Hypotensive and Uric Acid-Retaining Effects of Trichlormethiazide under Dietary Sodium Restriction in Spontaneously Hypertensive Rats

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Abstract—In order to evaluate both the hypotensive and uric acid-retaining effects of thiazide diuretics in an animal model with hypertension, the effects of trichlormethiazide were studied using spontaneously hypertensive rats (SHR) under dietary sodium restriction. Trichlormethiazide was dosed daily for two weeks at 0.05, 0.5, 3 and 10 mg/kg, p.o. All doses caused obvious natriuresis, while an increase of urine volume was observed only at 3 and 10 mg/kg. The hypotensive effect, which was estimated at day 6 and 13, was recognized at doses of more than 0.5 mg/kg. At the end of the dosing, the hematocrit value of all medicated groups rose, and both the uric acid excretory capacity, estimated by the clearance values of inulin and uric acid, and the plasma potassium level clearly decreased at 3 and 10 mg/kg. A detailed study using a dose of 3 mg/kg showed shifts of the cumulative sodium and potassium balances to negative directions against the control group. Thus, trichlormethiazide-treated SHR under dietary sodium restriction showed both a hypotensive effect which might be due to the natriuresis and a tendency toward undesirable side effects such as hypokalemia and hyperuricemia. As there is no practical method in animal studies for simultaneously proving hypotensive and uric acid-retaining effects of diuretic antihypertensives, the findings of the present study might aid in the evaluation of diuretics more useful than the thiazides.

An important problem in the use of thiazide diuretics as an antihypertensive agent is the undesirable side effect from renal handling of uric acid, which results in hyperuricemia (1, 2). The problem is generally known in clinical fields, but has not been adequately clarified in pharmacological studies with commonly used experimental animals because of species differences in the metabolism and excretion of uric acid (3). This has been restraining the development of useful diuretic antihypertensives which have less harmful aftereffects than thiazide diuretics.

We tried to devise an experimental procedure employing commonly used experimental animals to evaluate both the hypotensive and the uric acid-retaining effects of thiazide diuretics. We previously reported that the characteristics of diuretics acting on uric acid retention could be understood in renal clearance studies using normotensive rats (4, 5) and showed that consecutive administration of trichlormethiazide led to much greater diuresis with deterioration of the uric acid excretory capacity when dietary sodium was restricted than when a normal laboratory diet was given (4). A clinical study has also shown that the combination of a low sodium diet and thiazide diuretics induces hyperuricemia due to decreased uric acid clearance (6).

Diuretics together with dietary sodium restriction are often prescribed for patients with hypertension (7–9). However, the restriction of dietary sodium content has not yet been positively applied to evaluate the activity of diuretic antihypertensives using animal models with hypertension. This may
be due to the fact that the animals developing hypertension under high sodium intake are useful for determining the hypotensive effect of diuretics.

The present study used spontaneously hypertensive rats (SHR), which do not need a high sodium diet to maintain a hypertensive state. Dietary sodium was restricted and the diuretic, hypotensive and uric acid-retaining effects of a typical thiazide diuretic, trichlormethiazide, were examined. The animals showed various effects of trichlormethiazide as well documented in clinical studies, and we concluded that our procedure should be useful for evaluating diuretic antihypertensives.

**Materials and Methods**

**Animals and drug treatment:** Male CRJ-SHR of 15–16 weeks of age were used in all studies. They were housed in individual stainless steel metabolism cages (Shinano Seisakusho, Tokyo) in a room maintained at a constant temperature (22–23°C) and humidity (50–60%) and illuminated automatically from 8 AM to 8 PM.

The animals were provided with a low sodium diet (Oriental Yeast Co., Tokyo) and tap water from the beginning of drug administration. The diet was a solidified mixture of corn starch (38%), milk casein (25%), α-starch (10%), cellulose powder (8%), linoleic salad oil (6%), mineral mixture (6%), granulated sugar (5%) and a vitamin mixture (2%). The sodium and potassium contents of the diet (2.6 and 179.8 mEq/kg of food, respectively) were assayed by Japan Food Research Laboratories, Tokyo, while those of the drinking water (0.7 and 0.07 mEq/l of water, respectively) were determined using an atomic absorption spectrophotometer (Toshiba-Beckman, Tokyo, Model NF-1B) in our laboratory.

Trichlormethiazide (Shionogi, Osaka) was suspended in 1% gum arabic solution and orally administered at 2 ml/kg of body weight. Control rats were given only 1% gum arabic solution.

**Experimental protocol and analysis:** The time table of the experiment is given in Fig. 1. Trichlormethiazide administrations were continued for 14 days at 5 PM every day (day 0–13). Twenty-four-hour urine between daily drug treatments was collected from day 1 to 5 and from day 7 to 12. On days 0, 6 and 13, between 10 AM and 3 PM, the systolic blood pressure was measured indirectly using a tail pulse manometer (Natsume, Tokyo, Model KN 210) without anesthesia. Before the determination of blood pressure, the animals were warmed for 15 min at 37°C in order to readily detect the arterial pulse. Then, the rats were placed in a restraining holder maintained at 26°C and allowed to rest and acclimate during repeated measurements of blood pressure. After constant recordings of blood pressure, the average of ten measurements was calculated.

On the final day (day 14), a clearance study was performed in order to determine the changes in the renal handling of uric acid in accordance with the method described previously (5). An infusion medium was given at 2 ml/hr, and then three 20-min urine samples were collected and blood samples were collected at the midpoint. Data are shown as the average values of the three clearance periods. Immediately after cannulation for the clearance study, arterial blood (0.3 ml) was sampled to measure the hematocrit values and plasma electrolyte levels.

Such a clearance study and measurements of the hematocrit and plasma electrolyte concentrations were also performed on day 7 in an experiment to evaluate the detailed effects of trichlormethiazide at a daily dose of 3 mg/kg, p.o., as indicated in Fig. 1 by the dotted line. In the experiments, daily food and
water intake and urinary electrolyte outputs were measured to determine the changes of sodium and potassium balances. Sodium and potassium concentrations in plasma and urine were determined using an atomic absorption spectrophotometer (Toshiba-Beckman, Tokyo, Model NF-1B). Hematocrits were measured using heparinized hematocrit tubes.

The abbreviations used in this paper are:

- $P_{Na(K)}$, plasma sodium (potassium) concentration;
- $C_{In}$, inulin clearance;
- $P_{UA}$, plasma uric acid concentration;
- $U_{UA}$, urine-excreted amount of uric acid;
- $C_{UA}$, uric acid clearance;
- and $FE_{UA}$, fractional excretion of uric acid ($C_{UA}/C_{In}$). Statistical comparisons between the control and experimental groups were made utilizing Student’s $t$-test.

**Results**

Experiments were first done to survey the effects of trichlormethiazide in SHR fed a low sodium diet. The agent was given daily at doses of 0.05, 0.5, 3 and 10 mg/kg for 14 consecutive days.

As shown in Fig. 2, the trichlormethiazide treatment produced a gradual reduction in body weight according to the dose. The reduction was apparent even at 0.05 mg/kg, the lowest dose used in this study. On the other hand, a diuretic effect was observed at doses of more than 3 mg/kg and was greater at 10 mg/kg than 3 mg/kg. Urinary sodium output markedly decreased due to the dietary sodium restriction, but all groups given the agent excreted sodium much more than the control group.

Maintaining of SHR with a low sodium diet slightly lowered the systolic blood pressure as indicated in Table 1, and the hypotensive response to trichlormethiazide...
was observed at doses of more than 0.5 mg/kg. The correlation coefficient between the blood pressure and the dose of trichlormethiazide was $-0.62$ (n=34, P<0.01) at day 6 and $-0.69$ (n=34, P<0.01) at day 13. Although the blood pressure, which was reduced at day 6, tended toward recovery, the reduction at day 13 was still appreciable.

Table 1. Changes of systolic blood pressure in trichlormethiazide-treated SHR fed a low sodium diet

| Treatment period | 0       | 0.05    | 0.5     | 3       | 10      |
|------------------|---------|---------|---------|---------|---------|
|                   | No. of rats |        |         |         |         |
| Systolic blood pressure (mmHg) |         |         |         |         |         |
| Day 0            | 206±4   | 207±4   | 208±3   | 210±4   | 208±3   |
| Day 6            | 190±3   | 188±3   | 175±3** | 170±3** | 166±4** |
| Day 13           | 193±3   | 189±3   | 182±2** | 180±4** | 170±2** |

The results are expressed as means±S.E. **P<0.01 compared with the control group.

Table 2. Changes of hematocrit, plasma levels of sodium and potassium, and renal handling of uric acid in trichlormethiazide-treated SHR fed a low sodium diet

| No. of rats | 0       | 0.05    | 0.5     | 3       | 10      |
|-------------|---------|---------|---------|---------|---------|
| Hematocrit (%) | 48.1±0.5 | 50.2±0.8* | 52.5±0.5** | 52.2±1.2** | 52.5±0.7** |
| $P_{Na}$ (mEq/l) | 141.7±1.2 | 139.1±1.6 | 136.9±1.8* | 138.6±1.2 | 136.1±1.5* |
| $P_{K}$ (mEq/l) | 3.71±0.08 | 3.82±0.13 | 3.64±0.12 | 3.28±0.11** | 3.07±0.11** |
| $C_{In}$ (ml/kg min) | 10.34±0.87 | 8.50±0.48 | 8.44±0.82 | 7.08±0.87* | 6.03±0.63** |
| $P_{Na}$ (µg/ml) | 3.0±0.1 | 3.6±0.3* | 3.8±0.4 | 5.5±1.0* | 5.0±0.9* |
| $U_{Na}$(µg/k g min) | 10.7±0.6 | 9.9±0.7 | 9.0±0.9 | 6.6±0.9** | 6.3±1.1** |
| $C_{In}$ (ml/kg min) | 3.63±0.27 | 2.84±0.30 | 2.68±0.44 | 1.44±0.35** | 1.51±0.39** |
| $F_{E_{Na}}$ | 0.38±0.02 | 0.34±0.03 | 0.30±0.03 | 0.19±0.03** | 0.23±0.04** |

Trichlormethiazide was administered daily for 2 weeks. Results are expressed as means±S.E. *P<0.05, **P<0.01, compared with the control group.

Next, the influence of daily administration of trichlormethiazide at 3 mg/kg on sodium and potassium balances in SHR fed a low sodium diet was determined. Balance studies were performed for 5 consecutive days at the earlier stage (day 1–5) and for 6 consecutive days after the first measurement of blood pressure (day 7–12). The changes of food and water intakes, urine volume and water balance are given in Fig. 3. Upon treatment with trichlormethiazide, food intake was suppressed, while both daily urine volume and water intake increased. The percentage of the fluid excretion to fluid intake also increased in the drug-treated group compared with the control animals.

The balance data for sodium and potassium are given in Fig. 4. Daily sodium and potassium intakes were calculated as the sum of their amounts in the diet and drinking water. Sodium intake did not change in the treatment with trichlormethiazide because of the increased amount of drinking water, but potassium intake was lower in the trichlor-
methiazide-treated group than the control one. On the other hand, daily urinary sodium outputs were greater in the drug-treated group than in the control, while there was no significant change of daily urinary potassium outputs between the two groups. Cumulative balances of sodium and potassium were calculated as the difference between the intake and output amounts. To determine the cumulative balance at day 6, the intake and urinary output were calculated as the mean values of days 5 and 7 because the animals had been removed from their metabolism cages on day 6 for the blood pressure measurements. Daily sodium restriction alone produced a slight negative state of cumulative sodium balance and progressively enhanced the positive state of cumulative potassium balance. Treatment with trichlormethiazide obviously shifted the cumulative sodium balance to a more negative state against the control and lowered the positive state of the cumulative potassium balance. The negative shift of the sodium balance due to the drug was marked at the earlier stage and the difference was maintained during the dosing, while the shift of the potassium balance was gradual.

Changes in the hematocrit, plasma sodium and potassium levels and renal uric acid excretory capacity were studied at day 7 and 14 in the treatment with 3 mg/kg day of trichlormethiazide (Fig. 5). The hematocrit increased and the plasma sodium and potassium levels decreased at day 7. In the clearance study, decreases of inulin clearance and uric acid clearance were observed both at day 7 and 14, but the decrease of fractional excretion of uric acid was detected only at day 14.

Discussion
This study evaluated the influence of trichlormethiazide on fluid and electrolyte excretions, blood pressure and renal handling of uric acid using SHR under dietary sodium restriction. This work was based on the findings of previous studies: a) In SHR, high blood pressure is maintained even when dietary sodium is restricted (10). b) Diuretic treatment under sodium deprivation can be used for extracellular fluid volume depletion (11). c) Acute hypotensive actions of diuretics result from reduction of extracellular fluid volume (12). d) Treatment with diuretics combined with a low sodium diet decreases

Fig. 3. Effect of daily oral administration of trichlormethiazide at 3 mg/kg on food and water intakes, urine volume and water balance in SHR fed a low sodium diet. Control group (n=12), open circles; trichlormethiazide-treated group (n=12), closed circles. Results are expressed as means±S.E. Water balance is defined as (urinary output/intake) X100%. *P<0.05, **P<0.01, compared with the control group.
the uric acid clearance and induces hyperuricemia due to extracellular fluid volume contraction (6, 11, 13). e) Trichlormethiazide decreases the uric acid excretory capacity and leads to much greater diuresis in normotensive Wistar rats fed a low sodium diet than in those fed an ordinary diet (4).

Clear hypotensive, diuretic and uric acid-retaining effects of trichlormethiazide were demonstrated in the animals, although the doses causing these actions differed. The hypotensive and natriuretic effects of trichlormethiazide occurred at lower doses than those producing undesirable effects such as lowering of the plasma potassium level and uric acid excretory capacity. These results not only support the usefulness of SHR placed on a sodium-restricted regime in evaluating diuretic antihypertensives but also suggest that a lower dose of trichlormethiazide can be used to treat hypertension under dietary sodium restriction without inducing side effects.

It is interesting that the hypotensive effect of trichlormethiazide was demonstrated in SHR fed a low sodium diet at an earlier stage of the treatment in the present study, because the antihypertensive action of diuretics has not been clearly observed in SHR (14, 15), unless the animals are loaded with sodium chloride (16). The effect of trichlormethiazide was evaluated by an indirect tail cuff method under the prewarming at 37°C for 15 min, a condition which was recommended by the Committee on Care and Use of Spontaneously Hypertensive (SHR) Rats in the Institute of
Laboratory Animal Resources, Washington, D.C. (17). Although this method is generally used, there is some divergence of opinion on its utility. The animals need to be warmed in order to readily detect the arterial pulse. Yen et al. (18) reported that the thermal stress elevated the systolic blood pressure in SHR, which suggests that modification of drug action on blood pressure may be possible by warming the animals. Thus, the hypotensive effect of trichlormethiazide in sodium-restricted SHR should be further studied by various methods which include the direct recording of blood pressure in unanesthetized animals.

In this report, we discussed the mode of the hypotensive effect of trichlormethiazide from sodium depletion. Trichlormethiazide enhanced the urine output and sodium excretion, which was reflected by the elevation of the hematocrit. Moreover, water turnover increased and cumulative sodium balance shifted to a negative state compared to the control during the experiment at a dose of 3 mg/kg. Accordingly, the fluid volume contraction due to increased excretion of sodium, which is the major cation and the principal determinant of extracellular fluid, may contribute to trichlormethiazide-induced hypotension in SHR under sodium restriction. Further investigations are planned to analyze the relationship between the natriuretic potency and the hypotensive response of thiazide diuretics other than trichlormethiazide and of non-thiazide diuretics.

Weiner et al. (11) reported a fall of body weight, together with reduction of the glomerular filtration rate and elevation of the hematocrit value due to chronic volume depletion upon furosemide treatment of rats fed a sodium-free diet. Restriction of food intake is also known to lower blood pressure in the rat, especially in SHR (19). Consecutive administration of trichlormethiazide also decreased the body weight in SHR under sodium restriction in the present study. The fluid volume contraction and suppression of food intake induced by trichlormethiazide may participate in the loss of body weight. The correlation between the decrement of body weight and of systolic blood pressure was estimated using 27 animals treated with trichlormethiazide and 34 animals which included both the control and trichlormethiazide-treated groups (Fig. 2 and Table 1). The correlation was not found at day 6 (correlation coefficient: $r=0.234$, $n=27$, $P>0.05$ and $r=0.407$, $n=34$, $P>0.05$), but
was at day 13 (correlation coefficient: $r=0.549$, $n=27$, $P<0.01$ and $r=0.602$, $n=34$, $P<0.01$). Therefore, the hypotensive effect of trichlormethiazide in SHR fed a low sodium diet might be related to body weight loss with prolonged dosing.

Both the fall in the glomerular filtration rate as a result of the change in renal blood flow and the alteration of the uric acid transport system in the renal tubules by diuretic-induced volume contraction may play roles in the development of hyperuricemia during diuretic administration (2). These two mechanisms acting on renal uric acid retention were also presumed in the present study. With trichlormethiazide treatment at 3 mg/kg for a week, a decrease was found in uric acid clearance based on the fall in inulin clearance. Moreover, the decrease of uric acid excretion due to the change of transtubular uric acid transport capacity, which was indicated by a decrease in the fractional excretion of uric acid, was additive upon prolonged treatment for a week.

The relationship between such decreased uric acid excretion and the extracellular fluid volume contraction remains to be investigated. Suki et al. (6) reported that thiazide-induced renal uric acid retention did not occur in a person given a high sodium diet, which seemed to indicate resistance to the loss of body fluid under continued diuretic administration. Also, we previously reported that not only the decrease of inulin clearance but that of fractional excretion of uric acid under acute treatment with trichlormethiazide could be completely corrected by saline loading (5). A chronic sodium supplement to the present low sodium diet and acute saline loading may offer a useful approach for evaluating the importance of extracellular fluid volume contraction on trichlormethiazide-induced renal uric acid retention in sodium-restricted SHR.

Undesirable side effects, except for hyperuricemia, in treatment with diuretics are due to hypokalemia resulting from kaliuresis. Trichlormethiazide lowered the plasma potassium level and the positive state of cumulative potassium balance compared to the control, which indicates that the experimental procedure presented here can be useful for evaluating such a problem in the use of diuretic antihypertensives. Möhring and Möhring (20) indicated that the fecal excretions of sodium and potassium were only few percentages of their urinary excretions in rats fed a synthetic diet containing 150 mEq of sodium and potassium/kg of food. Accordingly, the "cumulative balance" in this report was determined from the difference between the amount of intake and that of the urinary output. As a result, the positive shift of the cumulative potassium balance proceeded gradually during the experiment not only in the control group but also in the experimental one. This indicates the retention of potassium in the body of both groups of animals despite evidence for the induction of hypokalemia by trichlormethiazide treatment. The reason for this phenomenon is unknown, but it suggests that fecal excretion of potassium should be considered for the low sodium diet used here.

As this study showed that SHR under continued dietary sodium restriction can be used to evaluate the effects of trichlormethiazide, a thiazide diuretic, during relatively short periods, we will try to evaluate the characteristics of various other diuretics in order to find diuretic antihypertensives which are more beneficial than the thiazides.

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