and cardioadrenergic activity. Other investigators have suggested that a high-sodium intake in some way contributes to the initiation and maintenance of hypertension and may be involved in cardiac hypertrophy. There is evidence of a relation between hypertension–induced cardiac hypertrophy and sodium intake, whose dietary increase leads to an increase in activity of the sympathetic nervous system that has been shown to be a determinant of left ventricular hypertrophy. Lindpaintner and Sen demonstrated the reversal of cardiac hypertrophy by dietary sodium restriction in renovascular hypertensive rats despite the persistence of hypertension. Similar observations were also made in patients with essential hypertension by Schmieder et al, who demonstrated that sodium homeostasis interferes with the development of left ventricular hypertrophy. Thus, sodium depletion by restriction in dietary sodium or diuretic administration should result in a reduction of cardiac hypertrophy, independent of its antihypertensive effect. In this respect, diuretics frequently have been used in the long-term treatment of hypertension, but these drugs usually did not reduce cardiac hypertrophy except in isolated cases. Since these initial studies were carried out, a body of evidence has been and is being accumulated showing that diuretics may indeed be effective in reducing the cardiac hypertrophy associated with arterial hypertension. The reasons for the discrepancies among these studies have not been fully clarified.

Thus, the present study was carried out to determine whether, in deoxycorticosterone acetate (DOCA)–salt hypertensive rats, chlorthalidone treatment reverses the development of ventricular hypertrophy and whether coadministration of chlorthalidone along with DOCA prevents or reduces the development of ventricular hypertrophy. In addition to assessing the level of ventricular hypertrophy by weight, we measured proteolytic activity and protein content of the ventricles of DOCA-salt–treated rats to determine if chlorthalidone treatment also affects these variables along with changes in fluid intake, urinary volume, and urinary and plasma sodium and potassium concentrations.

Methods  Male 45-day-old Wistar rats were uninephrectomized under ether anesthesia. Four days later the animals were treated with either DOCA (Sigma Chemical Co, St Louis, Mo; 8 mg/kg SC) or vehicle (soybean oil, 0.25 mL per animal). This treatment was repeated twice a week for 20 days in one group or for 40 days in another group. Rats treated with vehicle were provided either with plain drinking water (normal control) or, as all DOCA-treated animals, with drinking water containing 1% NaCl and 0.63% KCl (salt control). Simultaneously with DOCA treatment, half of the rats were given chlorthalidone (CIBA-GEIGY, Basel, Switzerland; 8 mg per animal per day added to food) during all 20 days of DOCA treatment (preventive regimen), and another group received chlorthalidone 20 days after DOCA treatment was initiated until the 40th day (therapeutic regimen). Fluid intake and urinary volume were mea-
Influence of Chlorthalidone Preventive and Therapeutic Regimens on Cardiovascular Parameters and Autonomic Tone in Deoxycorticosterone Acetate–Salt Rats

<table>
<thead>
<tr>
<th></th>
<th>Preventive Regimen</th>
<th>Therapeutic Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Control</td>
<td>Salt Control</td>
</tr>
<tr>
<td></td>
<td>(n=17)</td>
<td>(n=18)</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>110±2</td>
<td>107±2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>340±4</td>
<td>341±5</td>
</tr>
<tr>
<td>Vagal tone, bpm</td>
<td>107±8</td>
<td>105±6</td>
</tr>
<tr>
<td>Sympathetic tone, bpm</td>
<td>37±4</td>
<td>31±3</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>252±6</td>
<td>247±7</td>
</tr>
</tbody>
</table>

DOCA indicates deoxycorticosterone acetate; Chlor, chlorthalidone; and bpm, beats per minute. Vagal tone was measured as increase in heart rate caused by 1 mg/kg IV atropine methyl nitrate; sympathetic tone as decrease in heart rate caused by 2 mg/kg IV propranolol. Measurements were performed after DOCA treatment (8 mg/kg SC) twice weekly for 20 and 40 days. Chlorthalidone was given daily (8 mg PO simultaneously with DOCA in group treated for 20 days; in 40-day group, chlorthalidone was given daily over the last 20 days of DOCA treatment. Each value is mean±SEM.

†P<.05, ‡P<.01 compared with control values.
§P<.05, †P<.01 compared with DOCA-salt.

Assured daily over 24 hours for both treatment periods. Plasma samples (collected at the end of the treatment period) and urine were collected and frozen for estimation of sodium and potassium concentrations, which were measured in a flame photometer (Corning-EEL-100/13681, Evans Electroelenium Ltd, Essex, England).

At the end of the treatment period, the femoral artery and jugular vein were cannulated with a polyethylene catheter (PE-50 with tapered end filled with 40 U/mL heparinized saline) with rats under ether anesthesia. Six hours later, mean arterial pressure and heart rate measurements were made in conscious animals using a pressure transducer (1280C, Hewlett-Packard Co, Palo Alto, Calif) coupled to a polygraph (7754B, Hewlett-Packard). The animals in their original cages were allowed to acclimatize to the laboratory for approximately 1 hour while they were being connected for measurements. Subsequently, the levels of vagal and sympathetic drives to the heart were assessed by injections of atropine methyl nitrate (1 mg/kg IV) and propranolol (2 mg/kg IV), respectively. The level of vagal tone was measured as the increase in heart rate 10 minutes after injection of atropine. After full recovery of mean arterial pressure and heart rate basal values 2 hours after atropine, sympathetic tone to the heart was measured as the decrease in heart rate obtained 10 minutes after propranolol administration.

After hemodynamic variables had been recorded, animals were anesthetized with ether, and portions of blood were collected for estimation of plasma sodium and potassium concentrations. The heart was removed, and the right and left ventricles (including the septum) were separated and weighed. From each group, five to seven left ventricles plus septa and five to seven right ventricles were removed for biochemical analysis. The tissues were then rinsed with Ringer's lactate buffer and maintained on ice until dissection. They were then homogenized using an ice-cold Potter-Elvehiem glass homogenizer. The homogenates were then centrifuged at 1000g for 15 minutes, and the supernatants were saved for biochemical analysis. The total protein content was estimated (see Reference 21). The Barretti method was used for assaying cathepsin B activity by using the amount of free p-nitroaniline liberated from the acetyl-Phe-Arg-p-nitroanilide substrate (synthesized by Dr A.C.M. Paiva, Paulista School of Medicine, São Paulo, Brazil) as an index of cathepsin B activity. The activity was expressed as micromoles of p-nitroaniline released per hour.

Statistical analysis of data was performed by a two-way repeated-measures analysis of variance followed by a post hoc protected t test (Tukey's test). All experiments were conducted in conformity with the international guiding principles for biomedical research involving animals (CIOMS).

Results

Mean arterial pressure was found to be significantly higher (P<.01) higher (35% and 46% in the 20- and 40-day groups, respectively) in the DOCA-salt rats than in normal control and salt control animals (Table). The basal heart rate of DOCA-salt rats was significantly higher (9% and 6% in the 20- and 40-day groups, respectively) than in both control groups. The chlorthalidone preventive regimen significantly reduced the development and level of hypertension in the DOCA-salt rats. Although the chlorthalidone therapeutic regimen significantly reduced the DOCA-salt-induced hypertension (P<.01), the values still were higher than those observed in control groups (P<.05). Coadministration of chlorthalidone (preventive) significantly reduced the increase in heart rate caused by DOCA-salt, and when given after (therapeutic) DOCA-salt treatment returned heart rate back to control levels (Table).

Compared with normal and salt control animals, sympathetic tone (heart rate after propranolol) in the DOCA-salt–treated rats was found to be significantly increased by 30% and 55%, respectively, after the 20th and 40th day. Vagal tone (heart rate after atropine) was approximately 59% and 49% lower in DOCA-salt–treated animals after the 20th and 40th day compared with both control groups, respectively. Chlorthalidone coadministration with DOCA failed to prevent the increase in sympathetic tone, although this was not as large as with DOCA-salt alone. Furthermore, the addition of chlorthalidone in rats made hypertensive by DOCA-salt pretreatment (therapeutic) reduced the sympathetic tone to the heart to control levels. However, the vagal tone was only partially recovered in both regimens (preventive and therapeutic, see the Table).

During chlorthalidone treatment, liquid intake, urinary volume, and urinary and plasma sodium and
Bar graphs show effect of chlorthalidone treatment on ratios of left ventricular (LV) and right ventricular (RV) weights to body weight, protein content, and cathepsin B activity of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The reaction mixture to assay cathepsin B activity contained enzyme (25 mL supernatant); 225 µL of 0.05 mol/L sodium acetate buffer, pH 5.7, contained 1 mmol/L substrate, 1.0 mmol/L EDTA, 1 mmol/L dithiothreitol, and 0.1% Brij 35 (final concentration). The reaction was carried out for 25 minutes at 37 °C and stopped by addition of 0.5 mL of 10% trichloroacetic acid (wt/vol). The reaction mixture was centrifuged at 500g for 10 minutes; p-nitroaniline in the supernatant was determined colorimetrically (see Reference 23). Blank samples were prepared by reversing the order of addition of enzyme and trichloroacetic acid. Controls in the presence of kallikrein were made up for estimation of the effect of kallikrein activity (less than 5%). Each bar is mean±SEM. *P<.05, **P<.01 compared with control values (normal control and salt control); *P<.05, **P<.01 compared with DOCA-salt. Measurements were performed after DOCA treatment (8 mg/kg SC) twice a week for 20 (n=17) and 40 (n=18) days. Chlorthalidone was given daily (8 mg PO) simultaneously with DOCA in the 20-day group. In the 40-day group, it was given daily over the last 20 days of DOCA treatment.

Potassium concentrations were measured. The daily values of fluid intake and urinary volume observed in normal control rats increased from 34 and 8 mL to 55 and 23 mL, respectively, in salt control animals and to 70 and 43 mL, respectively, in the DOCA-salt group. Coadministration of chlorthalidone with DOCA maintained fluid intake and urinary volume at levels similar to those in the salt control rats, whereas addition of chlorthalidone to DOCA rats returned these levels to those observed in the salt control rats.

Both chlorthalidone regimens prevented the rise of or returned the significant increase in plasma sodium concentration (145±1 mEq/L) in the DOCA-salt groups to the levels observed in the control groups (140±1 mEq/L). Urinary excretion of sodium was significantly increased in the salt control group (220±6 mEq/L) and was at a level similar to that in the DOCA or DOCA plus chlorthalidone treatment groups compared with the normal control group (145±5 mEq/L). In all groups, ie, those receiving a sodium intake overload, urinary potassium was significantly reduced. Plasma potassium concentration was found not to differ from that of normal controls (4.4±0.2 mEq/L) in DOCA-salt and salt control rats; however, in both chlorthalidone regimens, plasma potassium concentration was significantly reduced (3.9±0.2 mEq/L).
DOCA-salt treatment resulted in a significant increase of the ratio of left ventricular (LV) and right ventricular (RV) weight to body weight at the 20th day (LV: from 2.2±0.03 mg/g in normal control to 3.09±0.05 mg/g in DOCA-salt groups; RV: from 0.55±0.01 mg/g in normal control to 0.62±0.02 mg/g in DOCA-salt groups) and 40th day (LV: from 2.36±0.05 mg/g in normal control to 3.40±0.13 mg/g in DOCA-salt groups; RV: from 0.51±0.02 mg/g in normal control to 0.61±0.03 mg/g in DOCA-salt groups) (Figure, top). The values obtained in the salt control group were not different from those obtained in the normal control group. Both chlorthalidone regimens partially reduced the development of hypertension (coadministration: LV, 2.50±0.06 mg/g and RV, 0.57±0.01 mg/g, P<0.01) or returned the hypertrophy in both ventricles close to their normal time-matched values (after chlorthalidone: LV, 2.6±0.08 mg/g and RV, 0.54±0.02 mg/g, P<0.05 compared with controls). Dry ventricular weights were not shown here because the results were the same as those observed with wet weights. As the muscles hypertrophied, the amount of protein per unit weight changed significantly (Figure). In DOCA-salt–treated rats, the protein content (milligrams per gram of wet tissue) of the LV had increased to 257±5 compared with 183±10 in the normal controls, whereas for the RV it had increased to 193±6 compared with 164±9 in the normal controls. As the hypertrophy was prevented or reversed by chlorthalidone treatment, there was a significant loss of proteins compared with the DOCA-salt group, and the values became similar to those of the normal and salt control groups (Figure).

There was no difference in the cathepsin B activity showed by the LV (0.87±0.09 U/mg protein) and RV (0.98±0.06 U/mg protein) of normal controls and salt controls; however, it was significantly decreased to 0.54±0.07 and 0.55±0.08 U/mg protein (P<0.01) in the 20- and 40-day DOCA-salt groups, respectively. The chlorthalidone preventive regimen was effective in significantly increasing cathepsin B activity to 1.77±0.27 and 2.73±0.09 U/mg protein in the LV and RV; the values observed in the therapeutic regimen were 1.23±0.11 and 1.41±0.1 U/mg protein, respectively (Figure, bottom).

**Discussion**

In the DOCA-salt–treated rats, a 20-day period of hypertension was sufficient to induce a significant increase in ventricular mass, and this was found to be associated with a significant decrease in the activity of the lysosomal protease cathepsin B in LV and RV homogenates. No such changes were observed in the normal and salt control groups. Chlorthalidone coadministration (preventive) or chlorthalidone (therapeutic) after 20 days of DOCA pretreatment was found to prevent or reduce the induced hypertension and related ventricular hypertrophy, compared with time-matched controls. These changes were associated with decreases in the DOCA-induced increase in cardiac sympathetic drive, heart rate, and plasma sodium. In addition, chlorthalidone was also found to significantly increase the activity of the lysosomal protease cathepsin B.

It is at present difficult to determine if one or a combination of the above variables altered by chlorthalidone treatment is responsible for the decrease of ventricular hypertrophy. However, in the case of changes in tissue cathepsin B activity, these results suggest that a decreased tissue cathepsin B activity is implicated in the development of hypertrophy, which has also been suggested for all the intracellular proteolytic systems, even though early studies had suggested that steroid hormones might promote proteolysis. However, only a slight increase or decrease in the rate of proteolysis in the heart has been reported for steroids. Although the exact mechanism of proteolysis regulation by steroid hormones in the heart is not yet known, these studies indicate that in DOCA-salt hypertension, the increase of cardiac protein is a consequence of a decreasing protein degradation. Taken together, these results suggest that if chlorthalidone treatment decreases the ventricular mass by increasing protein degradation, then the latter possibility occurs via a simple upregulation of protease activity; ie, the drug exerts a great influence on the cathepsin. The current results therefore demonstrate that diuretic treatment does decrease or prevent the development of ventricular hypertrophy. However, the precise mechanism remains to be determined.

**Acknowledgments**

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP), and Fundação Cecíliano Abel de Almeida (FCAA). We would like to thank Dr Andrew G. Ramage, from the Royal Free Hospital, University of London, for his suggestions to the manuscript.

**References**

16. Tarazi RC, Sen S, Fouad FM, Wicker P. Regression of myocardial hypertrophy: conditions and sequence of reversal in hypertensive...
Effects of chlorthalidone on ventricular hypertrophy in deoxycorticosterone acetate-salt hypertensive rats.
A M Cabral, F B Carvalhinho, E C Vasquez and M A Cicilini

Hypertension. 1994;23:I180
doi: 10.1161/01.HYP.23.1_Suppl.I180

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/23/1_Suppl/I180

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/