Uricosuric and Diuretic Activities of DR-3438 in Dogs and Rabbits

Satoru TANAKA, Akira KANDA* and Shin-ichiro ASHIDA

Research Institute, Daiichi Pharmaceutical Co., Ltd.,
1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134, Japan

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Abstract—The purpose of this study was to evaluate the uricosuric and diuretic properties of the new diuretic agent DR-3438. In the conventional clearance studies in urate-loaded dogs, intravenous injection of DR-3438 (3–30 mg/kg) resulted in dose-related increases in fractional excretion of urate (FEua), urine flow and sodium excretion. At doses causing similar natriuresis, tienilic acid (50 mg/kg, i.v.) markedly increased the FEua value, whereas indacrinone (1 mg/kg, i.v.) had no significant effect on it. Trichloromethiazide (1 mg/kg, i.v.) and furosemide (0.3 mg/kg, i.v.) tended to decrease the FEua. Thus, the uricosuric activity of DR-3438 (30 mg/kg) was 0.6-fold that of tienilic acid and 3.4-fold that of indacrinone. In contrast, in urate-loaded rabbits that exhibit net tubular secretion of urate, intravenous DR-3438 (30 mg/kg) produced a significant decrease in FEua. Stop-flow studies in dogs revealed that DR-3438 (30 mg/kg) blocks both urate reabsorption and p-aminohippurate secretion in the proximal segment of the nephron and strongly inhibits reabsorption of water, sodium and potassium in the distal segments. These results suggest that DR-3438 exerts uricosuric activity through blocking urate transport in the proximal tubules and diuretic and saluretic activities by inhibiting water and sodium reabsorption in the distal segment of the nephron.

A chronic use of the thiazide diuretics or the more potent loop diuretics is reported to occasionally result in a rise in serum uric acid levels (1–5). It is likely that asymptomatic hyperuricemia is a risk factor for the development of gouty arthritis, uric acid kidney stone and urate nephropathy and for accelerating or developing atherosclerosis (6). In addition, hyperuricemia is associated with a number of diseases including hypertension (6). For these reasons, diuretic agents with uricosuric activity are expected to be more beneficial drugs in the treatment of hypertension.

DR-3438 [(5-chloro-3-(2-methylphenyl)-4-oxo-4H-benzopyran-7-yl)oxy]acetic acid (Fig. 1) is a new diuretic agent with pronounced uricosuric activity (7). We previously showed that oral administration of the drug resulted in an increase in urine volume and urinary sodium excretion in rats and dogs, and it also increased the fractional excretion of urate (FEua) in a clearance study in rats. This drug caused a decrease in systolic arterial blood pressure in experimental hypertensive rats (7).

The present studies were undertaken to evaluate the uricosuric and diuretic properties of DR-3438 and to estimate the site of action of this drug with respect to its uricosuric and diuretic effects in dogs loaded with urate. Some studies were also performed to evaluate

* To whom all correspondence should be addressed.

Fig. 1. Chemical structure of DR-3438.
its effect on the urate transport in the nephron of urate-loaded rabbits because rabbits, but not humans and dogs, reveal net urate secretion in proximal tubules (8).

Materials and Methods

Drugs: DR-3438, indacrinone (MK-196) and trichloromethiazide were dissolved in 8.5% N-methylglucamine. Tienilic acid (ticrynafen) was dissolved in 4% triethanolamine. All drug solutions (pH 7-8) were administered intravenously at a volume of 0.3 ml/kg. The control group was given 8.5% N-methylglucamine alone at the same volume (pH 7-8). Trichloromethiazide (Fukuzyu Seiyaku) and furosemide (LASIX, Hoechst) were commercial preparations. The other compounds used were prepared in our laboratories.

Clearance experiments in dogs: Mongrel dogs of either sex, weighing 10 to 31 kg, were used. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and were given additional doses as necessary. Tracheotomy was performed and the animal was artificially ventilated. The right common carotid artery was cannulated with a polyethylene catheter for continuous monitoring of arterial blood pressure and for sampling of arterial blood. Both ureters were cannulated with a polyethylene catheter through a midline abdominal incision for collections of urine samples. The right femoral vein was catheterized for continuous monitoring of arterial blood pressure and for infusing loading-solution that consisted of 0.1% uric acid, 0.3% creatinine, 2.5% mannitol and 0.4% NaCl. After completion of the surgical preparation, all animals received a bolus injection of creatinine (50 mg/kg) followed by continuous infusion of loading-solution at a rate of 0.3 ml/kg/min via the right femoral vein. The temperature of the loading-solution was kept close to 37°C at pH 7.4. After about 90 min of equilibration, urine samples from 3 basal periods were collected before the intravenous injection of drugs. Arterial blood samples were obtained at the midpoint of each clearance period. Concentrations of urate, creatinine, Na⁺ and K⁺ in plasma and urine were determined. The glomerular filtration rate (GFR) was estimated by the creatinine clearance. Fractional excretion of uric acid (FEua) was calculated by dividing uric acid clearance by creatinine clearance. Results were expressed as the absolute change from the average of three basal values. The concentration of plasma urate was 2.4±0.1 mg/dl before the administration of drugs.

Clearance experiments in rabbits: Male New Zealand rabbits weighing 3.6 to 4.1 kg were used. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and were given additional doses as necessary. The clearance studies in rabbits were performed as in dog studies, but the infusion rate of the loading-solution was 0.4 ml/kg/min. Results in rabbits were calculated and expressed as they were in dog studies. The concentration of plasma urate was 3.2±0.4 mg/dl before the administration of drug.

Stop-flow experiments: The stop-flow experiment was performed by the method of Malvin et al. (9). Five mongrel dogs of either sex, weighing 13 to 20 kg, were used. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and were given additional doses as necessary. The surgical preparations were performed as in the clearance studies in dogs, but the left ureter was catheterized for collection of urine samples. All animals received intravenous injection of creatinine followed by continuous infusion of loading-solution that consisted of 0.1% uric acid, 0.2% creatinine, 5.0% mannitol, 0.5% para-aminohippuric acid (PAH) and 0.4% NaCl at the rate of 0.4 ml/kg/min. The temperature of the loading-solution was kept close to 37°C at pH 7.4. After about 90 min of equilibration, three samples of both blood and urine were obtained as a free-flow experiment with 3-min intervals, and then the left ureter was occluded for 6 min. At forty-five seconds before the release of the occlusion, inulin (50 mg/dog) was intravenously injected as the glomerular marker. Forty serial urine samples of approximately 0.5 ml each were collected. A blood sample was obtained at the midpoint of the occlusion period. At least 90 min after the first stop-flow trial, the second trial was similarly performed in the same dog. The second ureteral occlusion was performed 15 min after an intravenous administration of DR-3438 at 30 mg/kg. The stop-flow pattern
was reproduced well in the two trials. Intravenous injection of vehicle did not induce any marked change between the two stop-flow patterns. Concentrations of urate, creatinine, PAH, Na⁺ and K⁺ in plasma and urine were determined, and the U/P ratio of the concentration of each substance over the U/P ratio of the concentration of creatinine was calculated as a parameter of the stop-flow pattern.

**Analytical methods:** Concentrations of various substances in plasma and urine were determined by spectrophotometry as follows: urate by the uricase-peroxidase method (10) and creatinine by the Jaffe method (11). PAH by the p-dimethylaminocinnamaldehyde method (12) and inulin by the anthrone method (13). Sodium and potassium in plasma and urine were measured with electrodes (PVA-4, Photovolt).

**Statistical analysis:** All results are expressed as the mean±S.E.M. Statistical analysis was performed using ANOVA or Student's unpaired t-test to compare results of untreated groups with those of treated groups with drug. In addition, Student's paired t-test was used to compare basal values with those after the drug administration. A P value of less than 0.05 was considered as statistically significant.

**Results**

1. Effects in the clearance studies in dogs:

Base-line values of urine flow rate and FEua in the four groups treated with DR-3438 were 1.11±0.17 ml/min and 0.81±0.04 at the dose of 0 mg/kg, 1.50±0.23 ml/min and 0.76±0.06 at the dose of 3 mg/kg, 1.55±0.31 ml/min and 0.65±0.02 at the dose of 10 mg/kg, and 1.81±0.52 ml/min and 0.64±0.05 at the dose of 30 mg/kg, respectively. Intravenous bolus injection of DR-3438 at doses of 3–30 mg/kg resulted in significant (P<0.05–0.01) dose-dependent increases in urine flow and FEua, as shown in Fig. 2. Urinary excretion of sodium was also increased significantly (P<0.05–0.01) and dose-dependently by intravenous DR-3438 in a time-course similar to that observed in urine flow (data not shown). The onset of its diuretic, natriuretic and uricosuric responses were immediate. The peak effects of these responses were reached within 20 min of the administration of DR-3438. DR-3438 at the highest dose produced maximal increases by 470% in urine flow, by 1214% in sodium excretion and by 126% in FEua at this time. Thereafter, the diuretic and natriuretic responses to DR-3438 declined gradually to near baseline level within 90 min, whereas a significant (P<0.05) uricosuric response was observed 70 min after DR-3438. As shown in Table 1, DR-3438 (30 mg/kg) and tienilic acid (50 mg/kg) caused significant increases in total urine flow, total urinary excretion of sodium and total FEua during 90 min after administration of the drugs. Indacrinone (1 mg/kg) produced significant increases in total urine flow and total urinary excretion of sodium and caused a slight increase in total FEua during the same
period. The peak effect of tienilic acid on FEua was noted within 20 min of the administration and lasted for 90 min. The maximal FEua was induced within 10 min after indacrinone administration and followed by a gradual decline towards the baseline level for 90 min. The FEua value of DR-3438 (30 mg/kg) was 0.6-fold that of tienilic acid (50 mg/kg) and 3.4-fold that of indacrinone (1 mg/kg). Trichloromethiazide, on the other hand, slightly decreased total FEua and produced significant diuretic and saluretic responses. Furosemide also decreased total FEua and caused slight diuretic and significant saluretic effects. Baseline values of GFR were 47.0±4.4, 48.1±6.0, 60.1±10.0 and 57.3±5.2 ml/min among the four groups that received 0, 3, 10 and 30 mg/kg of DR-3438, respectively, and were not significant different in all subgroups of dogs. The administration of DR-3438 at the dose of 3 mg/kg had no significant effect on GFR. However, GFR was maximally decreased by 42% (P<0.01) 20 min after administration of DR-3438 at the dose of 10 mg/kg and gradually recovered to the basal level during 90 min; and, at the dose of 30 mg/kg, it was significantly (P<0.01) decreased by 32% within 20 min after the drug and was kept at about this level for 90 min.

Table 1. Effect of DR-3438 and other diuretics on total urine flow, total urinary excretion of sodium and fractional excretion of urate in urate-loaded dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg, i.v.)</th>
<th>N</th>
<th>Total JUF (ml/90 min)</th>
<th>Total JUNaV (Eq/90 min)</th>
<th>Total JFEua</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>6</td>
<td>30.0±0.3</td>
<td>-0.04±0.06</td>
<td>-0.10±0.34</td>
</tr>
<tr>
<td>DR-3438</td>
<td>50</td>
<td>4</td>
<td>25.3±5.4**</td>
<td>3.63±0.72**</td>
<td>1.06±0.27*</td>
</tr>
<tr>
<td>Tienilic acid</td>
<td>1</td>
<td>4</td>
<td>36.2±6.8**</td>
<td>4.24±0.86**</td>
<td>0.31±0.09</td>
</tr>
<tr>
<td>Indacrinone</td>
<td>1</td>
<td>3</td>
<td>12.6±1.1**</td>
<td>2.39±0.08**</td>
<td>-0.16±0.14</td>
</tr>
<tr>
<td>Trichloromethiazide</td>
<td>1</td>
<td>3</td>
<td>19.2±5.9</td>
<td>1.80±0.87*</td>
<td>-0.75±0.31</td>
</tr>
</tbody>
</table>

The effects are expressed as the absolute change from the basal value. Each value represents the mean±S.E.M. N: number of animals. UF: urine flow, UNaV: urinary excretion of sodium, FEua: fractional excretion of urate. *P<0.05, **P<0.01, significantly different from the control.
excretion of sodium was also increased significantly (P<0.01) and dose-dependently by intravenous DR-3438 in a similar time-course to that observed in urine flow. Baseline values of GFR in untreated- and treated-groups with DR-3438 were 13.8±3.0 and 13.7±3.7 ml/min, respectively. GFR was slightly increased 10 min after the administration of DR-3438 and thereafter was significantly (P<0.01) decreased by 27% by 20 min after DR-3438. The peak decreasing effect in GFR obtained 50 min (by 46%) after the drug.

3. Effect in the stop-flow studies: Intravenous administration of DR-3438 (30 mg/kg) caused similar changes in the quality of stop-flow patterns in all three dogs studied. Figure 4 illustrates a typical stop-flow pattern from an animal that received DR-3438.

Following the administration of the drug, the ratios of (U/P Na\(^+\))/(U/P Cr) and (U/P K\(^+\))/(U/P Cr) were increased in the distal and proximal segments of the nephron as compared with the control. On the other hand, the ratio of U/P Cr decreased in both segments. During the control period, a maximal decrease in the (U/P UA)/(U/P Cr) ratio was noted in the proximal segment, together with a maximal increase in the (U/P PAH)/(U/P Cr) ratio. These changes were markedly inhibited after administration of DR-3438.

Discussion

In humans and other primates, uric acid is the end product of purine metabolism. Most nonprimate mammals have the enzyme uricase that can degrade uric acid into the more soluble product allantoin. Although there is a significant difference in the purine metabolism between humans and most nonprimate mammals, there are several points of similarity in the renal handling of urate between them (8). It is known that urate is freely filtered at the glomerulus, since direct determinations of the concentration of urate in the glomerular ultrafiltrate and plasma indicate that this ratio is close to unity (14). In several species, for instance the human, rat and mongrel dog, the fraction of filtered urate in the urine is less than 100%, indicating net reabsorption of urate in the proximal tubule (8). Consequently, it seems appropriate to estimate the influence of the drug on urate transport in dog nephron of which the characteristics are similar to those of human nephron.

In the present clearance studies in urate-loaded dogs, tienilic acid exerted uricosuric activity, as demonstrated by a significant increase in FEua. A slight increase in the uricosuric effect was observed with indacrinone. On the other hand, trichloromethiazide and furosemide showed a tendency to exert antiuricosuric activity, as demonstrated by a slight decrease in FEua. These results are in accord with the effects of these drugs on urate handling in human kidney. Therefore, this finding indicates that the effect of a drug on renal urate handling in humans can be estimated by a urate clearance study in urate-loaded dogs, as described above. Under the same experimental conditions, DR-3438
exerted a significant and dose-dependent uricosuric activity accompanied with diuretic and natriuretic activities. The uricosuric effect of DR-3438 lasted longer than the diuretic effect. Moreover, the uricosuric activity of DR-3438 was 1.7 times less potent than that of tienilic acid and 3.4 times more potent than that of indacrinone. DR-3438 caused a significant decrease in GFR in dogs and rabbits. This phenomenon may have resulted from an elevation of intratubular pressure and a decrease in plasma volume caused by the significant diuretic effect of DR-3438. The drug has been demonstrated to produce a significant but transient increase in renal blood flow as measured with an electromagnetic probe in anesthetized dogs (A. Kanda et al., unpublished results). These findings suggest the possibility that the diuretic effect of DR-3438 may not depend on changes in GFR and renal blood flow.

Tienilic acid has been shown to enhance markedly the urinary excretion of uric acid in rats (15), dogs (15, 16) and humans (17–20). In our present studies, tienilic acid had the most potent uricosuric activity among all the diuretics tested here. Because of its potent uricosuric activity, tienilic acid was reported to cause renal stones due to a high uric acid concentration in the kidney (21–23). Therefore, we consider that DR-3438 may not exert side effects such as the renal stones observed with tienilic acid described above, because the uricosuric activity of DR-3438 was less than that of tienilic acid. Indacrinone has been reported to possess both natriuretic and uricosuric properties in animals (24) and humans (25). The uricosuric activity of indacrinone is too transient and is not sufficient at clinically used doses to prevent the rise in urate that is normally observed after diuretic therapy (26–28). In the present study, the uricosuric activity of indacrinone was not continuous and was less potent than that of DR-3438 in urate-loaded dogs. From these findings, we suppose that DR-3438 which possesses less potent uricosuric activity than tienilic acid and is more potent than indacrinone may cause useful uricosis in diuretic therapy. To ensure therapeutic efficacy, it is necessary to determine the beneficial ratio of the uricosuric activity to diuretic activity induced by a drug, because at present, the most beneficial ratio is yet unknown.

Mongrel dogs usually exhibit net reabsorption in the nephron as humans do, but on the contrary, rabbits exhibit net secretion under the urate-loaded condition (8). These are in accord with our results that FEu was less than 1 in urate-loaded dogs and was more than 1 in urate-loaded rabbits. DR-3438 caused an increase in FEu in dogs and a decrease in FEu in rabbits. Our results suggest that DR-3438 inhibits the reabsorption and secretion of urate in the proximal segment of the nephron.

In the stop-flow study, DR-3438 caused prominent depression in the reabsorption of the electrolytes and water in both proximal and distal parts of the nephron. However, it is difficult to determine the effect of drugs in the proximal part from stop-flow patterns, because the proximal patterns were influenced by the condition in the distal segment of the nephron as a shortcoming in the stop-flow method (29). It is therefore suggested that DR-3438 causes diuresis and natriuresis mainly in the distal part of the nephron. Urate reabsorption and PAH secretion were blocked in the proximal tubules after DR-3438 administration. Similar results were reported with tienilic acid by Lemieux et al. (15). These findings suggest that DR-3438 and tienilic acid have a similar mechanism of action. Additionally, we cannot use PAH clearance to evaluate renal plasma flow following administration of DR-3438 and tienilic acid because of the inhibiting effect of these drugs on PAH secretion.

In summary, our results suggest that DR-3438 induces the uricosuric effect in the proximal segment of the nephron, whereas it produces diuretic and natriuretic effects mainly in the distal segment, and that the uricosuric activity of DR-3438 may be between those of tienilic acid and indacrinone.

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