THE EFFECT OF CAFFEINE INGESTION ON PHARMACOKINETICS OF CAFFEINE AND ITS METABOLITES AFTER A SINGLE ADMINISTRATION IN PREGNANT RATS*

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The pharmacokinetics of caffeine and its metabolites were studied in pregnant rats in order to clarify the effects of maternal caffeine ingestion on the caffeine disposition. On gestational day 18 the single intravenous or oral 10 mg/kg dose of caffeine was administered to pregnant rats who had received drinking water containing 0.04% caffeine or water ad lib during the premating and/or pregnant periods. Concentrations of caffeine and its dimethylxanthines were simultaneously determined by high-performance liquid chromatography. The caffeine plasma concentration–time curves were analyzed by assumption of a one-compartment model. The apparent volume of distribution of caffeine in rats given caffeine only during pregnancy was decreased. The elimination rate constant (k_e) of caffeine in most of the rats taking caffeine during pregnancy was increased. The rats which had received caffeine throughout the premating and pregnant periods had a relatively high total body plasma clearance (CL) of caffeine. The k_e and the CL widely varied in the rats taking caffeine during pregnancy. The individual values of k_e or CL in pregnant rats were significantly correlated with the molar concentration ratios of the metabolites to caffeine in plasma at 8 h after administration of caffeine. It is concluded that the caffeine disposition is influenced by the different modes of maternal caffeine ingestion during the premating and/or pregnant periods.

Keywords — caffeine; theophylline; paraxanthine; caffeine-disposition; pharmacokinetics; pregnant rat

Caffeine is very commonly consumed in the world as a stimulant compound on the central nervous system. One retrospective survey indicated that the daily intake of much caffeine in pregnant women had induced adverse effects on the fetus such as spontaneous abortion, still-birth or premature delivery.1) In our previous paper a reduction of the fetal cerebral weight in rats was caused by the maternal caffeine ingestion during pregnancy, and we suggested that the adverse effect on the fetus depends on the mode of maternal caffeine ingestion during the premating and/or gestational periods.2)

The pharmacokinetic study of caffeine in the pregnant rat is one of the important approaches to clarify the toxicity of caffeine on the fetal development. Caffeine is metabolized into three dimethylxanthines, i.e. theobromine, theophylline and paraxanthine, in the hepatic microsomal monooxygenase system at the first biotransformation step. In the present study, considering the fact that the majority of women consume caffeine from coffee, tea or cola from the pregestational through the gestational periods, the female rats were given caffeine ad lib during the premating and/or the pregnant periods. As the characteristics of caffeine disposition are estimated by analyzing the temporal changes of

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caffeine and its metabolites in plasma following intravenous administration, the effects of con-
tinuous pretreatment with caffeine on the caf-
feine disposition were investigated by assaying
plasma caffeine and its dimethylxanthines levels
after intravenous administration of caffeine.
Since caffeine is usually given orally from caf-
feine-containing beverages in human beings, the
elimination of caffeine after oral administration
was also investigated in the pregnant rat. Fur-
thermore, the relations between the plasma con-
centration ratios of the metabolites to caffeine
and the elimination rate constant or the total
body plasma clearance were evaluated.

MATERIALS AND METHODS

Materials — Caffeine, theophylline and theo-
bromine were of analytical grade; and methyl al-
cohol, chloroform, isopropyl alcohol, tetrahydro-
furan and acetonitrile were of ultraviolet (UV)
grade. All the reagents were obtained from
Wako Pure Chemical Industries (Osaka, Japan).
Paraxanthine was obtained from Sigma Chemi-
cal Company (St. Louis, MO, U.S.A.), and 7-(2-
hydroxyethyl) theophylline from Tokyo Kasei
Kogyo Company (Tokyo, Japan).

Intravenous Study — The preparation of rat
models was carried out with the method de-
scribed in the previous paper. Twenty-seven
female albino rats of the Wistar strain were
maintained on a solid diet (MM-3, Funabashi
Corp., Chiba, Japan). During the premating
period of about 38 d the rats were divided into
the water and the caffeine groups. Caffeine was
given *ad lib* in their drinking water at 0.04% so-
lution. The day on which a sperm-positive vagi-
nal smear was obtained following mating was
considered as gestational day (g.d.) 0. Through-
out pregnancy the rats were divided into four
groups. The W-W (water-water) and the C-C
(caffeine-caffeine) groups were given water
only and water containing 0.04% caffeine, re-
spectively, as drinking water *ad lib* during the
overall periods. The W-C and the C-W groups
were given 0.04% caffeine during only the preg-
nant period and only the premating period, re-
spectively. Caffeine ingestion was discontinued
in the W-C and the C-C groups on g.d. 17, be-
cause caffeine and its dimethylxanthines in the
plasma obtained from the rat abstaining from
caffeine for 24 h could not be detected. On g.d.
18 the pregnant rats, weighing 290—385 g, aged
14—19 weeks, were placed on their back with-
out anesthesia and given intravenously 10
mg/kg of caffeine as 1% isotonic solution into
the internal jugular vein. Unanesthetized rats
were placed on their back and blood samples
(0.4—0.5 ml) were drawn from the internal jugular vein with a heparinized syringe at 2, 4, 6,
8, 10 and 12 h after the administration, because
the hematocrit value in a pregnant rat is lowered
by collecting more than 3 ml of blood. The
plasma was stored at —20 °C until assay.

Oral Study — After the premating period of
about 10 d, 14 female rats who had been divided
into the water and the 0.04% caffeine groups
were mated. During pregnancy the rats were
divided into 3 groups, *i.e.* the W-W, the W-C
and the C-C groups. On g.d. 18 the pregnant
rats, weighing 312—378 g, aged 13—21 weeks,
were given orally 10 mg/kg of caffeine as 0.2%
water solution by gastric tube. Blood samples
were collected without anesthesia at 2, 4, 6 and 8
h after the administration.

Assay Procedure — Concentrations of caf-
feine and its dimethylxanthines in rat plasma
were determined using a modified combination
of the solvent extraction procedure described by
Bonati *et al.* and a high-performance liquid
chromatographic method. To the mixture of
0.2 ml of rat plasma and 0.02 ml of the internal
standard solution containing 0.05 mg/ml of 7-
(2-hydroxyethyl) theophylline in 0.005 M
sodium acetate buffer (pH 5.0), 2.5 ml of chloro-
form-isopropyl alcohol (75:25, v/v) solution
was added. After being mixed on a vortex mixer
and then centrifuged at 1200 × *g* for 10 min, 2.0
ml of the organic phase was transferred into
another test tube and evaporated in a water bath
at 50 °C under a gentle nitrogen stream. The
residue was dissolved in 0.2 ml of 0.005 M
sodium acetate buffer, and 0.02 ml of the solu-
tion was injected into the high-performance liquid chromatograph (Model LC-3A; Shimadzu Corp., Kyoto, Japan). The analytical column was a 30 cm × 3.9 mm i.d. 10-μm μBondapak C18 column (Waters Assoc., Milford, MA, U.S.A.) connected with a 7 cm × 2.1 mm i.d. precolumn (Co: Pell ODS, Whatman, Clifton, NJ, U.S.A.). The spectrophotometric detector (Model SPD-2A; Shimadzu Corp., Kyoto, Japan) was operated at 280 nm and 0.01 a.u.f.s. The mobile phase was 0.005 M sodium acetate buffer (pH 5.0)–methyl alcohol-acetonitrile-tetrahydrofuran (92.5:3.0:2.8:1.7, v/v). The flow-rate of the mobile phase was set at 1.5 ml/min, yielding an operating pressure of 105 kg/cm². The data analysis for identification and integration of peaks was performed using a data processor (Model C-R1A; Shimadzu Corp., Kyoto, Japan).

Pharmacokinetic Analysis — The caffeine plasma concentration–time curves were analyzed following a one-compartment open model. The elimination rate constant (k_{el}) was calculated by least-squares log-linear regression analysis. The plasma elimination half-life (t_{1/2}) was determined using the equation of \( t_{1/2} = 0.693/k_{el} \). Since the absorption of caffeine is rapid,^{5,6} the apparent volume of distribution (V_d) was calculated by the equation:

\[
V_d = F \cdot \text{Dose/}C_0 , \quad \text{where } F \text{ is the absorbed fraction and } C_0 \text{ is the extrapolated concentration in plasma at zero time. As caffeine is completely absorbed,}^{5,6} \text{ } F \text{ was considered to be equal to 1.0 in the oral study. In the intravenous study, } V_d \text{ was determined using the equation of } V_d = \text{Dose}/C_0 . \text{ The total body plasma clearance (CL) was calculated by the following equation:}
\]

\[
CL = V_d \cdot k_{el}
\]

The metabolite pharmacokinetic analysis concerning caffeine was proposed by Tang-Liu et al.\(^7\) The rate of change in the amount of a metabolite in the body at any time was expressed by the following equation:

\[
dA_{M_i}/dt = f_{M_i} \cdot CL \cdot C_p - CL_{M_i} \cdot C_{M_i} \quad (i = 1, 2, 3)
\]

(1),

where \( A_{M_i} \) is the amount of the metabolite in the body, \( f_{M_i} \) is the fraction of the precursor converted to the metabolite, \( CL \) is the total body plasma clearance of the precursor, \( C_p \) is the plasma concentration of precursor, \( CL_{M_i} \) is the metabolic clearance and \( C_{M_i} \) is the plasma concentration of metabolite. When the precursor and the metabolites are distributed at equilibrium in the body and the plasma concentration of metabolite has reached the peak level after a single administration, it should be considered that \( dA_{M_i}/dt = 0 \). So the Eq. 1 gives:

\[
f_{M_i} \cdot CL \cdot C_p - CL_{M_i} \cdot C_{M_i} = 0
\]

(2)

consequently:

\[
CL = (CL_{M_i}/f_{M_i}) \cdot (C_{M_i}/C_p)
\]

(3)

If \( f_{M_i} \) and \( CL_{M_i} \) are constant or the variation of \( CL_{M_i}/f_{M_i} \) is neglected in any rat and the precursor is completely metabolized, \( CL \) should be proportional to the concentration ratio of the metabolite to the precursor \( (C_{M_i}/C_p) \) in the Eq. 3. Since caffeine is transformed into dimethylxanthines by the N-demethylation pathway, it is suitable to express the concentrations of caffeine and its metabolites as the molar concentrations in the Eqs. 1, 2 and 3.

Statistical Analysis — The results are expressed as the mean ± S.D. Subsequent to a one-way analysis of variance (ANOVA), all between-group comparisons were made utilizing the multiple comparison test of Scheffe. The analysis of covariation was used to detect the differences concerning the regression lines. A p value less than 0.05 was considered to be statistically significant.

RESULTS

Assay Procedure

The accuracy and precision of the method are summarized in Table I. The standard curves using the pooled plasma obtained from at least 10 rats were linear \((r = 0.997 - 0.999)\). The limits of detection were 0.1 \( \mu g/ml \) for theophylline and theobromine, and 0.2 \( \mu g/ml \) for paraxanthine and caffeine.

Intravenous Study

The plasma concentration–time curves of caffeine and its dimethylxanthines after intravenous administration of caffeine are shown in Fig.
1 (A) and (B). In each rat given intravenously 10 mg/kg of caffeine, the elimination of caffeine was linear in the semilogarithmic plot. The concentrations of the metabolites reached the peak

**TABLE I. Accuracy and Precision of the Assay**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Theobromine</th>
<th>Paraxanthine</th>
<th>Theophylline</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µg/ml)</td>
<td>(µg/ml) (%)</td>
<td>(µg/ml) (%)</td>
<td>(µg/ml) (%)</td>
<td>(µg/ml) (%)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.55 ± 0.17 30.6 8.4</td>
<td>0.46 ± 0.03 69.8.7</td>
<td>0.45 ± 0.04 85.12.4</td>
<td>0.48 ± 0.02 48.5.0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.11 ± 0.16 14.2 10.1</td>
<td>0.97 ± 0.05 50.27</td>
<td>0.97 ± 0.04 41.3.1</td>
<td>1.15 ± 0.15 12.7 13.2</td>
</tr>
<tr>
<td>2.0</td>
<td>2.13 ± 0.18 8.4 6.0</td>
<td>1.95 ± 0.06 3.0 2.7</td>
<td>1.93 ± 0.05 28.3.5</td>
<td>1.87 ± 0.11 5.9 7.0</td>
</tr>
<tr>
<td>5.0</td>
<td>5.13 ± 0.16 3.1 2.4</td>
<td>4.84 ± 0.19 3.9 3.4</td>
<td>4.91 ± 0.17 3.4 1.8</td>
<td>4.67 ± 0.36 7.8 7.0</td>
</tr>
<tr>
<td>10.0</td>
<td>10.27 ± 0.52 5.1 2.7</td>
<td>9.75 ± 0.30 3.1 2.5</td>
<td>9.90 ± 0.27 2.7 1.0</td>
<td>10.08 ± 0.27 2.7 0.8</td>
</tr>
<tr>
<td>20.0</td>
<td>20.65 ± 1.33 6.4 3.2</td>
<td>19.93 ± 1.18 5.9 0.4</td>
<td>20.21 ± 1.20 5.9 1.0</td>
<td>20.05 ± 1.13 5.6 0.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for five experiments assayed corresponding to each compound concentration. Coefficient of variation is expressed as C.V. Relative mean error is expressed as M.E. Concentration was calculated on the basis of the peak area ratios against the internal standard.

**FIG. 1. Plasma Concentration-Time Curves of Caffeine and Its Dimethylxanthines**

levels between 4 and 8 h after the administration of caffeine. Table II summarizes the pharmacokinetic parameters in pregnant rats after intravenous administration of caffeine. In the W-C group the \( V_d \) was significantly decreased. In the W-C and the C-C groups the \( k_{el} \) showed a tendency to increase. There was no significant difference in \( CL \) among the four groups, al-

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W-W (7)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>355±19</td>
</tr>
<tr>
<td>( V_d ) (ml/kg)</td>
<td>582±97</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>( k_{el} ) (h(^{-1}))</td>
<td>0.29±0.06</td>
</tr>
<tr>
<td>( CL ) (ml/h/kg)</td>
<td>169±47</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. for the number of rats indicated in parentheses. W-W: group taking water during the overall period, W-C: group taking caffeine during pregnancy only, C-W: group taking caffeine during premating period only, C-C: group taking caffeine during the overall period, \( V_d \): apparent volume of distribution, \( t_{1/2} \): plasma elimination half-life, \( k_{el} \): elimination rate constant, CL: total body plasma clearance.

\( a) p<0.05 \), significantly different from W-W, C-W and C-C groups.

<table>
<thead>
<tr>
<th>Plasma molar concentration ratios</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W-W (7)</td>
</tr>
<tr>
<td>6 h Theobromine</td>
<td>0.32±0.09</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td>Paraxanthine</td>
<td>0.32±0.08</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.24±0.07</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td>8 h Theobromine</td>
<td>0.75±0.27</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td>Paraxanthine</td>
<td>0.64±0.20</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.56±0.22</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. for the number of rats indicated in parentheses. Abbreviations are shown in Table II. Not significantly different among four groups using a one-way analysis of variance.
though a trend toward the increased CL was noted in the C-C group. In comparison of the W-W and the C-W groups, there was no significant difference in the pharmacokinetic parameters.

![Graphs showing correlations between molar concentration ratios of Paraxanthine and Theophylline to Caffeine in W-W and C-W groups.](image)

**FIG. 2. Correlations between Molar Concentration Ratios of the Metabolites to Caffeine in Pregnant Rat Plasma and k_{el} or CL**

Broken lines were determined using the data obtained from 14 rats of W-W (□) and C-W (○) groups. Solid lines were determined using the data obtained from 13 rats of W-C (■) and C-C (●) groups. The molar concentration ratios represent the values observed at 8 h after intravenous administration of caffeine. There was no different tendency between the results at 6 h and those at 8 h. r expresses the correlation coefficient (significant difference; p < 0.05).

**TABLE IV. Pharmacokinetic Parameters of Caffeine in Pregnant Rats after Oral Administration of Caffeine**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W-W (3)    W-C (6)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>330 ± 28    357 ± 23</td>
</tr>
<tr>
<td>V_d (ml/kg)</td>
<td>761 ± 211    640 ± 47</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>5.0 ± 1.0    3.3 ± 0.6</td>
</tr>
<tr>
<td>k_{el} (h^{-1})</td>
<td>0.14 ± 0.03   0.22 ± 0.05</td>
</tr>
<tr>
<td>CL (ml/h/kg)</td>
<td>108 ± 40     141 ± 36</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.D. for the number of rats indicated in parentheses. Abbreviations are shown in Table II.*

*a) p < 0.05, significantly different from W-W and W-C groups.*
ters. Table III shows the molar concentration ratios of the metabolites to caffeine in rat plasma at 6 and 8 h after intravenous administration of caffeine. No significant difference was found among the four groups, and there were individual variations in the W-C and the C-C groups. The individual values in the relationship between the molar concentration ratios of the metabolites to caffeine in rat plasma at 8 h after intravenous administration of caffeine and $k_e$ or $CL$ are plotted in Fig. 2. The data on theobromine were not shown in Fig. 2, because the results of theobromine were similar to those of the other dimethylxanthines. The values of the correlation coefficient varied from 0.50 to 0.92 ($p < 0.05$). There was no significant difference in the slopes of the regression lines between the caffeine-treated and the non-treated groups during pregnancy. There was a considerably wide variation of the caffeine disposition among individuals in the rats taking 0.04% caffeine throughout pregnancy.

**Oral Study**

The plasma concentration-time curves of caffeine and its dimethylxanthines after oral administration of caffeine are shown in Fig. 1 (C) and (D). The concentrations of the metabolites reached the peak levels between 4 and 8 h after oral administration of caffeine. Table IV summarizes the pharmacokinetic parameters in pregnant rats after oral administration of caffeine. The results in the oral study showed a similar tendency as in the intravenous study. In the W-C group the $V_d$ tended to decrease. In the C-C group $k_e$ and $CL$ were significantly increased. As shown in Fig. 3, there was a significant correlation between the molar concentration ratios of the metabolites to caffeine in rat

**FIG. 3.** Correlations between Molar Concentration Ratios of the Metabolites to Caffeine in Pregnant Rat Plasma and $k_e$ or $CL$

Solid lines were determined using the data obtained from 11 rats of W-C and C-C groups. The molar concentration ratios represent the values observed at 8 h after oral administration of caffeine. There was no different tendency between the results at 6 h and those at 8 h. $r$ expresses the correlation coefficient (significant difference; $p < 0.05$).

plasma at 8 h after oral administration of caffeine and $k_{el}$ or $CL$. The data on theobromine were not shown in Fig. 3, because the results of theobromine were similar to those of the other dimethylxanthines.

**DISCUSSION**

The plasma concentration of caffeine might obey two or three compartment model, if the plasma concentration of caffeine during the distribution phase was determined. But we could not determine the plasma concentration of caffeine during the distribution phase, because the available blood volume was limited in pregnant rats. As to caffeine, the distribution equilibrium in the tissues is achieved within 5 min in mice\(^{8,9}\) and rats\(^{6,8}\) So the distribution phase of caffeine might be short. Therefore, in the pharmacokinetic analysis of this present study, the pharmacokinetic parameters were obtained by using the linear decline during the elimination phase.\(^{10}\)

It is well known that caffeine-metabolizing capacity is dependent on the hepatic microsomal monooxygenase system.\(^{11,12}\) Mitoma et al.\(^{13,14}\) reported that the pretreatment with more than 40 mg/kg/d of caffeine for 3 d stimulated the drug-metabolizing activity in the rat. In the present study, the pregnant rats in the W-C and the C-C groups had taken about 50 mg/kg/d of caffeine when calculated without consideration of loss at drinking and the $k_{el}$ and the $CL$ were increased in most of these rats. This result may be caused by the induction of the hepatic microsomal monooxygenase system. It is theoretically considered that the molar concentration ratios of the metabolites to caffeine at the peaks of plasma metabolite levels should reflect the caffeine-metabolizing capacity, as mentioned in Materials and Methods. Practically, there were significantly positive correlations between the molar concentration ratios of the metabolites to caffeine in plasma at 8 h and $k_{el}$ or $CL$ in the W-C and the C-C groups. There were individual variations of the molar concentration ratios of the metabolites to caffeine in the W-C and the C-C groups, which mean that caffeine metabolism varied widely among the rats taking caffeine during pregnancy. Recently there have been several reports concerning the pharmacokinetic study of caffeine in pregnancy. Knutti et al.\(^{15}\) found that $t_{1/2}$ of caffeine in saliva was prolonged in pregnant women. The decreased caffeine elimination during late pregnancy was recognized in human\(^{16,17}\) and animal\(^{17}\) studies. It has been reported that the elimination of caffeine is impaired in women taking oral contraceptives.\(^{18,19}\) Brazier et al.\(^{20}\) suggested that the decreased elimination of caffeine may be related to the increased female steroidal hormones in pregnant women. It is considered that the continuous caffeine ingestion and the pregnancy induced the variation of caffeine-metabolizing capacity, as observed in the W-C and the C-C groups.

As to $k_{el}$ and $CL$, there were statistically significant differences between the C-C group and the other groups in the oral study, but not in the intravenous study. The above inconsistency in statistical analysis may not be due to the variation of $F$ of caffeine among the groups, as it is known that the absorption of caffeine is complete in the rat.\(^{6,11}\) In the oral study, the number of rats was small, the mean values of the $k_{el}$ and the $CL$ in the W-W group were exceptionally low, and the standard deviations of the $k_{el}$ and the $CL$ in the C-C group were small, as compared to the data obtained from the intravenous study. Therefore, the $k_{el}$ and the $CL$ in the C-C group were significantly increased in the oral study, but not in the intravenous study.

The causes of the decreased $V_d$ in the W-C group remain uncertain. As the plasma protein binding of caffeine is very low, its change does not affect $V_d$. The decreased $V_d$ may mean the lowered total body water. It is known that the total body water varied and the average water content is about 0.7 l/kg in adult rats.\(^{21}\) In the present study, the observed $V_d$ approximated to the total body water except in the W-C group. As to the causes of the lowered total body water, the change in the fat content and/or the diuretic
effect by the administration of caffeine only during pregnancy may be considered. On the other hand, it is reported that caffeine disposition exhibits dose-dependent pharmacokinetics at more than 10 mg/kg of caffeine in rat study. Therefore, $V_d$ may have been underestimated in the course of calculation. As to the change of $V_d$, further studies are necessary to clarify the change of water and fat contents.

Caffeine is metabolized into theobromine, theophylline, paraxanthine and 1,3,7-trimethyl-dihydrouric acid, among which there is no interconversion in the primary biotransformation step. In the present study, the concentrations of the metabolites in plasma have reached the peak levels between 4 and 8 h (Fig. 1). Since the $CL_i$ is slow, the concentrations of the metabolites between 4 and 8 h show nearly the peak levels. The distinct correlation between the molar concentration ratios of the metabolites to caffeine in plasma and $k_{el}$ or $CL$ was recognized in accordance with the Eq. 3, especially in the oral study (Figs. 2 and 3). Based on this study, the drug-metabolizing capacity might be predicted by determining the molar concentration ratios of the metabolites to caffeine in plasma collected at 8 h after oral administration of caffeine. The method to estimate the hepatic drug-metabolizing using the concentration ratios of a metabolite to its precursor has been studied with the rats pretreated with carbon tetrachloride or liver enzyme-inducing agents. Caffeine is rapidly absorbed, distributed into the total body water and almost completely metabolized by the hepatic microsomal monooxygenase system. Therefore, caffeine may also become a convenient indicator for the drug-metabolizing capacity.

The various modes of maternal caffeine ingestion during the premating and/or the pregnant periods changed some pharmacokinetic parameters. Caffeine is easily passed into the fetus. Since the maternal caffeine ingestion may affect the fetal cerebral development, further investigation on the pharmacodynamics of caffeine in the fetus is necessary.

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