Chronopharmacology of the New Uricosuric Diuretic S-8666 in Rats: (II) Examination in Aged Rats

Akio Fujimura, Hiroyuki Kajiyama, Kyo-ichi Ohashi and Akio Ebihara

Department of Clinical Pharmacology, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi 329-04, Japan

Received September 3, 1990 Accepted March 4, 1991

ABSTRACT—We have previously demonstrated a time-dependent variability in the diuretic effects of S-8666, a new loop diuretic with uricosuric activity, in young rats. The present study was undertaken to determine whether such a daily variation in the effects of the agent exists in aged rats. S-8666 (30 and 90 mg/kg) was orally given at 12:00 a.m. (day trial) or at 12:00 p.m. (night trial) in young (10-11 week old) and aged (23-24 month old) Wistar rats. Urine was collected for 8 hours after the agent; and urinary excretions of sodium, S-8666 and its active metabolite S-8680 were determined. Urinary excretions of volume and sodium following S-8666 at 12:00 a.m. were greater than those at 12:00 p.m. in the young and aged animals. Urinary excretions of S-8666 and S-8680 were also greater in the day trial compared to those in the night trial in both groups of rats. In the day and night trials, there were significant correlations between urinary S-8666 + S-8680 and the diuretic effects in both groups of rats. These findings indicate that the diuretic effects of S-8666 also vary with its time of dosing in aged rats. The time-dependent variations in urinary S-8666 and S-8680 might be involved in this phenomenon.

6,7-Dichloro-5-(N,N-dimethylsulfamoyl)-2,3-dihydro-2-benzofuran carboxylic acid (S-8666) is a new loop diuretic agent with uricosuric activity. The agent has two enantiomers. The (+)-enantiomer has uricosuric activity while the (−)-enantiomer shows diuretic activity (1, 2). Their demethylated metabolites, 5-monomethylsulfamoyl-6,7-dichloro-dihydrobenzofuran-2-carboxylic acid, [S-8680-(+)] and S-8680−(−)] are also active (1, 3).

There is increasing evidence demonstrating time-dependent changes in effectiveness and toxicity of cardiovascular agents (4-7). We already examined some chronopharmacological profiles of S-8666 in young Wistar rats. This study demonstrated that the effects of S-8666 are greater when it is administered at 12:00 a.m. which corresponds to the rats’ sleep period than when it is given at 12:00 p.m. which is their awake period (8).

Altered circadian rhythm in some physiological variables are reported in aged rodents compared to young ones. For example, the period of the free-running circadian activity cycle in the golden hamster as well as in the deer mouse become progressively shorter as the animals age (9). The amplitude of the circadian rhythm in motor activity, drinking and food intake are also reported to be reduced in aged rats (10, 11). Based on these findings, it is anticipated that the mode of a time-dependent change in the pharmacological effects of an agent might be altered in aged rodents. We actually demonstrated that the time-dependent variations in the effects of furosemide, another loop diuretic agent, dis-
appear in aged rats (12). To examine this hypothesis further, S-8666 was given orally at 12:00 a.m. or at 12:00 p.m. to young and aged groups of rats. The diuretic effects following the agent at 12:00 a.m. were compared to those given at 12:00 p.m. The urinary excretions of S-8666 and its active metabolite S-8680 were also determined.

MATERIALS AND METHODS

Young (10–11 weeks old, 300–350 g) and aged (23–24 months old, 600–830 g) male Wistar rats (Charles River Laboratory, Kanagawa, Japan) were maintained for more than 2 weeks under conditions of light from 7:00 a.m. to 7:00 p.m. and dark from 7:00 p.m. to 7:00 a.m. with free access to food and water. Three percent body weight (b.w.) of 1% NaCl solution was given by gavage into the stomach at 12:00 a.m. (or 12:00 p.m.) on day 1. Thirty and ninety mg/kg b.w. of S-8666 (Shionogi & Co., Ltd., Osaka, Japan) in 3% b.w. of 1% NaCl was given orally at 12:00 a.m. (or 12:00 p.m.) on day 4 and day 7, respectively. Urine was collected for 8 hours following the vehicle alone or the drug administration at 12:00 a.m. (or 12:00 p.m.). Food and water were deprived during the 8 hours after each administration. The administration of the drug was randomly assigned to 12:00 a.m. or 12:00 p.m. 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 concentrations of S-8666-(+) and S-8666-(−) and those of their active demethylated metabolites, S-8680-(+) and S-8680-(−), were measured by Shionogi & Co., Ltd. by high pressure liquid chromatography (1). The sensitivity of this assay was 0.2 μg/ml, and the coefficient of variation was 3.0%.

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

RESULTS

When 3% b.w. of NaCl solution was given as a S-8666 control, no significant difference was observed in urine volume or urinary excretion of sodium in the collection period following the 12:00 a.m. administration compared to the collection period beginning at 12:00 p.m. in the young or aged rats (Figs. 1 and 2). There were no significant differences in these basal values between the young and aged groups of rats.

Urine volume and urinary excretion of sodium increased dose-dependently after S-8666 in the young and aged groups of rats (Figs. 1 and 2). These parameters following 30 and 90 mg/kg b.w. of the agent were significantly greater at 12:00 a.m. than at 12:00 p.m. Urinary excretions of S-8666 and its active metabolite S-8680 in the day trial were greater than those in the night trial in both groups of animals (Tables 1 and 2).

There were significant correlations between urinary S-8666-(−) + S-8680-(−) and its diuretic effects in the day and night trials (Figs. 3 and 4). The slopes of the regression lines between the urinary agents and its effects did not differ among the two administration times in the young and aged groups of rats. The regression lines obtained in the day trial were significantly different from those in the night trial in the young rats, but not in the aged ones.

Significant correlations were observed in the urinary S-8666-(−) + S-8680-(−) and its diuretic effects in both groups of rats [urine volume: young rats, y = 1.7 × 10⁻³x + 27.6, r = 0.67 (P < 0.001), n = 48; aged rats, y = 2.4 × 10⁻³x + 28.8, r = 0.55 (P < 0.001), n = 48; urinary sodium: young rats, y = 3.1 × 10⁻⁴x + 5.0, r = 0.58 (P < 0.001), n = 48; aged rats, y = 4.0 × 10⁻⁴x + 4.2, r = 0.75 (P < 0.001), n = 48]. The slopes of the regression lines did not significantly differ among the young and aged groups of rats. In addition, the regression lines obtained in the young rats were not significantly different from those in the aged rats.
Fig. 1. Urine volume and urinary sodium excretion following oral administration of S-8666 at 12:00 a.m. (day trial) or at 12:00 p.m. (night trial) in young rats. Mean ± S.E., n = 12. Urine was collected for 8 hours after the animals were given 3% body weight (b.w.) of 1% NaCl solution (C) or S-8666 [30 mg (S-30) or 90 mg (S-90)/kg b.w.] in 3% b.w. of 1% NaCl.

Table 1. Urinary excretions of enantiomers of S-8666 (S-8666-(+) and S-8666(-)) and their active demethylated metabolites (S-8680-(+)) and S-8680(-)) following oral administration of the agent at 12:00 a.m. (day trial) or at 12:00 p.m. (night trial) in young rats

| Parameter                  | Day trial     | Night trial    |
|----------------------------|---------------|----------------|
|                            | S-30          | S-90           | S-30          | S-90           |
| S-8666-(+), µg/kg b.w./8 hr| 1142 ± 128**  | 3375 ± 447*    | 786 ± 78      | 2260 ± 360    |
| S-8680-(+), µg/kg b.w./8 hr| 2870 ± 202**  | 8295 ± 723*    | 1851 ± 358    | 6008 ± 651    |
| S-8666(-), µg/kg b.w./8 hr | 1833 ± 143*   | 5706 ± 589*    | 1282 ± 214    | 4270 ± 539    |
| S-8680(-), µg/kg b.w./8 hr | 729 ± 75*     | 2501 ± 270     | 548 ± 55      | 2139 ± 367    |

Mean ± S.E., n = 12. Urine was collected for 8 hours after administering S-8666 [30 mg (S-30) or 90 mg (S-90)/kg b.w.]. * = P < 0.05, ** = P < 0.01, compared to the night trial.
Fig. 2. Urine volume and urinary sodium excretion following oral administration of S-8666 at 12:00 a.m. (day trial) or at 12:00 p.m. (night trial) in aged rats. Mean ± S.E., n = 12. C = 3% b.w. of 1% NaCl solution only. S-30 = 30 mg/kg b.w. of S-8666 in 3% b.w. of 1% NaCl. S-90 = 90 mg/kg b.w. of S-8666 in 3% b.w. of 1% NaCl.

Table 2. Urinary excretions of enantiomers of S-8666 (S-8666-(+) and S-8666-(−)) and their active demethylated metabolites (S-8680-(+) and S-8680-(−)) following oral administration of the agent at 12:00 a.m. (day trial) or at 12:00 p.m. (night trial) in aged rats

| Parameter | Day trial | Night trial |
|-----------|-----------|-------------|
|           | S-30  | S-90       | S-30  | S-90       |
| S-8666-(+) | 1152 ± 155** | 2586 ± 242* | 708 ± 73  | 2171 ± 203 |
| μg/kg b.w./8 hr | 2569 ± 229** | 6086 ± 638* | 1690 ± 165 | 4622 ± 380 |
| S-8680-(+) | 2007 ± 232** | 5347 ± 452** | 1384 ± 131 | 3998 ± 301 |
| μg/kg b.w./8 hr | 601 ± 115* | 1690 ± 395* | 432 ± 80  | 1172 ± 233 |

Mean ± S.E., n = 12. Urine was collected for 8 hours after administering S-8666 [30 mg (S-30) or 90 mg (S-90)/kg b.w.]. * = P < 0.05, ** = P < 0.01, compared to the night trial.
Fig. 3. Relationship between urinary S-8666-(−) + S-8680-(−) and its diuretic effects in young rats. S-8666 was given orally at 12:00 a.m. (○) or 12:00 p.m. (●).
Fig. 4. Relationship between urinary S-8666-(−) + S-8680-(−) and its diuretic effects in aged rats. S-8666 was given orally at 12:00 a.m. (○) or 12:00 p.m. (●).
DISCUSSION

We have recently demonstrated that the diuretic effects of S-8666 are greater when it is administered at 12:00 a.m. than when it is administered at 12:00 p.m. in the young rats (8), which is confirmed in the present study. Circadian rhythms of physiological variables in general are considered to be altered with age (13). For example, the previous studies have demonstrated a dampening of the amplitudes of motor activity, drinking and food intake in aged rats (10, 11). Moreover, a daily variation of a pharmacological effect of an agent was suggested to disappear in aged rats (12). However, in the present study, the time-dependent variations in the effects of S-8666 persisted in the aged rats.

In the present study, the urinary amounts of S-8666-(−) and S-8680-(−), which have diuretic activities (1, 2), were greater in the day trial than in the night trial. In addition, there were positive correlations between the urinary excretion of S-8666-(−) + S-8680-(−) and its diuretic effects in the day and night trials. Since S-8666 is excreted in urine by active tubular secretion and subsequently inhibits chloride ion transport in the cortical thick ascending limb of Henle's loop (2, 14, 15), this time-dependent change in the diuretic effects of S-8666 might, at least in part, depend on the time-dependent variations in the urinary amount of S-8666-(−) and S-8680-(−) in the young and aged rats. Daily variations in the susceptibility of tissues to agents have been reported (16–18). The present study demonstrated that the slopes of the regression lines between the urinary agent and its diuretic effects did not differ between the day and night trials in both groups of rats. Moreover, the regression lines in the day trial were significantly different from those during the night trial in the young rats, but not in the aged ones. These observations indicate that the susceptibilities of renal tissues to S-8666 and S-8680 vary with the administration time in the young rats. However, although the daily variation in the susceptibility disappeared in the aged rats, the time-dependent changes in the effects of S-8666 remained in these animals. Therefore, the role of this mechanism might be minor in the time-dependent changes of the effects of S-8666 in the young animals.

We have previously published data indicating that the diuretic effects of furosemide and its urinary amount vary with the time of dosing in the young rats, but not in the aged ones (12, 19–21). These data concerning aged rats are apparently not in agreement with the present observations. Because the route of administration of S-8666 (oral) is different from that of furosemide (intra-vascular), a definite conclusion cannot be drawn.

The daily variations in the urinary amount of S-8666 and S-8680 were observed in the young and aged rats. This might be accounted for by either or both of the following mechanisms: 1) faster absorption rate after S-8666 at 12:00 a.m. compared to that at 12:00 p.m. Temporal variations of absorption rate have already been documented for several drugs (22); 2) higher excretion rate of S-8666 and S-8680 in the day trial compared to that in the night trial. This mechanism has been demonstrated by an intra-vascular injection study using furosemide (19–21). Although a circadian variation was demonstrated in renal glomerular function (23, 24), there has been no demonstration of rhythmicity in tubular function. Since S-8666 and furosemide are secreted by the renal tubule, a circadian variation might also exist in tubular secretory function. This hypothesis remains to be determined.

Rats metabolize uric acid to allantoin in the liver with uricase (25). Therefore, when the properties of uricosuric agents are evaluated using rats, the animals must be treated with the uricase inhibitor oxonate (26). Since we cannot rule out the possibility that oxonate alters the chronopharmacological profiles of S-8666, the rats were not treated with oxonate in the present study. Urinary excretions of S-8666-(+) and S-8680-(+), which have predominantly uricosuric activities, are greater when the agent is given at 12:00 a.m. than when it is administered at 12:00 p.m. Al-
though the urinary uric acid was not determined, these data indicate that the uricosuric activity of S-8666 in the day trial is greater than that in the night trial in the young rats.

In summary, the present study demonstrated that the time-dependent variations in the diuretic effects of S-8666 persist in the aged rats. However, it is not clear whether the mode of daily variation in the pharmacological effects of the agent in aged rats is identical to that in young rats. Further studies with more observation times are needed to clarify this issue.

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