SPECIES DIFFERENCES IN PHARMACOKINETICS OF A HEPATOPROTECTIVE AGENT, YH439, AND ITS METABOLITES, M4, M5, AND M7, AFTER INTRAVENOUS AND ORAL ADMINISTRATION TO RATS, RABBITS, AND DOGS

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ABSTRACT:
Pharmacokinetic parameters of YH439 and its metabolites, M4, M5, and M7, were compared after iv administration of YH439 to rats (1–10 mg/kg), rabbits (1–10 mg/kg), and dogs (1–20 mg/kg) and oral administration of YH439 to rats (50–500 mg/kg) and dogs (0.5–2 g per whole body weight). After oral administration of YH439 to rats, the F values were 3.67, 1.33, and 0.859% for YH439 oral doses of 100, 300, and 500 mg/kg, respectively. However, the F value increased significantly, 21.2%, after oral administration of YH439-contained mixed micelles (10 mg as free YH439) to rats due to increased water solubility of YH439. Species differences in the pharmacokinetics of YH439 and its metabolites were found. First, M7 was detected in both plasma and urine after both iv and oral administration of YH439 to dogs, whereas it was detected neither in rats nor in rabbits, indicating that considerable amount of M7 was formed from YH439 only in dogs. Second, the AUC (or $AUC_{M7}$) ratios of M4 to YH439 after iv administration of YH439 were 24.6–31.3, 42.2–49.2, and 2200–7640% for rats, rabbits, and dogs, respectively, indicating that formation of M4 after iv administration of YH439 was maximal in dogs. Third, the AUC (or $AUC_{M7}$) ratios of M5 to YH439 after iv administration of YH439 were 103–127, 2.93–3.31, and 92.4–158% for rats, rabbits, and dogs, respectively, indicating that formation of M5 after iv administration of YH439 was minimal in rabbits.

YH4391 (fig. 1), a malotilate analog, was developed as a hepatoprotective agent by Yuhan Research Center of Yuhan Corporation (Kunpo, South Korea). YH439 undergoes aromatic hydroxylation by cytochrome P450, followed by formation of M7 by esterase or M4 by amidase (fig. 1). M7 is further metabolized to M4 by amidase. YH439 is also metabolized to M5 by oxidation. The metabolites seemed to have negligible hepatoprotective activity compared with YH439 is also metabolized to M5 by oxidation. The metabolites seemed to have negligible hepatoprotective activity compared with YH439.

This study was supported in part by the Korea Ministry of Science and Technology (Han Project 4-1-1).

1 Abbreviations used are: YH439, [isopropyl 2-(1,3-dithioetane-2-yli-dene)2-[1-(1-([N-4-methylthiazol-2-yl]carbonylacetate)]; M7, [isopropyl 2-(1,3-dithioetane-2-yli-dene)2-[1-(1-([N-4-(1-carboxy-2-thiazoly]carbamoyl)]carbonyl)acetate]; M4, [isopropyl 2-(1,3-dithioetane-2-yli-dene)2-[1-carboxyethyl]thiourea]; M5, [isopropyl 2-(1,3-dithioetane-2-yli-dene)2-[1-(4-carboxy-2-thiazoly]carbamoyl])acetate]; HPLC, high-performance liquid chromatography; DMSO, dimethyl sulfoxide; AUC, total area under the plasma concentration-time curve from time 0 to time infinity; $AUC_{t}$, AUC from time 0 to the last measured time; CL, time-averaged total body clearance; AUMC, first moment of AUC; MRT, mean residence time; $V_{ss}$, apparent volume of distribution at steady state; $C_{t}$, time-averaged renal clearance; $CL_{ren}$, time-averaged renal clearance; $CL_{max}$, time-averaged nonrenal clearance; $A_E$, total amount excreted in urine up to time infinity; F, value, extent of dose absorbed into the general circulation.

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administration and blood sampling, respectively. Both cannulae were exter-
orized to the dorsal side of the neck, where each cannula terminated with long
Silastic tubing (Dow Corning, Midland, MI). The two Silastic tubes were
inserted in a wired coil to allow free movement of the rat. The exposed areas
were surgically sutured. Each rat was housed individually in a rat metabolic
cage (Daegong Scientific, Seoul, South Korea) and allowed to recover from
anesthesia for 4–5 hr before the study began. They were not restrained at any
time during the study. Heparinized 0.9% NaCl-injectable solution (20 units/ ml), 0.25 ml, was used to flush each cannula to prevent blood cloting.

Male New Zealand White rabbits (1.70–2.35 kg, Dai Han Laboratory of
Animal Development, Seoul, South Korea) were anesthetized with 50–100 mg
of iv ketamine (50 mg/ml) through the ear vein. The carotid artery and the
jugular vein of each rabbit were cannulated individually with Silastic tubing
for blood sampling and drug administration, respectively. The heparinized
0.9% NaCl-injectable solution was also used to flush each cannula after
surgery. The animals were allowed to recover for 4–5 hr from anesthesia
before study began. Urine samples were collected using a pediatric Foley
catheter (Sewoon, Seoul, South Korea), which was introduced into the urinary
bladder. Each rabbit was restrained individually in a rabbit cage during the
24-hr experimental period.

Five conditioned male unanesthetized beagle dogs (8.3–10.2 kg, Marshall
Farms, NY) were used. They were fasted overnight and restrained by means of
dogs (Alice King Chatham Medical Arts, Los Angeles, CA) during the
24-hr experimental period. An indwelling polypropylene urinary catheter (5
Fr., 22 in., Sovereign, St. Louis, MO) was introduced into the urinary bladder
via the urethra to collect urine, and an iv cannula (2 in., 22-gauge, Sovereign)
was placed into the cephalic vein of one leg (for the oral study) or both legs (for
the iv study) for blood sampling or drug infusion (for the iv study only). The
heparinized 0.9% NaCl-injectable solution (20 units/ml), 0.25 ml, was used to flush each cannula to prevent blood cloting.

Oral Administration to Rats. YH439 (suspended in 0.2% sodium salt of
carboxymethyl cellulose), 50 (N = 4), 100 (N = 8), 300 (N = 12), and 500
(N = 10) mg/kg, was administered orally to rats with a feeding tubing (total
oral volume was approximately 1.5 ml). Blood samples (0.12 ml) were
collected via the carotid artery at 0 (to serve as a control), 15, 30, 45, 60, 90,
120, 180, 240, 360, 480, and 720 min after oral administration. Other proce-
dures were similar to those of iv studies.

YH439-contained mixed micelles, 10 mg/kg as free YH439, were also orally
administered (total oral volume was approximately 2 ml) to rats (N = 13).
Blood samples (0.12 ml) were collected via the carotid artery at 0 (to serve as a control), 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 720 min after oral
administration. Other procedures were similar to those of iv studies.

Methyl [14C]YH439 (suspended in 0.85% sodium salt of carboxymethyl cellulose),
34.6 μCi (with 100 mg of unradiolabeled YH439/kg), was orally administered
(total oral volume was 4 ml/kg) at single dose or for seven consecutive days
to 18-hr fasted rats (N = 4, each). To detect radioactivity in the respiration, each
rat was housed individually in a respiration-metabolic cage (Harvard 52–0502,
Harvard Apparatus, Kent, UK). Air, 300 ml/min, was introduced into the cage
using a peristaltic pump (model 1210, Harvard Apparatus, Kent, UK). The
[14C] in the respiration was collected 0–24, 24–48, 48–72, 72–96, and
96–120 hr into a beaker containing 200 ml (20%, v/v) of monoethanolamine
solution. To detect radioactivity in urine and feces, each rat (N = 4) was
housed individually in a respiration-metabolic cage (Harvard 52–0502),
and urine and feces were collected separately 0–24, 24–48, 48–72, 72–96,
and 96–120 hr. Methanol, 50 ml, was used to extract radioactivity in the feces.

Methyl [14C]YH439, 34.6 μCi (with 20 mg of unradiolabeled YH439/kg), was also
orally administered (total oral volume was 4 ml/kg) to additional rats (N = 4)
after bile duct cannulation. Experiment was started after recovery from anes-
thesia. Each rat was kept in supine position for a 48-hr experimental period.

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thesia. Each rat was kept in supine position for a 48-hr experimental period.

Bile samples were collected 0–6, 6–12, 12–24, and 24–48 hr. Solvable, 2 ml,
was added to 0.1 ml of sample, and the mixture was then placed in a water-bath
shaker for 2 hr kept at 50°C to dissolve the mixture. One-hour standing after
addition of 0.3 ml of EDTA (200 mM) and 0.1 ml H2O2 (28%), the mixture
was placed in a water-bath shaker for 1 hr. After vortexing with addition of 5
ml of Atomlight, the radioactivity was measured using a liquid scintillation
counter (Beckman Instruments, LS-5801, Fullerton, CA).
Liver Distribution After Oral Administration to Rats. YH439 (suspended in 0.2% sodium salt of carboxymethyl cellulose), 300 mg/kg, was similarly orally administered to rats. Total oral volume was approximately 1.5 ml. At 0 (to serve as a control), 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 720 min (N = 5, each), as much blood as possible was collected via the carotid artery, and each rat was killed by cervical dislocation. At the same time, the liver was perfused with cold 0.9% NaCl-injectable solution, cut, and blotted with paper tissue. Approximately 1 g of the liver was homogenized (Ultra-Turrax T25, Janke & Kunkel, Ika-Labortechnik, Staufen, Germany) with 4 volumes of 0.9% NaCl-injectable solution and centrifuged for 10 min at 9000g. Plasma was stored at -80°C (for the calculation of AUC) for the calculation of AUC was estimated by dividing the last measured concentration by the terminal rate constant. The mean values of each clearance (Chiou, 1980), MRT (4), CL_r, and CL_NS were approximately 5.9, 6.4, 8.1, 9.9, and 10.9 min, respectively. The detectability limits of YH439, M4, M5, and M7 in human plasma were 50, 40, 50, and 50 ng/ml, respectively, and the values of M4, M5, and M7 in human urine were 40, 50, and 50 ng/ml, respectively. The interday and intraday coefficients of variation for YH439, M4, M5, and M7 in human plasma and urine were less than 8.05% and 9.99%, respectively.

Pharmacokinetic Analysis. The AUC or average oral AUC (AUC), was calculated by the trapezoidal rule—extrapolation method (Kim et al., 1993); this method employed the logarithmic trapezoidal rule for the calculation of area during the declining plasma level phase (Chiou, 1978) and the linear trapezoidal rule for the rising plasma level phase. The area from the last data point to time infinity (for the calculation of AUC) was estimated by dividing the last measured plasma concentration by the terminal rate constant. The standard method (Gibaldi and Perrier, 1982) was used to calculate the following pharmacokinetic parameters, CL, AUMC, MRT, V SS , CL_r, and CL_NS:

\[
CL = \frac{\text{dose}}{AUC} \quad (1)
\]

\[
AUMC = \int_0^\infty t \cdot C_p \, dt \quad (2)
\]

\[
MRT = \frac{AUMC}{AUC} - \frac{T}{2} \quad (3)
\]

\[
V_{SS} = CL \cdot MRT \quad (4)
\]

\[
CL_r = \frac{A_e}{AUC} \quad (5)
\]

\[
CL_{NS} = CL - CL_r \quad (6)
\]

where \(C_p\) is the plasma concentration of YH439, M4, M5, or M7 at time \(t\), is the infusion time, and \(A_{e}\) is the total amount of YH439, M4, M5, or M7 excreted in urine up to time infinity (this was assumed to be equal to the total amount of each compound excreted in the 24-hr urine, as a negligible amount of each compound could be found in urine collected thereafter).

The F value of YH439 after oral administration of the drug, 50, 100, 300, and 500 mg/kg, and YH439-containing mixed micelles (10 mg/kg as free YH439) to rats was determined by comparing the AUC to that after oral administration with the AUC after iv administration of YH439 (10 mg/kg) to rats because the pharmacokinetic parameters of YH439 were independent of iv dose ranges studied in rats.

The mean values of each clearance (Chiu, 1980), \(V_{ss}\) (Chiu, 1979a), and the \(t_{1/2}\) (Eatman et al., 1977) were calculated by the harmonic mean method.

Statistical Analysis. A p value of less than 0.05 was considered to be statistically significant using a Duncan’s multiple range test of SPSS posteriori ANOVA program among means for the unpaired (for rat and rabbit studies) and paired (for dog studies) data (Statistical Research Institute, College of
Results and Discussion

Intravenous Administration of YH439 and Its Metabolites to Rats. After a 15-min iv infusion of YH439 to rats, the plasma concentrations of YH439 seemed to decline in a parallel fashion in all four doses of YH439 (fig. 2). Note that the pharmacokinetic parameters of YH439 were independent of YH439 doses ranging from 2 to 10 mg/kg; the AUC values of YH439 increased proportionally to YH439 doses, and CL, CL_NR, t_1/2, V_SS, and MRT values of YH439 were not significantly different (table 1). The contribution of CL_R to CL of YH439 was negligible since negligible amount of YH439 was excreted as unchanged YH439 in the 24-hr urine (Ae (`,) less than 0.008% of four YH439 iv doses studied), suggesting that majority of the intravenously administered YH439 were metabolized in rats. Therefore, the CL values of YH439 in rats listed in table 1 could represent the CL_NR values of YH439. The pharmacokinetic parameters of YH439 after iv administration of the drug, 1 mg/kg, was not listed in table 1 because the plasma concentrations of YH439 were detected only up to 15 min postinfusion (fig. 2).

The formation of M4 after iv administration of 5 and 10 mg/kg of YH439 was rapid; the mean arterial plasma concentration of M4 reached its peak at the end of a 15-min iv infusion of YH439 and declined in a parallel fashion (fig. 2). The pharmacokinetic parameters of M4, AUC, t_1/2, and CL_R values after iv administration of YH439, 5 and 10 mg/kg, were also independent of YH439 doses (table 1).

The pharmacokinetic parameters of M4 were also measured after iv administration of M4 (5 mg/kg) to five rats. After a 15-min iv infusion of M4 to rats, the arterial plasma concentration of M4 was detected up to 1440 min and declined polyexponentially (data not shown) with a mean t_1/2 of 205 ± 22.5 min. The mean t_1/2 of M4, 205 min, based on plasma data up to 1440 min after iv administration of M4, was greater than values of 104 and 92.4 min, which were based on plasma data up to 60 and 120 min after iv administration of YH439, 5 and 10 mg/kg, respectively (fig. 2 and table 1). The mean t_1/2 of M4, 109 min, could be estimated based on only up to 180 min plasma data after iv administration of M4; the value of 109 min was very close to 92.4 – 104 min after iv administration of YH439, 5–10 mg/kg (table 1).

M4 was cleared (eliminated) slowly in rats compared with YH439; the value of CL (0.987 ± 0.331 ml/min/kg) was lower, and the values of MRT (242 ± 31.5 min) and t_1/2 (205 ± 22.5 min) of M4 were higher than those of YH439 (table 1). M4 was mainly eliminated by renal excretion; contribution of CL_R to CL of M4 was 74%. After iv administration of M4, neither M7 nor YH439 was detected in plasma, indicating that reversible metabolism between M4 and M7 as well as YH439 does not take place in rats. Reversible metabolism of drugs has been extensively reviewed (Cheng and Jusko, 1993).

Note that the AUC value (5070 ± 1480 μg·min/ml) of M4 after iv administration of M4, 5 mg/kg, was 393 and 238 times higher than...
that after iv administration of YH439, 5 mg/kg (12.9 μg·min·ml) and 10 mg/kg (21.3 μg·min·ml), respectively, to rats (table 1), suggesting that the formation of M4 from intravenously administered YH439 was negligible in rats. Approximately 1000 μg (74% of iv dose of M4) of M4 were excreted in the 24-hr urine as unchanged M4 after iv administration of M4 (5 mg/kg) to rats, but the values were negligible after iv administration of YH439 [5 mg/kg (10.4 μg) and 10 mg/kg (13.9 μg)] to rats (table 1). This again suggests negligible formation of M4 from intravenously administered YH439 to rats. Negligible formation of M4 from YH439 also supports the undetectability of M4 in plasma after iv administration of YH439 (1 and 2 mg/kg) to rats (fig. 2).

After iv administration of YH439 to rats, M7 was not detected in plasma (as well as in the urine) at all four YH439 doses studied. This could be due to either rapid and almost complete conversion of M7 to M4 as soon as M7 is formed from YH439 or negligible formation of M7 from the intermediate (bracket in fig. 1) after iv administration of YH439. To find out, M7, 5 mg/kg, was intravenously administered to six rats. After a 15-min iv infusion of M7, the plasma concentration of M7 was detected only up to 120 min (data not shown), and the values of t1/2, MRT, CL, and Ae CV , of M7 were 31.3 ± 10.3 min, 16.2 ± 3.11 min, 32.4 ± 8.05 ml·min/kg, and less than 2% of iv dose of M7, respectively. If M4 is formed rapidly and almost completely from M7 as soon as M7 is formed from YH439, high plasma concentrations of M4 could be expected after iv administration of M7 to rats. However, very low concentrations (0.05–0.1 μg/ml) of M4 were detected in plasma from the beginning to 90 min after iv administration of M7 (5 mg/kg) to rats (data not shown), suggesting that negligible amount of M4 is formed after iv administration of M7 to rats. Therefore, the undetectability of M7 in plasma (as well as in the urine) after iv administration of YH439 to rats could not be due to rapid and almost complete conversion of M7 to M4 as soon as M7 was formed from YH439. The AUC (or AUC CV ) values of M4 after iv administration of YH439, 5 mg/kg, and M7 (5 mg/kg) to rats should be comparable if all the intravenously administered YH439 is assumed to metabolize to M4 via M7. However, the AUC (or AUC CV ) value of M4 after iv administration of YH439, 5 mg/kg (12.9 ± 3.32 μg·min·ml), was greater than that of M7, 5 mg/kg (5.93 ± 0.495 μg·min·ml). Moreover, M7 was not detected in plasma at all four doses of YH439, and YH439 was also metabolized to M5 in rats. Above data suggested that negligible amount of M7 was formed from the intermediate (bracket in fig. 1), and almost all of M4 was formed directly from the intermediate (bracket in fig. 1) after iv administration of YH439 to rats. YH439 was not detected in plasma after iv administration of M7 to rats, again indicating that reversible metabolism between YH439 and M7 does not take place in rats.

Contrast to the rapid formation of M4 after iv administration of YH439 to rats (fig. 2), the formation of M5 after iv administration of YH439, 2–10 mg/kg, seemed to be slow; the mean arterial plasma concentration of M5 reached its peak at 60–120 min postinfusion of YH439 and declined in a parallel fashion among three doses (2–10 mg/kg) of YH439 (fig. 2). The AUC(CV ) values of M5 also seemed to increase proportionally to YH439 doses, 2–10 mg/kg, in rats (table 1).

The pharmacokinetic parameters of M5 was also measured after iv administration of M5 (5 mg/kg) to six rats. After a 15-min iv infusion of M5, the arterial plasma concentration of M5 was detected up to 240 min and declined polyexponentially (data not shown) with a mean t1/2 of 58.0 ± 8.41 min. The t1/2 of M5, 58.0 min, was close to the roughly estimated t1/2 of M5, 65.8 min, after iv administration of YH439, 10 mg/kg (fig. 2). The AUC value of M5 (494 ± 98.6 μg·min/ml) after iv administration of M5, 5 mg/kg, was approximately 11.6 times higher than AUC(CV ) value of M5 (42.5 μg·min/ml, table 1) after iv administration of YH439, 5 mg/kg, suggesting that M5 was not formed considerably after iv administration of YH439 to rats. Above data also support the undetectability of M5 in plasma after iv administration of YH439 (1 mg/kg) to rats (fig. 2). YH439 was also not detected in plasma after iv administration of M5 to rats, again indicating that reversible metabolism between M5 and YH439 does not take place in rats.

**Oral Administration of YH439 to Rats.** After oral administration of YH439 to rats, the plasma concentrations of YH439 seemed to maintain for up to 3–6 hr at YH439 doses ranging from 100 to 500 mg/kg (fig. 3). This might be due to continuous absorption of YH439 from rat GI tract. The AUC(CV ) and Ae(CV ) values of YH439 were almost constant (not significantly different) at YH439 oral doses ranging from 100 to 500 mg/kg (table 2). This could be due to constant amount of YH439 absorbed from rat GI tract. Therefore, it could be expected that the extent of oral absorption of YH439 from rat GI tract is low, and the F value of YH439 decreases with increasing oral doses of YH439; the F values were 3.67, 1.33, and 0.859% for YH439 oral doses of 100, 300, and 500 mg/kg, respectively.

The poor absorption of YH439 from rat GI tract was also proved by measuring the cumulative amount of total oral radioactivity excreted into the urine, bile, feces, and expiration after single oral administra-

<table>
<thead>
<tr>
<th>YH439</th>
<th>( \text{1 mg/kg (} N = 9) )</th>
<th>( \text{2 mg/kg (} N = 10) )</th>
<th>( \text{5 mg/kg (} N = 12) )</th>
<th>( \text{10 mg/kg (} N = 7) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg·min/ml)</td>
<td>16.6 ± 4.79</td>
<td>41.2 ± 15.4</td>
<td>86.6 ± 8.96</td>
<td>10.3 ± 4.12</td>
</tr>
<tr>
<td>( t_{1/2} ) (min)</td>
<td>52.9 ± 31.2</td>
<td>49.6 ± 17.1</td>
<td>60.1 ± 7.93</td>
<td>49.1 ± 10.0</td>
</tr>
<tr>
<td>CL (ml·min/kg)</td>
<td>121 ± 34.0</td>
<td>135 ± 54.0</td>
<td>115 ± 12.3</td>
<td>74.0 ± 29.0</td>
</tr>
<tr>
<td>( V_z ) (ml/kg)</td>
<td>5720 ± 2050</td>
<td>5670 ± 2970</td>
<td>5820 ± 1510</td>
<td>5820 ± 1510</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>51.8 ± 16.2</td>
<td>38.9 ± 14.7</td>
<td>45.1 ± 10.0</td>
<td>43.5 ± 10.0</td>
</tr>
<tr>
<td>CL(1/r) (ml/min/kg)</td>
<td>0.108 ± 0.00732</td>
<td>0.0256 ± 0.00365</td>
<td>0.00732 ± 0.00256</td>
<td>0.00732 ± 0.00256</td>
</tr>
<tr>
<td>CL(z) (ml/min/kg)</td>
<td>121 ± 34.0</td>
<td>135 ± 53.8</td>
<td>115 ± 12.2</td>
<td>115 ± 12.2</td>
</tr>
<tr>
<td>( A_{e(CV)} ) (μg)</td>
<td>UD*</td>
<td>1.70 ± 0.737</td>
<td>1.33 ± 0.704</td>
<td>1.33 ± 0.704</td>
</tr>
<tr>
<td>M4</td>
<td>AUC (μg·min/ml)</td>
<td>12.9 ± 3.32</td>
<td>21.3 ± 8.01</td>
<td>92.4 ± 27.5</td>
</tr>
<tr>
<td>( t_{1/2} ) (min)</td>
<td>104 ± 57.1</td>
<td>0.204 ± 0.175</td>
<td>0.129 ± 0.204</td>
<td>0.129 ± 0.204</td>
</tr>
<tr>
<td>CL(1/r) (ml/min/kg)</td>
<td>10.4 ± 3.84</td>
<td>13.9 ± 12.4</td>
<td>13.9 ± 12.4</td>
<td>13.9 ± 12.4</td>
</tr>
<tr>
<td>( A_{e(CV)} ) (μg)</td>
<td>UD*</td>
<td>13.8 ± 4.52</td>
<td>42.5 ± 12.5</td>
<td>110 ± 18.6</td>
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<tr>
<td>M5</td>
<td>AUC(CV) (μg·min/ml)</td>
<td>1.87 ± 0.443</td>
<td>1.23 ± 0.696</td>
<td>10.0 ± 7.97</td>
</tr>
</tbody>
</table>

* Under detection limit.
tion of \([^{14}\text{C}]\text{YH439}\), 34.6 \(\mu\text{Ci}\) (with 20 or 100 mg of unradiolabeled \text{YH439}/kg) to rats. Approximately 90\% of the total orally administered radioactivity was recovered up to the 120-hr feces after both single (86.2 \(\pm\) 8.51\%) and multiple (88.8 \(\pm\) 4.76\%) administration of the radiocompound to rats. Because the above data represent both the amount of radioactivity unabsorbed from GI tract and those excreted in bile (including GI-tract excretion), the total amount of radioactivity excreted in bile was measured; approximately 30\% (28.4\%) of the total orally administered radioactivity was recovered up to the 48-hr bile after single oral administration of the radiocompound to rats. Above data indicated that roughly 60\% of the total oral radioactivity (the value of 60\% is a minimum value, as the radioactivity excreted via bile could be reabsorbed from rat GI tract) was not absorbed from rat GI tract assuming that GI secretion of \text{YH439} is negligible. Based on above data, one could expect that approximately 40\% of \text{YH439} was absorbed from rat GI tract. However, the \(F\) values of \text{YH439} were 0.859 –3.67\%, indicating that most of the \text{YH439} absorbed after oral administration to rats could be metabolized to \text{M4} and \text{M5} by first-pass effect (liver and/or GI first-pass effect) as will be discussed below. Negligible amount of total oral radioactivity was recovered up to the 120-hr urine (less than 5.71 \(\pm\) 3.42\% of total oral radioactivity) and 120-hr expiration (less than 3.47 \(\pm\) 0.84\% of total oral activity) after both single and multiple oral administration of the radiocompound to rats. More than 95\% of total orally administered radioactivity eventually excreted in the 120-hr urine, feces, and expiration was excreted in 48 hr.

### TABLE 2

<table>
<thead>
<tr>
<th>(\text{YH439})</th>
<th>50 mg/kg ((N = 4))</th>
<th>100 mg/kg ((N = 8))</th>
<th>300 mg/kg ((N = 12))</th>
<th>500 mg/kg ((N = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{AUC}_{0-\text{t}}) ((\mu\text{g min/ml}))</td>
<td>31.8 (\pm) 3.73</td>
<td>34.6 (\pm) 12.1</td>
<td>37.2 (\pm) 14.2</td>
<td></td>
</tr>
<tr>
<td>(\text{Ae}_{\text{t}}) ((\mu\text{g}))</td>
<td>2.86 (\pm) 1.15</td>
<td>2.67 (\pm) 3.15</td>
<td>0.884 (\pm) 0.890</td>
<td>2.90 (\pm) 3.87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(\text{M4})</th>
<th>(\text{M5})</th>
<th>(\text{Mixed Micelles})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{AUC}_{0-12 \text{ hr}}) ((\mu\text{g min/ml}))</td>
<td>63.0 (\pm) 22.2</td>
<td>86.7 (\pm) 8.25</td>
</tr>
<tr>
<td>(\text{Ae}_{12 \text{ hr}}) ((\mu\text{g}))</td>
<td>5.23 (\pm) 1.76</td>
<td>8.05 (\pm) 3.48</td>
</tr>
<tr>
<td>(\text{AUC}_{0-12 \text{ hr}}) ((\mu\text{g min/ml}))</td>
<td>214 (\pm) 76.4</td>
<td>266 (\pm) 77.3</td>
</tr>
<tr>
<td>(\text{Ae}_{12 \text{ hr}}) ((\mu\text{g}))</td>
<td>9.27 (\pm) 3.48</td>
<td>49.3 (\pm) 25.4</td>
</tr>
</tbody>
</table>

### FIG. 3.

Mean arterial plasma concentration-time profiles of \text{YH439}, \text{M4}, and \text{M5} after oral administration of \text{YH439} 50 \((\bullet, N = 4)\), 100 \((\square, N = 8)\), 300 \((\square, N = 12)\), and 500 \((\square, N = 10)\) \(\mu\text{g/kg}\), and \text{YH439} after oral administration of \text{YH439}-contained mixed micelles \(10 \mu\text{g/kg as free YH439} (\bullet, N = 13)\) to rats. Bars represent standard deviation.
As shown in the iv study (fig. 2), the formation of M4 from YH439 after oral administration of YH439, 100–500 mg/kg, was also rapid; the mean plasma concentration of M4 reached its peak at 15 min and declined in a parallel fashion among YH439 doses ranging from 100 to 500 mg/kg (fig. 3). The AUC 0–12 hr and Ae (\(\text{\`}\)) values of M4 did not increase proportionally to YH439 oral doses from 100 mg/kg (table 2). This again could be due to constant amount of YH439 absorbed from rat GI tract. The AUC (or AUC_{0\rightarrow t}) ratios of M4 to YH439 after iv administration of YH439, 5–10 mg/kg, were 24.6–31.3% (based on table 1), but the corresponding values after oral administration of YH439, 100–500 mg/kg, were 198–355% (based on table 2), suggesting that M4 could be mainly formed after oral administration of YH439 by first-pass metabolism, such as liver and/or GI first-pass metabolism.

Note that after oral administration of YH439 (50–500 mg/kg) to rats, the mean plasma concentration of M5 kept increasing up to 720 min.

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Amount ((\mu g) per ml plasma or (\mu g) per g tissue)</th>
<th>T/P Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>YH439</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>18.4 ± 6.72</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>UD              (\text{`})</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>4.62 ± 0.430</td>
<td>0.272 ± 0.0806</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.411 ± 0.0938</td>
<td>0.0236 ± 0.00664</td>
</tr>
<tr>
<td>Brain</td>
<td>0.945 ± 0.0493</td>
<td>0.0562 ± 0.0194</td>
</tr>
<tr>
<td>Heart</td>
<td>0.105 ± 0.0607</td>
<td>0.00559 ± 0.00167</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.318 ± 0.0362</td>
<td>0.0186 ± 0.00533</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.63 ± 0.354</td>
<td>0.0964 ± 0.0324</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.310 ± 0.116</td>
<td>0.0192 ± 0.00911</td>
</tr>
<tr>
<td>Large intestine</td>
<td>1.36 ± 0.894</td>
<td>0.0821 ± 0.0550</td>
</tr>
<tr>
<td>Mesentery</td>
<td>0.333 ± 0.0937</td>
<td>0.0196 ± 0.00818</td>
</tr>
<tr>
<td>Muscle</td>
<td>UD              (\text{`})</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>UD              (\text{`})</td>
<td></td>
</tr>
</tbody>
</table>

* Tissue to plasma ratio.
\(\text{\`}\) Under detection limit.
min (fig. 3), and the AUC\(_{0–12}\) values of M5 were almost comparable (not significantly different) among YH439 oral doses from 50 to 500 mg/kg (table 2). This again could be due to constant amount of YH439 absorbed from rat GI tract (table 2). The AUC (or AUC\(_{0–3t}\)) ratios of M5 to YH439 after iv administration of YH439 (2–10 mg/kg) to rats were 83.1–127% (based on table 1), but the values after oral administration of YH439 (100–500 mg/kg) to rats were 817–855% (based on table 2, the actual values could be considerably higher than 817–855%, as the plasma concentrations of M5 were the highest at 12 hr after oral administration of YH439, and AUC\(_{0–12}\) values of M5 were employed instead of total AUC of M5 for estimation of the ratios). Above data suggested that M5 could be extensively formed from YH439 by first-pass metabolism. Therefore, the undetectability of YH439 in plasma at YH439 oral dose of 50 mg/kg (fig. 3) could be explained by the considerable formation of both M4 and M5 from YH439 by first-pass metabolism.

As mentioned earlier, the absorption of YH439 from rat GI tract was poor due to poor water solubility of the drug. Therefore, the F value could be expected to increase if the water solubility of YH439 is enhanced. To increase the F value of YH439, YH439-contained mixed micelles, 10 mg/kg as free YH439, were orally administered to 13 rats. Note that YH439 was detected in plasma after oral administration of YH439-contained mixed micelles, as low dose of free YH439, 10 mg/kg (fig. 3), and the AUC value of YH439 was 18.4 ± 6.64 μg·min/ml. The mean arterial plasma concentration of YH439 reached its peak at 45 min (absorption of YH439 was fast) and declined monoexponentially (fig. 3) with a t\(_{1/2}\) of 55.1 ± 20.8 min. The value, 55.1 min, was very close to 49.6–60.1 min (table 1) after iv administration of YH439 (2–10 mg/kg) to rats. The F value increased significantly after oral administration of YH439-contained mixed micelles; the estimated F value was 21.2%, which is consid-

### TABLE 4

Mean (±SD) amount (μg per ml plasma or μg per g tissue) of YH439, M4, and M5 (expressed in terms of YH439) recovered at 30 min after oral administration of YH439 (500 mg/kg) to rats (N = 5)

<table>
<thead>
<tr>
<th></th>
<th>YH439</th>
<th>M4</th>
<th>M5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount</td>
<td>Amount</td>
<td>T/P Ratio(^a)</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.742 ± 0.206</td>
<td>9.28 ± 1.65</td>
<td>0.0987 ± 0.0320</td>
</tr>
<tr>
<td>Lung</td>
<td>UD(^b)</td>
<td>0.0335 ± 0.00432</td>
<td>0.00372 ± 0.000940</td>
</tr>
<tr>
<td>Kidney</td>
<td>UD</td>
<td>0.112 ± 0.0129</td>
<td>0.00891 ± 0.0123</td>
</tr>
</tbody>
</table>

\(^a\) Tissue to plasma ratio.
\(^b\) Under detection limit.
erably greater than 0.859–3.67% after oral administration of free YH439, 100–500 mg/kg.

**Tissue Distribution in Rats.** As YH439 is a hepatoprotective agent and is developed for oral administration, the amount of YH439 and its metabolites recovered from the liver were compared with the plasma concentrations after oral administration of YH439 to rats. The mean arterial plasma concentration (μg/ml) and mean amount recovered from a gram of liver (μg per g of liver) of YH439, M4, and M5 after oral administration of YH439 (300 mg/kg) to rats are shown in fig. 4. Generally, the two values of YH439 seemed not to be significantly different for up to 240 min (fig. 4). However, the plasma concentrations of M4 were significantly higher than the amount in the liver (fig. 4). On the other hand, the amount of M5 (fig. 4) in the liver was considerably higher than the corresponding plasma concentrations.

**Tissue distribution of YH439 and its metabolites after iv administration of YH439 (10 mg/kg) to five rats are listed in table 3.** Although YH439 was widely distributed in most of rat tissues studied, its affinity to rat tissues studied was not considerable as reflected by the less-than-unity values of tissue to plasma (T/P) ratio in all tissues studied (table 3). M4 was detected in the liver, lung, heart, spleen,

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**TABLE 5**

Mean (±SD) pharmacokinetic parameters of YH439 and its metabolites, M4 and M5, after 1-min intravenous infusion of YH439 (1, 2, 5, and 10 mg/kg) to rabbits

<table>
<thead>
<tr>
<th></th>
<th>1 mg/kg (N = 9)</th>
<th>2 mg/kg (N = 10)</th>
<th>5 mg/kg (N = 8)</th>
<th>10 mg/kg (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YH439</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (μg·min/ml)</td>
<td>124 ± 28.0</td>
<td>37.3 ± 12.4</td>
<td>40.4 ± 9.63</td>
<td>27.4 ± 7.53</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>283 ± 6.01</td>
<td>120 ± 37.8</td>
<td>120 ± 0.431</td>
<td>120 ± 70.4</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>112 ± 5.42</td>
<td>125 ± 6.72</td>
<td>125 ± 0.882</td>
<td>125 ± 67.2</td>
</tr>
<tr>
<td>M4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (μg·min/ml)</td>
<td>3.63 ± 1.34</td>
<td>6.06 ± 2.16</td>
<td>6.06 ± 2.16</td>
<td>6.06 ± 2.16</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>22.8 ± 7.39</td>
<td>21.5 ± 5.14</td>
<td>21.5 ± 5.14</td>
<td>21.5 ± 5.14</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>1.98 ± 7.16</td>
<td>6.06 ± 2.16</td>
<td>6.06 ± 2.16</td>
<td>6.06 ± 2.16</td>
</tr>
<tr>
<td>M5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (μg·min/ml)</td>
<td>47.5 ± 34.3</td>
<td>120 ± 121</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Ae (mg)</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
</tbody>
</table>

* Under detection limit.

**FIG. 6.** Mean venous plasma concentration-time profiles of YH439, M4, M5, and M7 after 1-min iv infusion of YH439 [1 (●), 5 (○), 10 (■), and 20 (□) mg/kg] to dogs (N = 5 by crossover design). Bars represent standard deviation.
small intestine, and mesentery with T/P ratio of less-than-unity (table 3) indicating that the affinity of M4 to rat tissues studied was not considerable. This was supported by the relatively small value of VSS of M4, 242 ± 46.4 ml/kg, after iv administration of M4 (5 mg/kg) to five rats. M5 had high affinity to the liver and kidney as reflected by the greater-than-unity values of T/P but was not detected in other tissues studied (table 3).

Tissue distribution after oral administration of YH439 (500 mg/kg) to rats are listed in table 4. YH439 was not detected in rat tissues studied except in the liver (fig. 4) and plasma (table 4). The T/P values of M4 were all lower-than-unity in rat tissues studied (table 4) including the liver (fig. 4). However, the T/P values of M5 in the liver (fig. 4) and kidney (table 4) were higher than unity as shown after iv administration of YH439 (table 3). M7 was also not detected in all rat tissues studied.

Intravenous Administration of YH439 to Rabbits. After a 1-min iv infusion of YH439 to rabbits, the plasma concentrations of YH439 seemed to decline in a parallel fashion among four YH439 doses studied (fig. 5), with mean t1/2 values of 37.3 and 27.4 min at YH439 doses of 5 and 10 mg/kg, respectively (table 5). Note that the terminal phase of plasma concentrations of YH439 started from 60 min at YH439 doses of 5 and 10 mg/kg (fig. 5). Therefore, the pharmacokinetic parameters at YH439 iv doses of 1 and 2 mg/kg are not listed in table 5. The pharmacokinetic parameters of YH439, AUC, t1/2, CL, and MRT, after iv administration of YH439 (5–10 mg/kg) to rabbits were independent of YH439 doses studied (table 5), as shown in rats (table 1). YH439 was not detected in the 24-hr urine at all YH439 doses studied; therefore, the CL values of YH439 listed in table 5 could represent the CLNR values of YH439 as shown in rats (table 1).

After iv administration of YH439 to rabbits, the plasma concentrations of M4 also declined in a parallel fashion among the four YH439 doses (fig. 5), and the pharmacokinetic parameters of M4, AUC, t1/2, and CLR, were independent of iv doses of YH439 studied (table 5), as shown in rats (table 1). After iv administration of YH439 to rabbits, the plasma concentrations of M5 were detected only at YH439 doses of 5 and 10 mg/kg with lower concentrations (fig. 5). Note that the AUCinj values of M5 in rabbits were almost negligible (table 5) when compared with those at the same doses of YH439 in rats (table 1), although the AUC values of YH439 in rabbits were considerably higher (table 5) than those at the same doses of YH439 in rats (table 1). After iv administration of YH439, the AUC (or AUCinj) ratios of M5 to YH439 in rats were 103 and 127% (based on table 1) for YH439 iv doses of 5 and 10 mg/kg, respectively, but the ratios in

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**TABLE 6**

Mean (±SD) pharmacokinetic parameters of YH439 and its metabolites, M4, M5, and M7 after 1-min intravenous infusion of YH439 (1, 5, 10, and 20 mg/kg) to dogs (N = 5 by crossover design)

<table>
<thead>
<tr>
<th>YH439</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–24 hr (µg/ml)</td>
<td>120 ± 22.9</td>
<td>570 ± 85.0</td>
<td>917 ± 127</td>
<td>2160 ± 185</td>
</tr>
<tr>
<td>CLR (ml/min/kg)</td>
<td>0.0771</td>
<td>0.0326</td>
<td>0.0143</td>
<td>0.00491</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>440 ± 63.0</td>
<td>467 ± 74.3</td>
<td>473 ± 53.9</td>
<td>394 ± 73.9</td>
</tr>
<tr>
<td>M7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC0–24 hr (µg/ml)</td>
<td>125 ± 38.6</td>
<td>324 ± 136</td>
<td>324 ± 136</td>
<td>905 ± 262</td>
</tr>
<tr>
<td>CLR (ml/min/kg)</td>
<td>0.297 ± 0.397</td>
<td>0.371 ± 0.293</td>
<td>0.475 ± 0.345</td>
<td>0.460 ± 0.203</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>19.4 ± 17.2</td>
<td>19.4 ± 17.2</td>
<td>19.4 ± 17.2</td>
<td>19.4 ± 17.2</td>
</tr>
</tbody>
</table>

---

* Each dose was significantly different (*p < 0.05) from 10 mg/kg.

---

**FIG. 7.** Mean venous plasma concentration-time profiles of M4 and M7 after oral administration of YH439 (5.0 g per whole body weight) to dogs (N = 5 by crossover design).

Bars represent standard deviation.
rabbits were only 2.93–3.31% (based on table 5). However, the ratios of M4 to YH439 were higher in rabbits (42.2–49.2%, based on table 5) than those in rats (24.6–31.3%, based on table 1). Above data indicated that after iv administration of YH439, larger amount of M4 is formed from YH439 in rabbits than that in rats and vice versa for M5. As shown in rats (table 1), the pharmacokinetic parameters of YH439 and its metabolites were independent of YH439 doses after iv administration of YH439 to rabbits (table 5). M7 was not detected in the biological samples after iv administration of YH439 to rabbits, suggesting that M7 is not formed from YH439 in rabbits as shown in rats. The oral studies of YH439 in rabbits were not performed because rabbit, being a regurgitating animal, is not a good animal model for oral study (Weisbroth et al., 1974).

Intravenous Administration of YH439 to Dogs. After a 1-min iv infusion of YH439 (10 and 20 mg/kg) to dogs, the mean venous plasma concentration of YH439 reached its peak at approximately 90 min and declined parallel to each other (fig. 6). This result was quite unexpected as the plasma concentrations of drugs generally reached their peak just after the end of infusion of the drugs (1 min in the present dog study). This could be due to precipitation of the injected YH439 (which has poor water solubility) and subsequent redissolution of YH439 in blood. Such second peak phenomena in plasma concentrations after iv administration of some other poorly water-soluble drugs such as lidocaine (Shinider and Way, 1968) and diisopyramide (Hinderling and Garrett, 1976) were also explained by precipitation-redissolution of the drugs in blood (Chiou, 1979b). The plasma concentrations of YH439 after iv doses of YH439 (1 and 5 mg/kg) to dogs were detected only up to 5 min postinfusion with low concentrations (not included in fig. 6). Unlike in rats (table 1) and rabbits (table 5), the AUC_{0→∞} value of YH439 at YH439 iv dose of 20 mg/kg (98.4 μg/min/ml) increased more proportionally to that of 10 mg/kg (12.0 μg/min/ml, table 6). YH439 was not detected in the 24-hr dog urine at all four doses of YH439 studied.

Unlike in rats (fig. 2) and rabbits (fig. 5), the formation of M4 from iv doses of YH439 was slow; the mean venous plasma concentration of M4 reached its peak at approximately 4 hr and declined in a parallel fashion among the four iv doses of YH439 (fig. 6). As shown in rats (table 1) and rabbits (table 5), the pharmacokinetic parameters of M4, AUC, t_{1/2}, CL, and Ae after iv administration of YH439 to dogs seemed to be independent of YH439 doses (table 6). Note that the AUC values of M4 in dogs were significantly higher than those of YH439 at all four doses of YH439 studied (AUC ratios of M4 to YH439 were 7640 and 2200% for YH439 doses of 10 and 20 mg/kg, respectively, based on table 6), and the ratios were considerably higher than those after iv administration of YH439 to rats (24.6–31.3%, based on table 1) and rabbits (42.2–49.2%, based on table 5), suggesting that the formation of M4 from YH439 increased significantly in dogs compared with those in both rats and rabbits. Above data also support the lower concentration of YH439 in plasma after iv administration of YH439 (1 and 5 mg/kg) to dogs (fig. 6). The CLR of M4 were almost negligible in all four iv doses of YH439 (table 6).

After iv administration of YH439 to dogs, the mean venous plasma concentration of M5 reached its peak at approximately 3–4 hr and declined parallel to each other at YH439 doses of 10 and 20 mg/kg (fig. 6). The amounts of M5 excreted in urine as unchanged M5 (less than 0.0503% of various iv doses of YH439 when expressed in terms of YH439) were almost negligible (table 6). The AUC value of M5 at YH439 dose of 20 mg/kg (90.9 μg/min/ml) increased more proportionally to that of 10 mg/kg (18.9 μg/min/ml, table 6).

It is quite interesting to note that unlike in rats and rabbits, M7 was detected in dog plasma after iv administration of YH439, 5–20 mg/kg (fig. 6). After iv administration of YH439 to dogs, the mean venous plasma concentration of M5 reached its peak at approximately 3–4 hr and declined in a parallel fashion among YH439 doses of 5, 10, and 20 mg/kg (fig. 6). The amount of urinary excretion of unchanged M7 was the highest among YH439, M4, M5, and M7; the percentages of iv dose of YH439 excreted in 24 hr urine as unchanged M7 were 1.07, 1.01, 1.55, and 2.77% (expressed in terms of YH439) at YH439 doses of 1.5, 10, and 20 mg/kg, respectively (table 6). The AUC_{0→24 h} value of M7 in dogs were 2700 and 920% of AUC values of YH439 at YH439 iv doses of 10 and 20 mg/kg, respectively, indicating that considerable amount of M7 is formed from YH439. The low concentrations of YH439 in plasma after iv administration of YH439 at 1 and 5 mg/kg could be due to considerable formation of M4 and M7 from YH439 in dogs.

Oral Administration of YH439 to Dogs. After oral administration of YH439 to dogs, YH439 was detected neither in plasma nor in urine in all high oral doses of YH439 studied, and this was quite surprising because YH439 was detected in plasma after small iv dose (10 mg/kg) of YH439 (fig. 6). This could be due to a small extent of oral absorption of YH439 from dog GI tract due to its poor water solubility and/or considerable first-pass metabolism of YH439 to form M4 and M7 (fig. 6) as discussed in the iv study (fig. 5).

After oral administration of YH439 to dogs, the mean venous plasma concentration of M4 reached its peak at approximately 3–4 hr and declined in a parallel fashion among three oral doses of YH439 studied (fig. 7). Although the AUC_{0→∞} values of M4 tended to increase with YH439 oral doses, they were not significantly different among the three different oral doses of YH439 (table 7), which has sharp contrast to the AUC_{0→∞} values of M4 after four different iv doses of YH439 in dogs (table 6). Moreover, the AUC_{0→∞} values of M4 and the amount of M4 excreted in the 24-hr urine as unchanged M4 after oral administration of YH439 (0.5–2 g) were very close to the values after iv administration of YH439 (1 mg/kg) to dogs (table 6). Above data also support the almost constant amount of YH439 absorbed from dog GI tract after oral admin-

TABLE 7
Mean (±SD) pharmacokinetic parameters of M4 and M7 after oral administration of YH439 (0.5, 1, and 2 g per whole body weight) to dogs (N = 5 by crossover design)

<table>
<thead>
<tr>
<th></th>
<th>0.5 g</th>
<th>1 g</th>
<th>2 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0→∞} (μg/min/ml)</td>
<td>133 ± 37.9</td>
<td>162 ± 94.0</td>
<td>242 ± 120</td>
</tr>
<tr>
<td>CL_{ss} (ml/min/kg)</td>
<td>0.00457 ± 0.00112</td>
<td>0.00831 ± 0.00417</td>
<td>0.00929 ± 0.00270</td>
</tr>
<tr>
<td>Ae_{ss} (μg)</td>
<td>5.88 ± 1.94</td>
<td>13.6 ± 8.43</td>
<td>20.3 ± 6.56</td>
</tr>
<tr>
<td>M7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0→∞} (μg/min/ml)</td>
<td>47.9 ± 72.0</td>
<td>73.5 ± 57.7</td>
<td></td>
</tr>
<tr>
<td>CL_{ss} (ml/min/kg)</td>
<td>0.777 ± 0.514</td>
<td>0.362 ± 0.340</td>
<td></td>
</tr>
<tr>
<td>Ae_{ss} (μg)</td>
<td>286 ± 411</td>
<td>260 ± 116</td>
<td></td>
</tr>
</tbody>
</table>

*0.5 g was significantly different (*p < 0.05) from 1 and 2 g.
*0.5 g was significantly different (*p < 0.05) from 2 g.
Pharmacokinetics of a New Hepatoprotective Agent in Animals


