Oral pharmacokinetics of acetaminophen to evaluate gastric emptying profiles of Shiba goats

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ABSTRACT. The pharmacokinetics of acetaminophen was investigated following oral dosing to Shiba goats in order to evaluate the properties of gastric emptying. Acetaminophen was intravenously and orally administered at 30 mg/kg body weight to goats using a crossover design with a 3-week washout period. The stability of acetaminophen in rumen juice was also assessed. Acetaminophen concentrations were measured by HPLC. Since acetaminophen was stable in rumen juice for 24 hr, the extremely low bioavailability (16%) was attributed to its hepatic extensive first-pass effect. The mean absorption time and absorption half-life were unexpectedly short (4.93 and 3.35 hr, respectively), indicating its marked absorption from the forestomach, which may have been due to its smaller molecular weight. Therefore, acetaminophen was considered to be unsuitable for evaluating gastric emptying in Shiba goats.

KEYWORDS: acetaminophen, gastric emptying, goat, oral absorption, pharmacokinetics
Laboratory Animals' approved by the Ethics Committee of the Faculty of Agriculture, Tokyo University of Agriculture and Technology (approval number 76/25). These goats were housed in pens at an ambient temperature and with good ventilation. Animals were fed hay cubes (#1A Cubes, Eckenberg Farms Inc., Mattawa, WA, U.S.A.) at 0.8 kg/head twice a day, and water was available ad libitum.

The oral pharmacokinetics of AAP, its stability in rumen juice and the octanol-buffer (pH 6.5) partition coefficient were investigated in the present study. In the pharmacokinetic study, AAP was administered into the left jugular vein or orally at a dose of 30 mg/kg body weight to five male goats using a crossover design with at least a 3-week washout period. Blood samples (3 ml) were collected from the right jugular vein immediately prior to and 0.5, 1, 2, 3, 4, 6, 9 and 12 hr following an intravenous injection of AAP, and 0.5, 1, 2, 4, 6, 9, 12 and 16 hr after its oral administration. Plasma samples were separated by the centrifugation of blood at 1,600 g for 10 min and stored at −20°C until later analyses.

The stability of AAP in the rumen juice was determined as described previously [4]. Briefly, 40 ml of rumen fluid was collected from two goats using a catheter, pooled and processed for incubation immediately after its collection. Two hundred microliters of the AAP solution (1 mg/ml) was added to 1.8 ml of the rumen juice to give a final concentration of 100 µg/ml per ml of the incubation mixture. Five samples were prepared from this mixture and incubated in a thermostatic shaking water bath at 39°C for 24 hr under anaerobic conditions. The incubated mixture was then centrifuged at 20,000 g for 10 min, and the supernatant was collected.

The octanol-buffer partition coefficient of AAP was determined by the shake flask method as recommended by the Organization for Economic Cooperation and Development [13]. Before partitioning, the two solvents were mutually saturated at 25°C for 24 hr. Solutions of AAP (10 µg/ml) were prepared in octanol-saturated phosphate buffer (50 mM, pH 6.5). These solutions were then equilibrated at 25°C with an equivalent, double and half volume of buffer-saturated octanol. Two separating funnels were used in all three runs. After equilibration, the buffer phase was collected and centrifuged at 20,000 g for 10 min, and the supernatant was collected.

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The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of a pump (LC-10AD), UV detector (SPD-6A), integrator (Chromatopac C-R7A plus) and loop injector (model 7125). The mobile phase was a mixture of 0.1 M acetate buffer (pH 4) and acetonitrile (90:10, v/v). Triethylamine 150 µl/l mobile was added. Analytical separation was accomplished using a reversed-phase ODS column (TSKgel ODS-120T®, 4.6 µm × 250 mm, TOSOH Co., Tokyo, Japan). The flow rate was 1 ml/min. The wavelength of the detector was 248 nm. Sample preparation and analyses were conducted at room temperature. AAP was found to be accurately resolved as a single sharp peak with a retention time of 5–6 min. The recovery of AAP from plasma samples was 100.1 ± 2.65% at 1 µg/ml (mean ± SD, n=5), while that from rumen juice samples was 97.0 ± 2.03% at 25 µg/ml (mean ± SD, n=5). The inter-day CV values ranged from 2.24 to 3.20% for plasma samples and from 1.44 to 3.05% for rumen juice samples (n=5, 3 times).

The plasma concentration-time curves of AAP after the intravenous injection fit well with the two compartment model. Therefore, the curves obtained after the intravenous injection \((C_{p_{iv}}(t))\) and oral administration \((C_{p_{po}}(t))\) were described by Eq. 1 and 2, respectively.

\[
C_{p_{iv}}(t) = \frac{Dose}{V} \left( \frac{\alpha}{\alpha - \beta} \cdot e^{-\alpha t} + \frac{k_{21}-\beta}{\alpha - \beta} \cdot e^{-\beta t} \right) \quad \text{(Eq. 1)}
\]

\[
C_{p_{po}}(t) = \frac{Dose \cdot F \cdot k_a}{V} \left( \frac{k_{21} - k_3}{(k_3 - \alpha) (\alpha - \beta)} \cdot e^{-(\alpha - k_3)t} + \frac{k_{21} - \beta}{(k_3 - \beta) (\alpha - \beta)} \cdot e^{-\beta t} + \frac{k_{21} - k_3}{(\alpha - k_3) (\beta - k_3)} \cdot e^{-k_3 t} \right) \quad \text{(Eq. 2)}
\]

Equations 1 and 2 were simultaneously fit to the plasma concentration-time curves of AAP after it was intravenously and orally administered to the same goats, respectively, in order to calculate pharmacokinetic parameters by the nonlinear least-squares method using the curve fitting program, MULTI [19].

Several pharmacokinetic parameters were calculated by a non-compartmental analysis. The area under the concentration versus time curve (AUC) was calculated using the trapezoidal method (from time zero to the last sampling time) and integration (from the last sampling time to infinity). Total body clearance (\(C_{L_{tot}}\)), bioavailability (\(F\)), mean residence time (\(MRT\)), MAT and the distribution volume at steady state (\(V_{dss}\)) were calculated by conventional methods.

The plasma concentrations of AAP rapidly increased and peaked 0.90 ± 0.22 hr after being orally administered, and this was followed by its slow elimination. On the other hand, plasma concentrations were eliminated rapidly after the intravenous injection with short half-lives (1.14 ± 0.46 hr), as presented in Fig. 1. The calculated average values with SD of MAT and absorption half-life \((t_{1/2a})\) of AAP were 4.93 ± 0.87 and 3.35 ± 0.50 hr, respectively (Table 1). These values are similar to those of DF (6.75 ± 2.74 and 4.13 ± 1.94 hr, respectively) in a previous study using Shiba goats [4]. These results suggest that AAP was absorbed more from the forestomach, similar to DF. The partition coefficient of
AAP was markedly lower than that of DF at the pH of rumen fluid (pH 6.5), as shown in Table 2. This result indicated that factors other than lipophilicity predominantly influenced the absorption of AAP from the forestomach, for example, molecular size, as has already been suggested by Morishita et al. [10]. They compared the gastrointestinal absorption of several sulfonamides in rats and found that sulfanilamide had a fast absorption rate that was unexplainable from its smaller partition coefficient than other sulfonamides. They concluded that the fast absorption of sulfanilamide may have been due to its small molecular weight (172.21). Because the molecular weight of AAP (151.2) is similar to that of sulfanilamide and is markedly smaller than that of DF (318.1), as listed in Table 2, the faster absorption of AAP may have been due to its smaller molecular weight, similar to sulfanilamide.

Fast oral absorption of AAP has been found in dairy cows by Grünberg et al. [7]. In their experiment, peak concentrations of AAP were observed less than 2 hr after oral administration. This fact may suggest that AAP is markedly absorbed from forestomach also in daily cows like in Shiba goats.

The bioavailability of AAP was less than 20%. The recoveries of AAP from rumen juice samples at 100 µg/ml (n=5) after a 12- and 24-hr incubation at 39°C were 90.5 ± 1.5 and 88.7 ± 0.8% (mean ± SD), respectively. Since AAP is stable in rumen juice, its low bioavailability after its oral administration may have been due to its extensive first-pass effect in the liver. This may also be attributed to the large metabolic capacity of Shiba goats [1, 17].

In conclusion, the results of the present study suggested that AAP was markedly absorbed from the forestomach of Shiba goats, which may have been due to its small molecular weight. Therefore, AAP was considered unsuitable for evaluating gastric emptying in Shiba goats.

**REFERENCES**


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**Table 1. Pharmacokinetic parameters of AAP in male Shiba goats determined after single intravenous and oral administration of 30 mg/kg body weight**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AAP</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</td>
<td>0.986 ± 0.507</td>
<td>0.294 ± 0.128</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>0.900 ± 0.224</td>
<td>0.194 ± 0.073</td>
</tr>
<tr>
<td>α (hr&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>3.33 ± 2.10</td>
<td>0.273 ± 0.114</td>
</tr>
<tr>
<td>β (hr&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.693 ± 0.267</td>
<td>0.453 ± 0.187</td>
</tr>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt; (hr&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.210 ± 0.032</td>
<td>0.173 ± 0.019</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2ka&lt;/sub&gt; (hr)</td>
<td>3.37 ± 0.48</td>
<td>2.10 ± 0.45</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2β&lt;/sub&gt; (hr)</td>
<td>1.14 ± 0.49</td>
<td>0.86 ± 0.26</td>
</tr>
<tr>
<td>F (%)</td>
<td>17.0 ± 8.3</td>
<td>29.10 ± 2.74</td>
</tr>
<tr>
<td>F* (%)</td>
<td>100</td>
<td>91.8 ± 9.5</td>
</tr>
<tr>
<td>CL (l/hr/kg)</td>
<td>6.05 ± 2.74</td>
<td>91.8 ± 9.5</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt; (l/kg)</td>
<td>4.93 ± 0.867</td>
<td>6.05 ± 2.74</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;p.o.&lt;/sub&gt; (hr)</td>
<td>5.46 ± 0.86</td>
<td>318.1</td>
</tr>
<tr>
<td>MAT (hr)</td>
<td>0.617 ± 0.15</td>
<td>0.194 ± 0.073</td>
</tr>
</tbody>
</table>

**Table 2. Absorption profiles and physicochemical properties of AAP and DF**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AAP</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK&lt;sub&gt;a&lt;/sub&gt;</td>
<td>9.56</td>
<td>4</td>
</tr>
<tr>
<td>F&lt;sub&gt;*&lt;/sub&gt; (%)</td>
<td>100</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td>2.07 ± 0.17</td>
<td>91.8 ± 9.5</td>
</tr>
<tr>
<td>P*</td>
<td>2.07</td>
<td>29.10</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>151.2</td>
<td>318.1</td>
</tr>
<tr>
<td>MAT (hr)</td>
<td>4.93 ± 0.867</td>
<td>6.05 ± 2.74</td>
</tr>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt; (hr&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.210 ± 0.032</td>
<td>0.194 ± 0.073</td>
</tr>
</tbody>
</table>

pK<sub>a</sub>: Dissociation constant. Referred from reference [9] (AAP) and [16] (DF). F<sub>*</sub>: The ratio of the unionized fraction (calculated at pH 6.5). P: Apparent partition coefficient between octanol and phosphate buffer at pH 6.5. P*: Intrinsic partition coefficient between octanol and phosphate buffer calculated from apparent partition coefficient and pK<sub>a</sub> in the table. MAT: Mean absorption time. k<sub>a</sub>: Absorption rate constant.

a) Measured by the same method used for AAP in the present study.

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**Fig. 1. Plasma concentration–time curve of AAP (30 mg/kg body weight) after its single intravenous (open circles) and oral administration (closed circles) to goats. Concentrations are presented as the logarithm of mean and SD (n=5).**