ANTIHYPERTENSIVE EFFECT OF TRICHLORMETHIAZIDE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—Antihypertensive and diuretic effects of trichlormethiazide (TCM) were investigated in the spontaneously hypertensive rats (SHR). The antihypertensive effect of TCM in an acute experiment was observed in male SHR only at a dose over 10 mg/kg given intraperitoneally and not in female SHR and normotensive Kyoto Wistar rats. In a subacute experiment (6 weeks), TCM retarded the development of hypertension in the male SHR loaded with 1% saline solution at an oral dose over 1 mg kg⁻¹ day⁻¹ and such had a diuretic effect. Oral administration of TCM and hydrochlorothiazide (HCT) at 10 mg kg⁻¹ day⁻¹ retarded the development of hypertension in the saline loaded female SHR to the same degree, but the relationship between antihypertensive and diuretic effects of both compounds was obscure. Except for decreases of water contents in the thoracic artery and wet weights of hearts, the electrolyte, uric acid, catecholamine and 5-hydroxytryptamine contents in the serum or/and organs were not affected by either TCM or HCT. It is concluded that the antihypertensive effect of TCM and HCT can be observed in SHR with a saline-load, and that the effect may be due to diuretic actions in the male. The relationship was not apparent in female SHR.

Benzothiadiazine derivatives are still the drug of first choice for treatment of various hypertensive diseases, as they have 1) a high efficacy, 2) synergistic effect with other antihypertensive agents, 3) no severe side effects (1, 2). However, the antihypertensive mechanisms of benzothiadiazine derivatives have not been fully explained, though decreases of circulating blood volume, peripheral resistance, and responsiveness of peripheral arteries have been referred to as possible mechanisms (3).

Trichlormethiazide (3-dichlormethyl-6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide: TCM) is cited as group II diuretics which act on the cortical diluting segment (3), and the antihypertensive effect is clinically well known (4, 5). In a previous paper (6), it was observed that the inhibitory effect of TCM on the development of DOC/saline hypertension in Wistar rats was principally associated with its diuretic effect.

Although the antihypertensive effect of benzothiadiazine derivatives has not been clearly observed in spontaneously hypertensive rats (SHR) (7), we found that such can be demonstrated in SHR after a saline load. The present study was an attempt to clarify whether or not the antihypertensive effect of TCM is associated with its diuretic (saluretic) effect and also to monitor changes of electrolyte and water contents in specific organs such as the heart and thoracic aorta. Effects of TCM on the catecholamine (CA) and 5-hydroxytryptamine (5-HT) contents of the cerebrum, brainstem, and adrenals were also investigated,
as the development of hypertension in SHR is known to be related to enhanced sympathetic nervous activity (8, 9).

MATERIALS AND METHODS

Experimental animals

A strain of SHR developed by Okamoto and Aoki (10) and bred in the Shionogi breeding laboratory (Aburahi, Shiga) was used.

1) Acute experiment: SHR of both sexes (F 28) and normotensive Kyoto Wistar rats (KWR), 3 to 6 months old, were used. Effects of i.p. administration of TCM on the systolic blood pressure of SHR and KWR was compared with effects of reserpine and hydralazine.

2) Subacute experiment: SHR of both sexes (F 26), 6 weeks old, were grouped as given in Table 1 and maintained on a stock chow diet CA-1 (Nihon Clea Co., Tokyo, Na 2.4 mg/g) with 1% saline solution for drinking, except for one group which was given tap water. Effects of daily oral administration of TCM and hydrochlorothiazide (HCT) on the systolic blood pressure and heart rate were compared with the control groups for 6 weeks.

Measurement of systolic blood pressure

A physiograph and electrosphygmomanometer (DMP-4B and PE-300, Narco Biosystems, Inc., Houston) were used to measure the systolic blood pressure of the tail artery and heart rate in the conscious SHR. Before blood pressure determination, the rats were warmed for 2 min at 50°C in the subacute experiment. In the acute experiment, the warming temperature was increased to 60°C in order to readily detect the arterial pulse of older rats. To obtain the systolic blood pressure of one rat, no less than five determinations were performed and averaged.

Urine collection and determination of electrolytes and uric acid concentration

During the subacute experiment, urinary volume and electrolytes were determined four times. On the 2nd, 16th, 30th, and 44th days, rats were housed for 6 hr in individual metabolism cages and the urine collected. Preceding the examination, there was no restric-

| Groups | Sex    | n | Treatment          | Diet  | Drinking water |
|--------|--------|---|-------------------|-------|----------------|
| G-I    |        | 7 | 1% gum arabic     |       | 1% saline      |
| G-II   | male   | 7 | TCM 0.1 mg kg⁻¹ day⁻¹ | CA-1  | 1% saline      |
| G-III  |        | 7 | TCM 1 mg kg⁻¹ day⁻¹ | (N.C.)| 1% saline      |
| G-IV   |        | 8 | TCM 10 mg kg⁻¹ day⁻²|       | 1% saline      |
| G-V    |        | 7 | 1% gum arabic     |       | 1% saline      |
| G-VI   | female | 6 | TCM 10 mg kg⁻¹ day⁻¹| CA-1  | 1% saline      |
| G-VII  |        | 7 | HCT 10 mg kg⁻¹ day⁻¹| (N.C.)| 1% saline      |
| G-VIII |        | 6 | 1% gum arabic     |       | tap water      |

TCM: trichlormethiazide, HCT: hydrochlorothiazide, N.C.: Nihon Clea. In groups I, V, VIII, 1 ml kg⁻¹ of 1% gum arabic solution was orally administered daily.
tion on the intake of food and water. Drinking water consumed per day in each group was also measured 10 times. At the end of the experiment, all SHR were decapitated and the wet weights of the isolated hearts, kidneys, and adrenals were measured. Dry organ weight was obtained after the organs had been left in an incubator at 100°C for one week. Water contents of organs were determined as the difference between wet and dry weights. Electrolytes of the serum and isolated organs were determined using an atomic absorption spectrophotometer (NF1B, Toshiba Beckman, Tokyo). Uric acid concentration of the serum was determined using an autoanalyzer (Autoanalyzer-1, Technicon, Tarrytown) following the Crowly method (11). The hematocrit value was obtained by centrifugation (1.1×10^4 rpm, 5 min) of blood-filled capillaries.

_Determination of CA and 5-HT concentration_

After SHR had been sacrificed in the subacute experiment, the cerebrum, brainstem, and adrenals were isolated and frozen with dry ice. CA and 5-HT were extracted following the methods of Chang (12) and Gordon and Meldrum (13), respectively, and the levels were determined using the method of Korf and Valkenburgh-Sikkema (14).

The results are presented as the mean±S.E. The statistical significance of the differences between the groups was determined using Student's t-test.

**Compounds**

Trichlormethiazide (Shionogi, Osaka), hydrochlorothiazide (Takeda, Osaka), reserpine (Daiichi, Tokyo), and hydralazine hydrochloride (Takeda, Osaka) were used. Doses of compounds were expressed in terms of bases. TCM and HCT were given to rats either p.o. or i.p. as a 1% suspension of gum arabic.

**RESULTS**

**Acute experiment**

Effect on systolic blood pressure in SHR: Intraperitoneal injection of TCM at a dose of 10 or 30 mg/kg caused a significant antihypertensive effect at 4 hr after administration in male SHR (Fig. 1). These antihypertensive effects were not dose-dependent and lasted about 48 hr. No effects were observed on SHR heart rate with TCM. Marked antihypertensive effects of hydralazine and reserpine differed in the time course of their effects. Significant tachycardia induced by hydralazine was observed for several hours.

In female SHR, however, TCM showed no antihypertensive effect even at 30 mg/kg, i.p. Systolic blood pressure and heart rate of female SHR were 169±5.9 mmHg and 445.7±18.3 beats/min, respectively (n=5).

Effect on systolic blood pressure in KWR: No hypotensive effect was observed with i.p. administration of TCM even at a dose of 30 mg/kg, though a marked hypotensive effect with tachycardia of hydralazine and a moderate one with reserpine were recorded (Fig. 2).

**Subacute experiment**

Effect on development of hypertension in SHR: In non-treated male SHR with a saline-load (G-1), the systolic blood pressure increased to about 160 mmHg in 8 weeks and
to 200 mmHg in 11 weeks. The heart rate gradually decreased with the development of hypertension. Two weeks after TCM administration at 1 and 10 mg/kg, a significant antihypertensive effect was observed (Fig. 3). In 3 weeks, even the smallest dosage of TCM (0.1 mg/kg) temporarily produced a significant antihypertensive effect. Although TCM in a dose of 1 and 10 mg/kg significantly inhibited the development of hypertension throughout the experimental period from 2 weeks, the systolic blood pressure of the TCM group was elevated to about 180 mmHg at the end of the experiment. No dose-related inhibition was observed between the 1 and 10 mg/kg dosages of TCM.

The hypertension which developed in female SHR was comparatively milder than that in male SHR and systolic blood pressure reached about 185 mmHg at 12 weeks in G-V. In the last 2 weeks of the experiment, the systolic blood pressure of rats given tap water for drinking (G-VIII) was significantly lower than that of rats given 1% saline (G-V). TCM and HCT at 10 mg/kg inhibited the development of hypertension in female SHR throughout the experimental periods from one week after treatment (Fig. 4).
FIG. 2. Effects of i.p. administration of trichlormethiazide (TCM), reserpine, and hydralazine on systolic blood pressure and heart rate in male Wistar Kyoto rats. Vertical bars represent standard errors. No. of rats: 5. Statistically significant compared with control (0 hr): *p<0.05; **p<0.01; ***p<0.001.

No significant effects were observed with the administration of TCM and HCT on the heart rate and body weight in SHR of both sexes.

Effects on urinary volume and electrolytes: In male SHR, the diuretic effect of TCM decreased in the following order: 1 mg/kg>10 mg/kg>0.1 mg/kg (Table 2). The increase of sodium excretion paralleled that of urine volume, but potassium excretion was slightly more marked at 10 mg/kg of TCM. Higher excreted sodium/potassium ratios were observed at 0.1 and 1 mg/kg of TCM.

In female SHR, the diuretic effects of TCM and HCT were not so marked as those in male SHR. Significant diuretic effects of both compounds appeared on the 44th day after treatment (Table 3). In G-VIII, urinary volume and sodium excretion were lower, but potassium excretion was higher than in G-V.

Effect on drinking water consumption: The consumption of drinking water in each group was measured ten times at random and averaged with S.E. Drinking water consumed (ml) per rat in a day was: G-I, 84.2±4.1; G-II, 84.0±5.1; G-III, 87.1±5.4; G-IV, 78.9±4.5; G-V, 63.4±3.1; G-VI, 59.0±2.3; G-VII, 63.1±3.1; G-VIII, 36.5±1.3. TCM and
HCT had no effects on the consumption of drinking water in both sexes of SHR. The volume of drinking water consumed in the saline-loaded SHR was double that of SHR given tap water for drinking.

Effects on electrolyte and water contents in the serum, myocardium and thoracic aorta: TCM and HCT had no effects on the electrolyte contents of serum, myocardium, and thoracic aorta, but the water contents of the thoracic aorta were slightly reduced by TCM and HCT. In G-VIII, the sodium concentration in the serum and thoracic aorta, and the water contents in the thoracic aorta were lower than in G-V.

Effects on organ weights and hematocrit value: In male SHR, TCM had no effect on the wet weight of kidneys, the adrenals, and the hematocrit value except for heart weight. It is well known that a parallel relationship exists between blood pressure and heart weight. TCM, in a dose of 1 and 10 mg/kg, significantly reduced the wet weight of the heart and such was also reduced by TCM and HCT administration in female SHR. In G-VIII, the wet weights of the hearts and kidneys were significantly lower than in G-V.

Effects on CA and 5-HT contents of the cerebrum, brainstem, and adrenals: Norepinephrine (NE), dopamine (DA), and 5-HT contents in the cerebrum and brainstem were
Fig. 4. Effects of trichlormethiazide (TCM) and hydrochlorothiazide (HCT) on systolic blood pressure, heart rate, and body weight in female SHR. Vertical bars represent standard errors. No. of rats: Control (7), TCM (6), HCT (7), tap water (6). Statistically significant compared with paired control: *p<0.05; **p<0.01; ***p<0.001.

### TABLE 2. Effects of oral administration (44 days) of trichlormethiazide on the urinary volume and electrolytes in male SHR

| Groups | Urinary volume (ml/6 hr) | Days after TCM administration |
|--------|--------------------------|------------------------------|
|        |                          | 2 | 16 | 30 | 44 |
| G-I    | 4.4±0.61               | 6.8±0.8 | 7.2±0.5 | 6.3±1.0 |
| G-II   | 4.1±0.4               | 7.8±0.6 | 8.5±0.8 | 13.2±1.0*** |
| G-III  | 6.4±0.6*              | 10.9±1.3* | 12.4±1.4** | 14.9±0.6*** |
| G-IV   | 5.8±0.3              | 9.7±0.9* | 9.6±0.9* | 12.3±0.8*** |
| G-I    | 0.65±0.09            | 0.99±0.12 | 0.98±0.10 | 0.87±0.16 |
| Na⁺ (mEq) |                | 1.33±0.12 | 1.29±0.12 | 2.04±0.15*** |
| G-II   | 0.73±0.06            | 1.33±0.12 | 1.29±0.12 | 2.04±0.15*** |
| G-III  | 1.15±0.11**          | 1.74±0.19** | 1.93±0.22** | 2.28±0.12*** |
| G-IV   | 0.92±0.07*           | 1.54±0.13** | 1.21±0.12 | 1.86±0.14*** |
| K⁺ (mEq) |                | 0.21±0.02 | 0.13±0.02 | 0.13±0.03 |
| G-I    | 0.20±0.04            | 0.21±0.02 | 0.13±0.02 | 0.23±0.02* |
| G-II   | 0.17±0.02            | 0.19±0.02 | 0.15±0.02 | 0.23±0.02* |
| G-III  | 0.24±0.03            | 0.25±0.02 | 0.20±0.03 | 0.23±0.03* |
| G-IV   | 0.23±0.02            | 0.33±0.03* | 0.18±0.03 | 0.29±0.03** |
| Na/K   |                | 3.5±0.3 | 4.6±0.4 | 8.2±1.2 | 7.3±1.1 |
| G-I    | 4.5±0.4              | 7.1±0.3*** | 8.9±0.5 | 8.9±0.2 |
| G-II   | 5.0±0.5*             | 6.9±0.4** | 10.0±0.5 | 10.3±0.9* |
| G-III  | 4.0±0.2              | 4.8±0.3 | 7.4±0.7 | 6.6±0.5 |

1) Mean±S.E. Statistically significant compared with G-I (*: p<0.05, **: p<0.01, ***: p<0.001). See Table 1 for specification of groups I-IV.
not influenced by TCM and HCT administration in both sexes of SHR. TCM had no consistent effect on the CA contents in the adrenals. No significant differences between G-V and G-VIII in the CA and 5-HT contents were observed.

**TABLE 3. Effects of oral administration (44 days) of trichlormethiazide and hydrochlorothiazide on the urinary volume and electrolytes in female SHR**

| Groups | Urinary volume (ml/6 hr) | Days after TCM or HCT administration |
|--------|--------------------------|-------------------------------------|
|        |                          | 2        | 16       | 30       | 44       |
| G-V    | 3.2 ± 0.6**              | 3.4 ± 0.6| 5.3 ± 0.7| 5.1 ± 0.6|
| G-VI   | 4.5 ± 0.6**              | 6.1 ± 1.1| 6.9 ± 0.5| 8.2 ± 0.5**|
| G-VII  | 3.5 ± 0.4**              | 4.4 ± 0.4| 7.1 ± 0.5*| 7.1 ± 0.5*|
| G-VIII | 3.1 ± 0.3**              | 3.0 ± 0.5| 3.5 ± 0.3*| 3.4 ± 0.3*|

| Na+ (mEq) | 0.55 ± 0.10 | 0.44 ± 0.12 | 0.78 ± 0.14 | 0.69 ± 0.10 |
| K+ (mEq)  | 0.75 ± 0.10  | 0.88 ± 0.20  | 0.94 ± 0.09  | 1.20 ± 0.11** |
| Na/K      | 0.08 ± 0.02  | 0.08 ± 0.02  | 0.09 ± 0.02  | 0.10 ± 0.01  |

1) Mean ± S.E. Statistically significant compared with G-V (*: p < 0.05, **: p < 0.01, ***: p < 0.001). See Table 1 for specification of groups V-VIII.

DISCUSSION

In the acute experiment, the antihypertensive effect of intraperitoneally administered TCM was observed only in male SHR. The antihypertensive doses of TCM (10 mg/kg, i.p.) were, however, much higher than the diuretic doses given the male Charles River rats (0.01–0.04 mg/kg, p.o.) (15). In the subacute experiment, the consistent antihypertensive effect of TCM was accompanied by diuretic and sodium excreting effects in male SHR with a saline-load. In female SHR, however, diuretic effects of TCM and HCT were only observed in the later phase of experiment, though the antihypertensive effect of both com-
pounds appeared one week after treatment. Thus, saline-loading in SHR revealed the antihypertensive effect of TCM.

Although the antihypertensive effect of TCM was confirmed in the subacute experiment in both sexes of SHR, the relationship between the antihypertensive and diuretic effects of TCM was not so clear in female SHR. Lower systolic blood pressure of female SHR may prevent a clear observation of the relationship.

Decreases in circulating blood volume due to diuresis, peripheral resistance and responsiveness of peripheral arteries are possible mechanisms of the antihypertensive effects of benzothiadiazine derivatives (3). In the present experiment, the antihypertensive effect of TCM was noted with the diuretic effect in SHR with a saline-load. Antihypertensive effect of TCM in the DOC/saline hypertensive rats was also observed together with its diuretic effect (6). To confirm the antihypertensive mechanism of TCM, measurement of circulating blood volume and cardiac output in hypertensive rats must be done, as Leth observed a lasting contraction of plasma volume and extracellular fluid volume in humans on HCT administration (16).

Rubin et al. (17, 18) attributed the spasmolytic effect of TCM to one of its antihypertensive mechanisms when they observed the nonspecific spasmolytic effects of TCM and diazoxide in the isolated thoracic artery of rabbits.

Increase in NE contents in adrenals (19–21) and a decrease of NE turnover rate in the brainstem (22, 23) have been reported in SHR. But, TCM and HCT had no effects on the NE contents in the cerebrum, brainstem, and adrenals. Such side effects as hypokalemia and hyperuricemia reported with long term clinical treatment with thiazide diuretics (2) were not observed in the present experiment.

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