Chronopharmacology of the New Uricosuric Diuretic S-8666 in Rats

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ABSTRACT—A new loop diuretic with uricosuric activity, 6,7-dichloro-5-(N,N-dimethylsulfamoyl)-2,3-dihydro-2-benzofuran carboxylic acid (S-8666), was given orally at 12:00 a.m. or 12:00 p.m. in rats. The diuretic of S-8666 and the urinary excretions of the drug and its active metabolite S-8680 (N-demethyl S-8666) were greater at 12:00 a.m. than at 12:00 p.m.. Thus, the present study indicates that the diuretic effects of S-8666 varies with its administration time. Time-dependent variations in the amount of urinary excretions of S-8666 and S-8680 might be involved in the mechanisms for this phenomenon.

6,7-Dichloro-5-(N,N-dimethylsulfamoyl)-2,3-dihydro-2-benzofuran carboxylic acid (S-8666) is a new uricosuric diuretic agent with two enantiomers. The (+)-enantiomer has predominantly uricosuric activity, while the (–)-enantiomer shows diuretic activity (1, 2). Each enantiomer has an active metabolite. The demethylated metabolite of S-8666-(+), S-8680-(+) has uricosuric activity, and that of S-8666-(–), S-8680-(–), has diuretic activity (1, 3). The major site of the diuretic action of S-8666 is the cortical thick ascending limb of Henle's loop (CAL) (4) which is common to that of furosemide, a widely used loop diuretic agent.

There is increasing evidence demonstrating time-dependent changes in the effectiveness and toxicity of cardiovascular agents. We already examined chronopharmacological profiles of furosemide in rats. These studies demonstrated that the effects of furosemide are greater when it is administered during the rats' sleep period than when it is administered during their awake period (5, 6). This time-dependent change in the diuretic effects of

furosemide depends on the time-dependent variations in the urinary amount of furosemide (5, 6). Since, similar to furosemide (7), S-8666 is excreted in the urine by active tubular secretion, and subsequently inhibits chloride ion transport in the CAL (2, 4), it is assumed that the diuretic effects of the agent also vary with its time of dosing. The present study was therefore undertaken to extend our original observations to another loop diuretic agent. S-8666 was given orally at 12:00 a.m. or 12:00 p.m. to 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.

Male Wistar rats (Charles River Laboratory, Kanagawa, Japan) (10–11 weeks old, 300–350 g) 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. Four 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 4% 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 vehicle alone or the drug administration at 12:00 a.m. (or 12:00 p.m.). Food and water were deprived for 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.

A loop diuretic agent inhibits the Na-K-2Cl cotransport system in the CAL. Potassium and chloride are secreted at the distal tubule (8, 9). In addition, potassium is reabsorbed at the medullary collecting duct, and it is subsequently secreted at the medullary thick ascending limb of Henle's loop (10). These observations indicate that the urinary amounts of potassium and chloride are not appropriate parameters for estimating the diuretic activity of a loop diuretic agent. Therefore urinary sodium was measured to evaluate the diuretic effect of S-8666 in the present study. Urinary sodium concentration was determined by flame photometry (Flame Photometer 775-A, Hitachi, Tokyo, Japan). Urinary concentrations of S-8666-(+) and S-8666(-) and their active demethylated metabolites, S-8680-(+) and S-8680(-), were measured by Shionogi & Co., Ltd., using high pressure liquid chromatography (1). The sensitivity of this assay was $0.2 \mu\text{g/ml}$ and the coefficient of variation was 3.0%. The results are expressed as the means $\pm$ 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 an 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. (Fig. 1). Urine volume and urinary sodium excretion increased dose-dependently after S-8666 in the day and night trials. 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 excretion of S-8666 was significantly greater in the day trial than in the night trial (Table 1). Urinary excretion of its active metabolite S-8680 also had a tendency to be greater at 12:00 a.m. than at 12:00 p.m. Urinary excretion of S-8666-(+) was smaller than that of S-8666(-), while the value of S-8680-(+) was greater than that of S-8680(-) in the present study.

The present study demonstrates that S-8666 produces an increased sodium and water diuresis when administered at 12:00 a.m. corresponding to the rats' sleep period compared to that administered at 12:00 p.m., which is their awake period. This finding is similar to the chronopharmacological profiles of furosemide in rats (5, 6). The present study

Fig. 1. Urine volume and urinary sodium excretion following oral administration of S-8666 at 12:00 a.m. (day trial) or 12:00 p.m. (night trial) in rats. Mean $\pm$ S.E., $n = 16$. Urine was collected for 8 hours after 4% 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 4% 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 rats

Parameter	Day trial		Night trial	
	S-30	S-90	S-30	S-90
S-8666-(+), $\mu\text{g/kg b.w./8 hr}$	984 $\pm$ 100*	3206 $\pm$ 351**	719 $\pm$ 67	1990 $\pm$ 297
S-8680-(+), $\mu\text{g/kg b.w./8 hr}$	2704 $\pm$ 178	7783 $\pm$ 599**	2666 $\pm$ 287	6241 $\pm$ 863
S-8666-(-), $\mu\text{g/kg b.w./8 hr}$	1629 $\pm$ 118*	5343 $\pm$ 472**	1177 $\pm$ 97	3734 $\pm$ 604
S-8680-(-), $\mu\text{g/kg b.w./8 hr}$	793 $\pm$ 61	2349 $\pm$ 223 [§]	703 $\pm$ 75	1907 $\pm$ 300

Mean $\pm$ S.E., n = 16. Urine was collected for 8 hours after S-8666 [30 mg (S-30) or 90 mg (S-90)/kg b.w.]. [§] = P < 0.10, * = P < 0.05, ** = P < 0.01, compared to the night trial

also shows that the urinary excretions of S-8666-(-) and its active metabolite S-8680-(-) are greater in the day trial than in the night trial. Since the main site of action of S-8666 is the luminal side of the thick ascending limb of Henle's loop (4), these time-dependent changes 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-(-). These daily variations in the urinary amount of the agent 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 (11); 2) higher excretion rate in the day trial compared to that in the night trial. This mechanism has been demonstrated by an intra-vascular injection study using furosemide (5, 6). Although a circadian variation is demonstrated in renal glomerular function (12, 13), a rhythmicity in renal tubular function has not been demonstrated. Since S-8666 and furosemide are secreted by renal tubules, a circadian variation might also exist in tubular secretory function.

Rats metabolize uric acid to allantoin in the liver with uricase (14). Therefore, when prop-

erties of uricosuric agents are evaluated using rats, the animals must be treated with the uricase inhibitor oxonate (15). Since the possibility that oxonate alters the chronopharmacological profiles of S-8666 can not be ruled out, 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. Although the amount of urinary uric acid was not determined, these data indicate that the uricosuric activity of S-8666 is also greater in the day trial than that in the night trial.

Urinary excretion of S-8666-(+) was smaller than that of S-8666-(-), while the excretion of the metabolite of S-8666-(+) was greater than that of the metabolite of S-8666-(-). Since the renal clearance of S-8666-(+) and S-8666-(-) did not differ (2), the metabolic clearance of S-8666-(+) might be greater than that of S-8666-(-). Further studies are needed to evaluate this hypothesis.

In summary, the present study demonstrated the time-dependent variations in the diuretic effects of S-8666 and the urinary excretion of S-8666-(-) and S-8680-(-). Since the amount of urinary S-8666-(+) and S-8680-

(+), which exert predominantly uricosuric action, varied with the administration time, the uricosuric activity of S-8666 might also be influenced by its time of dosing.

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