Chronopharmacology of Trichlormethiazide in Rats: (III) Influence on Serum Triglyceride and Glucose

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ABSTRACT — Trichlormethiazide was given orally to rats at 10 a.m. or 10 p.m. for 14 days. The diuretic effects of the agent at 10 a.m. were greater than those at 10 p.m. on day 14. Serum concentrations of triglyceride and glucose increased in both trials. The increments in these parameters were enhanced following trichlormethiazide at 10 a.m. These data indicate that the diuretic effects of trichlormethiazide and its untoward influences on serum metabolic parameters might vary with the administration time during a repeated therapy.

Trichlormethiazide, a diuretic agent, is widely prescribed for the treatment of hypertension. However, untoward effects on blood biochemical parameters are observed in some patients during long-term treatment with the agent (1). There is increasing evidence demonstrating time-dependent changes in the effectiveness and toxicity of cardiovascular agents (2, 3). We already examined the chronopharmacological profiles of trichlormethiazide following a single oral administration in rats (4). This study has demonstrated that the effects of trichlormethiazide are greater when it is administered during their resting period than when it is administered during their active period. Antihypertensive agents including trichlormethiazide are usually prescribed on a basis of chronic use. Therefore, it is interesting to examine whether the diuretic effects of trichlormethiazide and its influence on biochemical parameters vary with the time of administration during a repeated therapy.

To address this issue, trichlormethiazide was given orally to rats once a day in the day or night for 14 days. The diuretic effects of the agent and its influence on serum electrolytes, lipids and glucose were compared between the day and night trials.

Male Wistar rats (Charles River Laboratory, Kanagawa, Japan) at 10 weeks of age were maintained for more than 2 weeks under conditions of light from 7 a.m. to 7 p.m. and dark from 7 p.m. to 7 a.m. with free access to food and water. During the control period (day 0), 1 ml of 1% NaCl solution (vehicle) was given into stomach at 10 a.m. (day trial, n = 28) or at 10 p.m. (night trial, n = 28). Water intake and urine volume were measured for 24 hours after administration of the vehicle. Thereafter, each group of rats was divided into two subgroups. One group (n = 14) received 4 mg/kg of trichlormethiazide in 1 ml of vehicle at 10 a.m. (or 10 p.m.) for 14 days. The other group (n = 14) received 1 ml of vehicle alone at 10 a.m. (or 10 p.m.) for 14 days and served as a trichlormethiazide control. The parameters obtained during the control period also were measured for 24 hours following the final dosage of trichlormethiazide or vehicle alone (day 14). Blood samples at 24 hours after the final
administration also were obtained under pentobarbital anesthesia.

Serum and urinary concentrations of electrolytes and serum total cholesterol and triglyceride were measured using an autoanalyzer (736, Hitachi, Tokyo, Japan). Serum glucose concentration was determined by the glucose oxidase method (Glucoroder, Shino Test, Tokyo, Japan). Urinary trichlormethiazide concentration was measured by high performance liquid chromatography (5). The sensitivity of this assay was 10 ng/ml.

The results are expressed as the mean ± S.D. Data were analyzed by analysis of variance.

During the control period, no significant difference was observed in any parameter between the vehicle and trichlormethiazide groups in the day or night trial (Fig. 1). The mean water intake increased slightly by the repeated dosage of trichlormethiazide in both trials. The 24-hour urine volume increased slightly in the night trial and increased significantly in the day trial on day 14. Urinary

![Image of graph showing water intake, urine volume, and urinary excretion of sodium and trichlormethiazide following repeated administration.](image-url)

**Fig. 1.** Water intake, urine volume and urinary excretion of sodium and trichlormethiazide following repeated administration of trichlormethiazide at 10 a.m. (day trial) or at 10 p.m. (night trial). The 24-hour water intake was determined and urine was collected during the control period (day 0) and following the final dosage of trichlormethiazide (day 14). The values of the mean ± S.D. are shown. □ = vehicle group. ▣ = trichlormethiazide group. Vehicle (1 ml of 1% NaCl solution) or trichlormethiazide (4 mg/kg in 1 ml of vehicle) was given once a day at 10 a.m. (n = 14 in each group) or at 10 p.m. (n = 14 in each group) for 14 days.
sodium excretion following trichlormethiazide increased significantly in the day and night trials. The urinary excretion of the agent in the day trial was significantly higher than that in the night trial.

Serum concentration of chloride decreased significantly by the repeated dosage of trichlormethiazide (Table 1). There was no significant difference in this parameter between the day and night trials. Serum concentrations of triglyceride and glucose in the vehicle group were significantly lower in the day trials than in the night ones. These parameters increased following the repeated dosage of trichlormethiazide in both trials. The mean increments in the day trial were higher than those in the night trial [triglyceride (mg/dl): day trial = 96, night trial = 10; glucose (mg/dl): day trial = 22, night trial = 14]. No untoward influences were observed in other biochemical parameters (sodium, potassium or total cholesterol) by the repeated dosage of trichlormethiazide.

We have recently published data indicating that the urine volume and urinary sodium excretion following a single dosage of trichlormethiazide are greater when the drug is given at daytime compared to those when it is given at night-time in Wistar rats (4). The present study extends these observations to a repeated administration study over a period of 14 days. The present study showed that the 24-hour urinary excretion of trichlormethiazide in the day trial is greater than that in the night trial. Since the main site of action of trichlormethiazide is the luminal side of the distal convoluted tubule (6), the time-dependent change in the diuretic effects of trichlormethiazide increased significantly in the day and night trials. The urinary excretion of the agent in the day trial was significantly higher than that in the night trial.

Table 1. Serum concentrations of electrolytes, cholesterol, triglyceride and glucose following repeated administration of trichlormethiazide at 10 a.m. (day trial) or at 10 p.m. (night trial) in rats

| Parameter          | Day trial                     | Night trial                   |
|--------------------|-------------------------------|-------------------------------|
|                    | vehicle (n = 14) | trichlormethiazide (n = 14) | vehicle (n = 14) | trichlormethiazide (n = 14) |
| Sodium mEq/l       | 142 ± 1                     | 143 ± 2                      | 139 ± 1          | 140 ± 2                      |
| Potassium mEq/l    | 4.0 ± 0.4                   | 3.9 ± 0.3                   | 4.2 ± 0.4        | 4.0 ± 0.4                   |
| Chloride mEq/l     | 100 ± 1                     | 98 ± 1                      | 101 ± 1          | 99 ± 1                      |
| Total cholesterol mg/dl | 61 ± 4                     | 63 ± 4                      | 62 ± 5           | 61 ± 7                      |
| Triglyceride mg/dl | 71 ± 16                     | 167 ± 24                   | 145 ± 23        | 155 ± 32                   |
| Glucose mg/dl      | 135 ± 14                    | 157 ± 10                   | 171 ± 8         | 185 ± 16                   |

Vehicle (1 ml of 1% NaCl solution) or trichlormethiazide (4 mg/kg in 1 ml of vehicle) was given once a day at 10 a.m. or at 10 p.m. for 14 days. Twenty-four hours after the final dosage, blood samples were obtained. The values of the mean ± S.D. are given.
methiazide might, at least in part, depend on the time-dependent variation in the urinary amount of the agent.

Previous studies have demonstrated that serum triglyceride concentrations are greater after the onset of feeding in the night-time, while no diurnal variation is observed in serum cholesterol concentrations in rats (7, 8). These data might explain the difference in serum lipids between the day and night trials observed in the vehicle-treated animals. Serum concentrations of cholesterol and triglyceride sometimes increase during a prolonged treatment with thiazide (1, 9, 10). Although LDL-cholesterol and VLDL-triglyceride are mainly elevated by thiazide, the mechanisms of these events are unknown. Since hypercholesterolemia and also probably hypertriglyceridemia are the risk factors in coronary artery and other diseases, a deterioration in lipid metabolism should be prevented. The present animal study demonstrated that the increase in serum triglyceride concentration is enhanced following the repeated dosage of trichlormethiazide at daytime. This finding indicates that the untoward influence of trichlormethiazide on lipid metabolism might vary with its time of administration.

Hyperglycemia is also observed in some patients receiving prolonged treatment with thiazide (1). The present study showed that the elevation in blood glucose concentration is greater in the day trial which indicates that the diabetogenic action of trichlormethiazide might also depend on its administration time. One well-known untoward effect of thiazide is hypokalemia, which may at times suppress insulin release and impair glucose tolerance (11). There is, however, evidence that a diuretic agent may cause a decrease in glucose tolerance independent of a hypokalemic action (12). Although serum potassium concentration slightly decreased in the day and night trials, the present study did not provide any evidence explaining the time-dependent influence of trichlormethiazide on glucose tolerance.

Serum chloride concentration significantly decreased by trichlormethiazide therapy which might be, at least in part, induced by an increased excretion of chloride in the urine (13). No significant difference was observed in this parameter between the day and night trials. In addition, the influences of the agent on serum concentrations of sodium and total cholesterol of the two trials did not differ.

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