Daily Variation in the Effects of Furosemide in Rats

Akio Fujimura, Tsuyoshi Shiga, Toshiaki Sudoh, Kyo-ichi Ohashi and Akio Ebihara

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

Received June 8, 1992 Accepted September 3, 1992

ABSTRACT—Daily variation in the effects of furosemide, a loop diuretic agent, was examined in Wistar rats maintained under conditions of light from 7 a.m. to 7 p.m. and dark from 7 p.m. to 7 a.m. Furosemide (30 mg/kg) was given orally at 12 p.m., 4 a.m., 8 a.m., 12 a.m., 4 p.m. or 8 p.m. Urine was collected for 8 hr after furosemide administration, and urinary excretions of sodium and furosemide were determined. There were significant daily variations in the urine volume and urinary excretions of sodium and furosemide with a peak at 8 a.m. and a trough at 12 p.m. Significant correlations were observed between the urinary amount of furosemide and its diuretic effects (urine volume and urinary sodium excretion). These results suggest that the diuretic effects of furosemide show daily variations which are, at least in part, caused by the daily variation in the urinary excretion of furosemide.

Keywords: Furosemide, Diuretic effect, Daily variation

There is increasing evidence demonstrating time-dependent changes in the effectiveness of cardiovascular agents (1). We already examined some chronopharmacological profiles of furosemide, a loop diuretic agent, in rats (2, 3). These studies showed that the effects of furosemide are greater when it is administered at 12 a.m. during their resting period than when it is administered at 12 p.m. during their active period.

Furosemide is used for the treatment of patients with congestive heart failure and hypertension. As the duration of the diuretic effect of furosemide is relatively short, the agent is often given to these patients three or four times a day (4). Therefore, it is interesting to examine whether the diuretic effects of furosemide show the daily variation. To address this issue, the diuretic effects of furosemide and its urinary excretion were determined following furosemide at six different administration times.

MATERIALS AND METHODS

Male Wistar rats (Charles River Laboratory, Kanagawa, Japan), weighing 300 to 350 g, 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.

Six sets of trials were done in the present study. The administration of furosemide was randomly assigned to 12 p.m., 4 a.m., 8 a.m., 12 a.m., 4 p.m. or 8 p.m. Three percent body weight (b.w.) of 1% NaCl solution was given by gavage into the stomach in each trial. Twenty-four hours after the vehicle alone, 30 mg/kg of furosemide in 3% b.w. of vehicle was given orally. Urine was collected for 8 hr following the vehicle alone or the drug administration. Food and water were deprived during the 8 hr after each administration. The washout period between two sets of trials was 2 or 3 days.

The urinary sodium concentration was determined by flame photometry (775-A, Hitachi, Tokyo, Japan). The urinary furosemide concentration was measured by high pressure liquid chromatography (5).

The results are expressed as the means ± S.E. Analysis of variance with the randomized block design method was used to determine whether there was a significant daily variation in each parameter. The difference between two administration times was evaluated by the Wholly-Significant-Difference method.

RESULTS

There were significant daily variations in urine volume and urinary sodium excretion following 3% b.w. of NaCl solution (Figs. 1 and 2). Their peak and trough values were obtained at the 4 a.m. and 4 p.m. trials, respectively. Significant daily variations also were
observed in urine volume and urinary sodium excretion following furosemide and its urinary excretion with the peak at 8 a.m. and the trough at 12 p.m. (Figs. 1, 2 and 3). There were significant correlations between urinary furosemide and its effects (urine volume and urinary sodium) in each trial (Table 1).

DISCUSSION

We have recently published data indicating that furosemide produces an increased sodium and water diuresis when administered at 12 a.m. (mid-light) compared to that administered at 12 p.m. (mid-dark) (2, 3). The present study showed that the urine volume and urinary sodium excretion following furosemide have significant daily variation with a peak at 8 a.m. (early-light) and a trough at 12 p.m. (mid-dark). There was also similar daily variation in the urinary furosemide excretion. In addition, significant positive correlations were observed between the urinary furosemide excre-
tion and its diuretic effects in each trial. It is well-known that furosemide is excreted in the urine and thereafter, exerts its diuretic effects at the thick ascending limb of the loop of Henle (6). Therefore, the daily variations in the effects of furosemide might, at least in part, be caused by the daily variation in the amount of urinary furosemide.

Rats are nocturnal and they wake during the night-time and sleep during the daytime. As the daily variation in the activity of the adrenergic nervous system reflected by urinary norepinephrine excretion are in part dependent on sleep-wake cycles (7), it is suspected that the activity of this system is enhanced during the night-time in this nocturnal rodent. In fact, the turnover of norepinephrine in a peripheral organ (heart) during the night-time was faster than that during the daytime in rats (8). Our previous study has demonstrated that the urinary excretion of furosemide was smaller during the night-time in rats, and this time-dependent phenomenon was eliminated by pretreatment with 6-hydroxydopamine (9), which produces a selective destruction of adrenergic nerve endings. Such a time-dependent change in the urinary excretion of furosemide also was blunted in renal-denervated rats (A. Fujimura et al., submitted). Based on these findings, it is assumed that the adrenergic nervous system including the renal one contributes to the time-dependent change in the urinary excretion of furosemide. As the activity of this system during the night-time is suspected to be enhanced compared to that during the daytime in rats, it is hypoth-

Fig. 3. Daily variation in the urinary excretion of furosemide after the agent in rats. Mean ± S.E., n = 14.

<table>
<thead>
<tr>
<th>Relation</th>
<th>Time</th>
<th>Regression line</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary furosemide</td>
<td>12 p.m.</td>
<td>y = 0.027x + 18.6</td>
<td>0.66</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>4 a.m.</td>
<td>y = 0.046x + 11.4</td>
<td>0.72</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>8 a.m.</td>
<td>y = 0.028x + 22.5</td>
<td>0.63</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Urine volume</td>
<td>12 a.m.</td>
<td>y = 0.014x + 32.8</td>
<td>0.58</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>4 p.m.</td>
<td>y = 0.022x + 22.9</td>
<td>0.69</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>8 p.m.</td>
<td>y = 0.018x + 20.6</td>
<td>0.66</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

n = 14 for each administration time.
esized that the activated adrenergic system exerts an inhibitory influence on the renal excretion of furosemide. Therefore, we think that the daily variation in the activity of the adrenergic nervous system is involved in the mechanism of the daily variation in the urinary excretion of furosemide.

Furosemide is mainly excreted in the urine by renal tubular secretion, but a small fraction of the agent is excreted by glomerular filtration (6). Tubular as well as glomerular functions are considered to have daily variations (10). However, the role of glomerular filtration in the daily variation of the urinary furosemide excretion appears unlikely because the glomerular filtration rate estimated by creatinine clearance is greater during the night-time in rats (11) which, in turn, might lead to the increased excretion of furosemide during this period. Time-dependent changes in the absorption rate have been reported for several agents (12). As the chronopharmacokinetic study was not done in the present study, it remains possible that the daily variation in the absorption rate of furosemide is involved in the mechanism of the daily variation in the urinary furosemide excretion.

In the present study, urine volume and urinary sodium excretion following NaCl solution alone also showed daily variations with the peak at 4 a.m. (late-dark) and the trough at 4 p.m. (late-light). Rats eat and drink mainly during the night-time, and subsequent urine volume and urinary sodium excretion have daily variations with the peak during the late-dark period and the trough during a late-light period (13, 14). These daily variations in food and water consumption might contribute to the daily variation in the diuresis following NaCl solution. Finally, many hormonal factors involved in the regulation of water and electrolyte homeostasis exhibit daily variations (15–17). These factors might also influence the diuresis following NaCl solution and the diuretic effects of furosemide.

REFERENCES