Regular Article

Studies on the Metabolic Fate of M17055, a Novel Diuretic (5):
Pharmacokinetics and Pharmacodynamics of Unchanged Drug in Rat and Dog After Intravenous Administration of M17055

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Summary: The pharmacokinetics and pharmacodynamics of M17055, a novel diuretic were studied after a single intravenous administration to rats and dogs, the two species used in the pharmacological and toxicological studies. No gender dependent response to systemic exposure was observed at the high dose level in rats, in agreement with the determined LD50. A gender difference in urinary excretion of M17055, however, was clearly observed in rats. The slower elimination and the lower total body clearance (CLtot) values of M17055 in dogs reflect the difference of the no-effect level (NOEL) between rats (0.1 mg/kg) and dogs (0.03 mg/kg) well. The diuretic response was well correlated with the urinary M17055 excretion rate by fitting to a sigmoid Emax model in both rats and dogs. The derived ER50 value of M17055 in dogs was approximately 10 times less than that reported for furosemide, suggesting that the intrinsic potency of M17055 is equal to or higher than those of other powerful loop diuretics. Although diuretic sensitivity was considered to be lower in dogs than in rats, the higher amount of M17055 reaching the dog kidney is likely to compensate for this. The diuretic response in female rats was predictable by using the pharmacodynamic parameters derived from male rats.

These results show that the apparent high diuretic potency and the other pivotal observations for M17055 found in the pharmacological and toxicological studies can be rationalized by the pharmacokinetic and pharmacodynamic properties of the unchanged compound.

Key words: M17055; diuretic; systemic exposure; urinary excretion rate; pharmacokinetics; pharmacodynamics

Introduction

M17055, 7-chloro-2,3-dihydro-1-(2-methylbenzoyl)-4(1H)-quinolinone 4-oxide-O-sulfonic acid potassium salt, was discovered as a novel diuretic derivative of quinolinone oxime sulfonic acid1 and exhibits potent diuretic activity when administered to rats, mice and dogs.2 The mechanism of action is reported to be that M17055 acts not only on the thick ascending limb of Henle's loop but also on the distal segments via inhibition of electrogenic Na+ transport.3

In the in-vivo pharmacological and toxicological evaluation of M17055, our colleagues demonstrated that the dose causing half-maximal effect (ED50) of M17055 was much lower than that of furosemide, and the no-effect level (NOEL) and ED50 were estimated to be higher in rats than in dogs. The half-lethal dose (LD50) estimated in rats showed no gender dependence. Studies on the pharmacokinetics and disposition of M17055 were also conducted by using [14C]M17055,4–7 in which we reported that the systemic exposure and the urinary excreted amount of labeled compound was higher in dogs than in rats. The relative amount of the circulating metabolites was found to be high in rats but low in dogs. However, the issue, whether the main pharmacological and toxicological properties can be attributed to the kinetics and dynamics of M17055 itself, still remains.

The aim of this study was to investigate systemic exposure of the unchanged compound, and the relationship between its amount at the site of action and the diuretic response after intravenous administration of M17055 to rats and dogs, in order to interpret the pharmacological and toxicological events for this drug can-
Materials and Methods

Chemicals and animals: M17055 (Fig. 1) was supplied by the Pharmaceutical Laboratory of Mochida Pharmaceutical Co., Ltd. (Shizuoka, Japan), as described in a previous report.4 M13268 (7-chloro-2,3-dihydro-1-(3-methylbenzoyl)-4(1H)-quinolinone 4-oxime-O-sulfonic acid potassium salt) was synthesized in our laboratories and used as the internal standard for HPLC analysis. The other reagents and solvents used in this study were commercially available products of analytical grade or chromatographic grade.

Wistar rats of 8 weeks of age weighing 187–221 g for males and 132–157 g for females from SLC (Shizuoka, Japan), and Beagle dogs of 1–3 years of age weighing 10.2–11.0 kg for males and 10.2 kg for females from Hazleton Research Animals Inc. (TX, USA) were used in this study. The animals were kept in an animal room at the constant temperature (23 ± 2°C) and humidity (55 ± 15%), with 12 hr of light per day and were allowed free access to water and were given a commercially available diet ad libitum.

Dosing and sample preparation: M17055 was dissolved in 75 mM phosphate buffer (pH 7.0) to make up each dose formulation at the concentrations of 3, 10 and 30 mg/mL for rats and 5 mg/mL for dogs. These formulations were administered intravenously to rats at doses of 3, 10 and 30 mg/kg and to dogs at a dose of 1 mg/kg.

Blood samples were withdrawn via the jugular vein of each three rats at each time point (sparse sampling), or via the sephalic vein of each three dogs at various time points (full sampling) with heparinized syringes, and were centrifuged (1,700 g) for 15 min at 4°C to obtain the plasma samples. Urine was collected continuously up to 24 hr after administration. The collection intervals of urine samples for the pharmacodynamic analysis were as follows.

Male rats: 0–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–12 and 12–24 hr
Female dogs: every hour up to 12 hr and 12–24 hr

Procedure for determination of M17055 in plasma and urine: Fifty microliters of the internal standard (M13268) solution in 20 mM phosphate buffer (PB) were added to 500 μL of the plasma samples. After washing twice with ethyl acetate, 6% tetra-n-butylammonium bromide (t-BAB, w/v) in water or 20 mM PB was added to the aqueous layer. Subsequently, M17055 was extracted twice with ethyl acetate. The organic layer was transferred to other tubes and washed once with 1% t-BAB in 20 mM PB. After removing the organic solvent under reduced pressure, the dried extract was reconstituted in the mobile phase buffer for HPLC and submitted for quantitative analysis. Urine was centrifuged to remove debris. The derived supernatant was filtered with a filter cartridge (Sunprep4 (T)-HV, Millipore Corporation, MA, USA) and the filtered aliquots were submitted for quantitative analysis using HPLC.

HPLC conditions for quantitative analysis: A JAS- CO LC-800 or TR1-ROTAR-VI HPLC system (Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with either an 805-GI (Japan Spectroscopic), Maxima 820J or Millennium integrator (Millipore) was used for the determination of M17055. Analyses were performed on an Inertsil ODS-2 (4.6 × 250 mm, GL Science, Tokyo, Japan) or a YMC Pack A-312 ODS (6 × 150 mm, YMC, Tokyo, Japan) column at 1.0 mL/min using isocratic elution. Mobile phases used were 48% acetonitrile/52% 20 mM PB (pH 7.0) containing 10 mM t-BAB for rat plasma and urine, 45% acetonitrile/55% 20 mM PB (pH 7.0) containing 5 mM t-BAB for dog plasma, and 37% acetonitrile/63% 20 mM PB (pH 7.0) for dog urine, respectively. The detection wavelength and the column temperature were set at 270 nm and 35°C, respectively.

Pharmacokinetic and pharmacodynamic analyses: The elimination half-life (T 1/2), the area under the plasma concentration-time curve from time 0 to infinity (AUC0-∞), the volume of distribution at steady state (Vdss) and the total body clearance (CLtot) were estimated by the nonlinear least squares program PCNONLIN (Scientific Consulting Inc.) with a 2-compartment model. The renal clearance (CLR) was calculated by dividing the amount excreted unchanged in urine up to 24 hr (Ae(0–24hr)) by AUC0-∞. The non-renal clearance (CLnr) was calculated by subtracting CLR from CLtot. The gender differences between the mean values for Ae and CLR were evaluated by Student’s t-test. Pharmacodynamic analysis was assessed by relating the logarithm of the urinary M17055 excretion rate (ng/min/kg) to the response quantified as the urine flow rate (μL/min/kg).8–11

The sigmoid-shaped curves were analyzed using the
Fig. 2. Plasma concentrations of M17055 after a single intravenous administration of M17055 to rats. Gray circles, open squares and closed triangles express the observed values of individual animals at doses of 3, 10 and 30 mg/kg, respectively. The biphasic lines express the regression curves.

Table 1. Pharmacokinetic parameters of M17055 after a single intravenous administration of M17055 to male and female rats at doses of 3, 10 and 30 mg/kg

<table>
<thead>
<tr>
<th>Dose</th>
<th>Gender</th>
<th>T1/2α (hr)</th>
<th>T1/2β (hr)</th>
<th>AUC0-∞ (ng·hr/mL)</th>
<th>CLtot (mL/min/kg)</th>
<th>Vdss (mL/kg)</th>
<th>Ae(C0-24h) (% of dose)</th>
<th>CLr (mL/min/kg)</th>
<th>CLnr (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/kg</td>
<td>Male</td>
<td>0.099</td>
<td>0.354</td>
<td>1788</td>
<td>28.0</td>
<td>135</td>
<td>8.0 ± 2.5</td>
<td>25.7 ± 0.7</td>
<td>28.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.098</td>
<td>0.315</td>
<td>1376</td>
<td>36.3</td>
<td>144</td>
<td>21.6 ± 2.5*</td>
<td>34.7 ± 0.5</td>
<td>31.0 ± 1.0</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>Male</td>
<td>0.166</td>
<td>1.37</td>
<td>4218</td>
<td>39.5</td>
<td>430</td>
<td>12.1 ± 1.2</td>
<td>4.77 ± 0.48</td>
<td>34.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.107</td>
<td>0.534</td>
<td>4300</td>
<td>38.8</td>
<td>3082</td>
<td>20.1 ± 2.6*</td>
<td>7.78 ± 1.01*</td>
<td>34.7 ± 0.5</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>Male</td>
<td>0.206</td>
<td>1.48</td>
<td>23907</td>
<td>20.9</td>
<td>26631</td>
<td>9.5 ± 2.0</td>
<td>18.9 ± 0.4</td>
<td>14.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.108</td>
<td>0.858</td>
<td>3756</td>
<td>18.8</td>
<td>66</td>
<td>22.1 ± 3.3*</td>
<td>18.9 ± 0.4</td>
<td>14.6 ± 0.6</td>
</tr>
</tbody>
</table>

Each parameter was estimated with a data set consisting of all sparse-sampling points at each dose. Standard error for estimation. Each value represents the mean ± S.D. of three animals. *Significantly different from male rats (P < 0.01).

nonlinear least squares program, WinNonlin (Pharsight Corporation) with a sigmoid E_max model12) expressed by the following equation. The initial values of ER50 and E_max for regression were obtained by graphic analysis. The initial value of γ was 1.0.

\[ E = E_0 + (E_{\text{max}} - E_0) \times \frac{R^\gamma}{R^\gamma + ER_{50}} \]

R: Urinary M17055 excretion rate
E_0: Basal response of urine flow rate (fixed as the prior 24 hr value)
γ: Slope factor
ER50: Urinary M17055 excretion rate causing half-maximal response
E_max: maximal response of urine flow rate

The pharmacodynamic parameters derived from the data in male rats at 3 mg/kg were used for the prediction of overall diuretic response at each dose level in male and female rats.

Results

Pharmacokinetics of M17055 in rat: The plasma concentration-time data and the best-fit curves obtained by nonlinear regression analysis are shown in Fig. 2. The derived pharmacokinetic parameters are summarized in Table 1. CLtot values were found similar for male and female and decreased at the highest dose in
both sexes. Larger Ae and CLr values were observed in female rats than in male rats. In contrast, CLnr values were similar for male and female rats.

Pharmacokinetics of M17055 in dog: The plasma concentration-time data and the best-fit curves obtained by nonlinear regression analysis are shown in Fig. 3. The derived pharmacokinetic parameters are summarized in Table 2. Similar pharmacokinetic parameters were found in male and female dogs. T1/2 values were longer and CLtot values were 4 or 5 times less than those in rats.

A larger proportion of M17055 than that in rats was excreted into urine in both male and female dogs within 24 hr of administration.

Pharmacodynamic analysis: The relationship between the urinary M17055 excretion rate and the diuretic response (shown as the urine flow rate) in rats and dogs is shown in Fig. 4. A good fit was achieved by using the sigmoid Emax model in both rats and dogs. The main difference in the pharmacodynamic parameters between rats and dogs was revealed in the ER50 values, indicating that the urinary M17055 excretion rate in female dogs needed to be approximately 2.8 times that in male rats to cause a half-maximal response (Table 3).

The overall diuretic responses in male and female rats are shown in Fig. 5. The urinary volume corrected for body weight within 24 hr after intravenous administration was larger in female rats than in male rats at each dose level. The observed values of urinary volume in male and female rats at each dose level well fitted to the predicted values derived from the pharmacodynamic parameters, except for the value in female rats at 30 mg/kg.

Discussion

In the present study, we investigated the pharmacokinetics and the pharmacodynamics of M17055 in rats and dogs to evaluate the rate and extent of systemic exposure, and also the relationship between the amount of this compound reaching the principal site of action and the diuretic response.

When M17055 was administered intravenously to rats, AUC0–∞ and CLtot were similar for male and female rats especially at the high dose level, suggesting that any gender difference of M17055 for systemic exposure is slight in rats. The result agrees well with that in the acute toxicological studies (LD50: 383 mg/kg for males and 350 mg/kg for females). Ae and CLr values in female rats were approximately double those in male rats. When CLr were corrected for plasma unbound fraction (2.5%), the renal clearance of the plasma unbound M17055 much exceeded the glomerular filtration rate (5.24 mL/min/kg) in both male and female rats. Thus the gender difference in the urinary excretion and renal clearance of the unchanged compound may be caused by a gender difference in the renal secretory mechanism, which has often been found in rodents.

In contrast, non-renal clearance showed a practically constant value for male and female rats at each dose level, suggesting that a gender-dependent factor does not exist for non-renal clearance (primary metabolic elimination) of M17055 in rats. The nonlinear kinetics ob-
Fig. 4. Relationship between urinary M17055 excretion rate and diuretic response (shown as urinary flow rate) after intravenous administration of M17055 to rats and dogs. The sigmoid-shaped lines express the regression curves. Closed and open circles express the observed values of individual animals at all time points after intravenous administration of M17055 to male rats (dose: 3 mg/kg) and female dogs (dose: 1 mg/kg), respectively.

Table 3. Pharmacodynamic parameters of M17055 after intravenous administration of M17055 to rats and dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Dog</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>E&lt;sub&gt;max&lt;/sub&gt; (μL/min/kg)</td>
<td>466</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td>391</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER&lt;sub&gt;50&lt;/sub&gt; (ng/min/kg)</td>
<td>216</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
<td>608</td>
<td>133</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ</td>
<td>1.0</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td>1.4</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aStandard error for estimation.

Fig. 5. Urinary volume excreted within 24 hr after intravenous administration of M17055 to rats at doses 3, 10 and 30 mg/kg. Each bar represents the mean ± S.D. of three animals. The dashed line expresses the basal response. ◇: Predicted value derived from pharmacodynamic parameters and urinary M17055 excretion rate.

...serve in rats at the highest dose may be caused by the saturation of both hepatic drug metabolism and renal tubular secretion, considering the reduced CL<sub>r</sub> and CL<sub>n</sub>r values.

The slower elimination and the lower CL<sub>tot</sub> values of M17055 in dogs reflect the difference of the NOEL level between rats (0.1 mg/kg) and dogs (0.03 mg/kg) estimated in our chronic toxicological studies. Derived pharmacokinetic parameters, including CL<sub>r</sub>, demonstrate that the gender difference of M17055 kinetics in dogs is slight. The longer T<sub>1/2</sub> and the higher Ae values indicate that M17055 reaches to the site of action more efficiently in dogs than in rats.

The pharmacological potency of most loop diuretics has been evaluated by correlating the diuretic response (generally quantified as the sodium excretion rate) with the drug excretion rate into the urine with a sigmoid-shaped dose-response curve. In our pharmacodynamic analysis, the diuretic response of M17055 was evaluated by the urine flow rate, because the increase of urine flow is the primary basis for determining the drug’s therapeutic efficacy for edema, which is the main target of clinical application of M17055. The derived ER<sub>50</sub> value of M17055 in dogs, which reflects the intrinsic potency for loop diuretics, was about 10 times less than that reported for furosemide, a conventional loop diuretic. Based on the ratio of ER<sub>50</sub> values reported for other loop diuretics on the clinical stage, piretanide, azosemide and torasemide are considered to have similar potency and be 5–8 times more potent than furosemide. Thus the result indicates that the...
Pharmacokinetics and Pharmacodynamics of M17055

The diuretic potency of M17055 is equal to or stronger than those of other powerful loop diuretics. The $T_{1/2\beta}$ value for M17055 in dogs was about 3 times longer than that reported for furosemide (about 0.9 hr),\textsuperscript{19} suggesting its long-lasting potency. In fact, M17055 induced the clear increase of urinary volume in dogs up to 4 hr post dose, while furosemide induced it up to 2 hr.\textsuperscript{19} Although the fraction excreted in urine of M17055 in dog was somewhat less than that of furosemide (60% of the dose),\textsuperscript{19} the parameters derived in the present study would support an apparent diuretic activity of M17055 in dogs that was about 10 times more potent than that of furosemide.\textsuperscript{21}

On the other hand, the ER$_{50}$ values suggest that M17055 is essentially more potent in rat than in dog, in contrast to the result of an in vivo pharmacological study, which demonstrates that M17055 has a stronger effect in dog (ED$_{50}$: 0.1 mg/kg) than in rat (ED$_{50}$: 0.3 mg/kg) when administered intravenously. The fraction of M17055 excreted in the urine accounted for 37.9% of the dose in female dogs, which corresponds to a level that is 4.7 times greater than that (8.0% of the dose level) found in male rats. Moreover, the $T_{1/2\beta}$ was considerably longer in dogs than in rats. Thus, the discrepancy between the result of the in-vivo pharmacological study and that of the pharmacodynamic analysis introduced in the present study, was attributed to the marked species difference of the rate and the amount of the drug reaching to the site of action between rats and dogs.

The larger urinary volume in female rats than in male rats observed at each dose level reflects a gender difference in the urinary M17055 excretion as well. The diuretic response in female rats was predictable by using the pharmacodynamic parameters derived from male rats. These results suggest that the gender difference in the diuretic response is predominantly controlled by a pharmacokinetic factor. The unexpectedly low diuretic response observed in female rats at the highest dose may reflect an acute tolerance, in order to maintain homeostasis of body fluid, as is often observed for other loop diuretics.\textsuperscript{20,21}

In conclusion, M17055 is considered to have much higher potency than furosemide based on the pharmacodynamic parameters, and the pivotal observations found in the pharmacological and toxicological studies for M17055 in rats and dogs can be rationalized by the pharmacokinetic and pharmacodynamic properties of the unchanged compound observed in the present study.

References


