Chronopharmacology of Trichlormethiazide in Rats

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ABSTRACT—Trichlormethiazide was given orally at 1200 hrs or 2400 hrs to rats. Its diuretic effects were greater at 1200 hrs than at 2400 hrs. There were significant correlations between urinary trichlormethiazide and its effects in both trials. The regression lines of two trials did differ. These findings indicate that the effects of trichlormethiazide vary with its administration time. Time-dependent variations in urinary trichlormethiazide and susceptibility to the agent might be involved in this phenomenon.

There is increasing evidence demonstrating time-dependent changes in the effectiveness of cardiovascular agents (1). We already examined the chronopharmacological profiles of furosemide, a loop diuretic agent, in human subjects and rats. These studies demonstrated that the effects of furosemide are greater when it is administered during their resting period than when it is administered during their active period (2–4). Similar chronopharmacological properties are also reported for thiazide diuretics such as hydrochlorothiazide and trichlormethiazide in humans. The diuretic effects of these agents following the evening dosage are greater than those after the morning dosage (5, 6). However, hydrochlorothiazide produces an increased diuresis when administered during the active period than when administered during the resting period in rats (7). It is interesting to examine whether this discrepancy in the chronopharmacological profile of hydrochlorothiazide between humans and rats is common to thiazide-type diuretics.

To address this issue, trichlormethiazide was given orally at 1200 hrs or 2400 hrs to rats. The diuretic effects following the agent at 1200 hrs were compared to those given at 2400 hrs. The urinary excretion of trichlormethiazide was also determined.

Male Wistar rats (Charles River Laboratory, Kanagawa, Japan) (10–11 week old, 300–350 g) were maintained for more than 2 weeks under conditions of light from 0700 hrs to 1900 hrs and dark from 1900 hrs to 0700 hrs with free access to food and water.

Four percent body weight (b.w.) of 1% NaCl solution was given by gavage into the stomach at 1200 hrs (or 2400 hrs) on day 1. Trichlormethiazide (Shionogi & Co., Ltd., Osaka, Japan) (0.5 and 2.0 mg/kg b.w. in 4% b.w. of 1% NaCl) was given orally at 1200 hrs (or 2400 hrs) on day 4 and day 7, respectively. Urine was collected for 8 hours following the vehicle alone or the drug administration at 1200 hrs (or 2400 hrs). The animals were deprived of food and water during the 8 hours after each administration. The administration of the drug was randomly assigned to 1200 hrs or 2400 hrs. The washout period between the two sets of experiments was 7 days.

Urinary sodium concentration was determined by flame photometry (Flame Photometer 775-A, Hitachi, Tokyo, Japan). Urinary chloride concentration was determined by an autoanalyzer (736, Hitachi, Tokyo, Japan). Urinary concentration of trichlormethiazide was measured by Shionogi & Co., Ltd., using
high pressure liquid chromatography. The sensitivity of this assay was 10 ng/ml.

The results are expressed as the means ± S.E. Data were analyzed by analysis of variance and the Wholly-Significant-Difference Method.

When 4% b.w. of NaCl solution was given as a trichlormethiazide control, no significant difference was observed in the urine volume and urinary excretion of sodium or chloride in the collection period following the 1200 hrs administration (day trial) as compared to the collection period beginning at 2400 hrs (night trial) (Table 1). Urine volume and urinary excretion of sodium and chloride increased dose-dependently after trichlormethiazide in the day and night trials. These parameters following administration of 0.5 and 2.0 mg/kg b.w. of the agent were significantly greater at 1200 hrs than at 2400 hrs.

Urinary excretion of trichlormethiazide was significantly greater in the day trial than in the night trial (Table 1). There were significant correlations between the urinary output of trichlormethiazide and its effects (urine volume and urinary excretion of sodium and chloride) in the day and night trials (Fig. 1). The slopes of the regression lines between the urinary trichlormethiazide and its effects did not differ among the two administration times. Moreover, in each parameter, the regression lines obtained in the day trial were significantly different from those in the night trial.

The present study demonstrates that the diuretic effects of trichlormethiazide are greater when it is administered at 1200 hrs corresponding to the rats' sleep period than when it is administered at 2400 hrs which is their awake period. This finding is similar to the chronopharmacological profile of trichlormethiazide in human subjects (6). Therefore, although the chronopharmacological properties of hydrochlorothiazide in rats are different from those in human subjects (5, 7), this phenomenon might not be common to thiazide type diuretics.

The present study shows that the urinary excretion of trichlormethiazide in the day trial is greater than that in the night trial. In addition, there are positive correlations between the urinary excretion of the agent and its diuretic effects. Since the main site of action

| Table 1. Urine volume and urinary excretion of sodium, chloride and trichlormethiazide following oral administration of the agent at 1200 or 2400 hrs |
|---------------------------------|-----------------|-----------------|-----------------|
| Parameter                        | Administration time. hrs |               |               |               |
|                                 | 1200             | 2400            | 1200           | 2400           |
|                                 | vehicle (1% NaCl) | t 0.5 mg        | t 2.0 mg       | vehicle (1% NaCl) | t 0.5 mg | t 2.0 mg |
| Urine volume ml/kg/8 hrs        | 33.6 ± 2.2        | 58.9 ± 1.2 **   | 65.2 ± 1.8 **  | 31.4 ± 2.0      | 45.2 ± 2.3 | 51.7 ± 2.0 |
| Urinary sodium mEq/kg/8 hrs     | 4.9 ± 0.5         | 9.2 ± 0.2 **    | 10.5 ± 0.3 **  | 5.2 ± 0.3       | 7.7 ± 0.2  | 8.5 ± 0.2  |
| Urinary chloride mEq/kg/8 hrs   | 4.5 ± 0.4         | 10.9 ± 0.3 **   | 11.7 ± 0.3 **  | 4.9 ± 0.5       | 8.6 ± 0.2  | 10.3 ± 0.2  |
| Urinary trichlormethiazide μg/kg/8 hrs | 212 ± 9 **        | 606 ± 21 **     | 124 ± 10       | 467 ± 17       |

Urine was collected during 8 hours after trichlormethiazide. t 0.5 mg or 2.0 mg = trichlormethiazide at 0.5 or 2.0 mg/kg. (mean ± S.E., n = 15). ** = P < 0.01 compared to 2400 hrs.
Fig. 1. Relationship between urinary trichlormethiazide and urinary excretion of volume, sodium and chloride in rats. Trichlormethiazide (0.5 and 2.0 mg/kg) was given orally at 1200 hrs (○) or 2400 hrs (●). Urine was collected during 8 hours after the agent.
of trichlormethiazide is the luminal side of the distal convoluted tubule (8), these time-dependent changes in the diuretic effects of trichlormethiazide might, at least in part, depend on the time-dependent variations in the urinary amount of the agent. These daily variations in the urinary excretion of trichlormethiazide might be accounted for by either or both of the following mechanisms: 1) faster absorption rate after the agent is given 1200 hrs compared to that when it is given at 2400 hrs. Temporal variations of absorption rate have already been documented for several drugs (9); 2) higher excretion rate in the day trial compared to that in the night trial. This mechanism has been demonstrated by an intravascular injection study using furosemide (3, 4). Although a circadian variation is demonstrated in renal glomerular function (10, 11), a rhythmicity concerning renal tubular function is quite unknown. Since trichlormethiazide and furosemide are secreted by renal tubules, a circadian variation might also exist in tubular secretory function. Further studies are needed to evaluate this hypothesis. The present study demonstrates that the slopes of the regression lines between the urinary amount of trichlormethiazide and its diuretic effects did not differ between the day and night trials. In addition, the regression lines in the day trial were different from those in the night trial. These observations indicate that the susceptibility of renal tissues to trichlormethiazide varies with its time of administration. This mechanism might also be involved in the time-dependent changes in the effects of trichlormethiazide. Finally, many hormonal factors involved in the regulation of water and electrolyte homeostasis exhibit circadian changes. Among these factors, plasma concentrations of angiotensin II, aldosterone and corticosterone peak during the early active phase (11-14); and consequently, the diuretic effect of trichlormethiazide might be reduced.

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REFERENCES

