NOTE *Pharmacology*

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.

KEY WORDS: acetaminophen, gastric emptying, goat, oral absorption, pharmacokinetics

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Although the entire gastrointestinal tract is capable of drug absorption, the main site of absorption of orally administered drugs is the proximal part of the gut. Several factors have been shown to influence the absorption of drugs from the gastrointestinal tract, with the gastric emptying rate being identified as important [5, 6, 16, 17]. The rate of gastric emptying determines the time taken to reach the absorption site, and thus, significantly affects the rate and extent of drug absorption. Delays in the gastric emptying time was previously reported to significantly decrease the rate of absorption of acetaminophen (AAP) and aspirin, whereas stimulating the gastric emptying accelerated the absorption of these drugs [11, 12].

Gastric emptying in ruminants necessitates drug transit from the rumen through the reticulum, omasum and abomasum, leading to the long residence of orally administered drugs in the forestomach. Therefore, the rate of drug absorption in ruminants may be the slowest of all animals due to the time required for drug particles to pass through the four-chambered stomach [1]. This finding explains why drugs with a very short half-life by the intravenous route, such as salicylic acid (1 hr), may nevertheless give sustained plasma concentrations in ruminants when administered by the oral route [5, 17].

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Although the oral route is considered to be inappropriate for ruminants, we previously demonstrated the effectiveness of this route for diclofenac (DF) and sulphamonomethoxine (SMM) in Shiba goats [4]; the mean absorption time (MAT) of DF (6.05 hr) was less than half that of SMM (15.1 hr). These findings suggested that the short MAT of DF was due to its marked absorption from the forestomach while the long MAT of SMM was due to a long gastric emptying time; however, gastric emptying time needs to be estimated in order to confirm this.

AAP is mainly absorbed from the small intestine of humans and most animal species, and not from the stomach [2, 14, 15, 18]. The AAP absorption test, which involves measuring plasma AAP concentrations in short time intervals following its oral administration, is considered a reliable method to evaluate gastric emptying rates in the stomachs of humans [2] as well as ponies and horses [3, 9]. Therefore, the present study was undertaken to examine the pharmacokinetics of AAP after oral dosing in order to evaluate the properties of gastric emptying in Shiba goats.

AAP was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). AAP was dissolved in 70% propylene glycol at a high temperature (approximately 70°C) for its intravenous administration. AAP was dissolved in 90% ethanol, mixed with three hay cubes and then allowed to dry before its oral administration. These solutions were prepared at a concentration of 200 mg/m*l*. All other reagents and chemicals used in this study were of HPLC or analytical grade.

The present study was performed using five clinically healthy male Shiba goats, weighing 21–44 kg and aged 2–3 years. All goats were maintained in accordance with the recommendations of the ‘Guide for the Care and Use of

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 pharmacokinetics 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 m*l*) 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 m*l* 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/m*l*) was added to 1.8 m*l* of the rumen juice to give a final concentration of 100 μg per m*l* 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/m*l*) 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 1,600 *g* for 10 min. The concentration of AAP in the buffer phase was then determined. The concentration of AAP in the octanol phase was obtained by mass balance. The apparent and intrinsic octanol/buffer partition coefficients were then determined from these data.

AAP concentrations in plasma, rumen juice and buffer samples were determined by HPLC with UV detection, as described previously [8] with some modifications. Briefly, 200 μl of perchloric acid (0.15 M) was added to 200 μl of the plasma or rumen juice samples and stirred. The mixtures were centrifuged at 20,000 *g* for 10 min. The supernatants were obtained and filtered using a 0.45-μm HPLC filter (Chromatodisc®, 4P, Kurabo Biomedical Industries, Ltd., Osaka, Japan). Fifty microliters of the filtrate was injected into the HPLC column.

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 (TSK-gel ODS-120T®, 4.6 μm × 250 mm, TOSOH Co., Tokyo, Japan). The flow rate was 1 m*l*/min. The wavelength of the detector was 248 nm. Sample preparation and analysis 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/m*l* (mean ± SD, n=5), while that from rumen juice samples was 97.0 ± 2.03% at 25 μg/m*l* (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 (Cp_{iv} (t)) and oral administration (Cp_{po} (t)) were described by Eq. 1 and 2, respectively.

$$\mathrm{Cp}_{iv}(t) = \frac{\mathrm{Dose}}{\mathrm{V}}\left\{\frac{\propto -\mathrm{k}_{21}}{\alpha-\beta}\cdot \mathrm{e}^{-\alpha\cdot \mathrm{t}} + \frac{\mathrm{k}_{21-\beta}}{\alpha-\beta}\cdot \mathrm{e}^{-\beta\cdot \mathrm{t}}\right\} \quad \text{(Eq. 1)}$$

$$\mathrm{Cp}_{po}(t) = \frac{\mathrm{Dose}\cdot \mathrm{F}\cdot k_a}{\mathrm{V}}\left\{\begin{array}{l}\dfrac{\mathrm{k}_{21-\alpha}}{(\mathrm{k_a}-\propto)(\beta-\alpha)}\cdot \mathrm{e}^{-\alpha\cdot \mathrm{t}} \\ +\dfrac{\mathrm{k}_{21}-\beta}{(\mathrm{k_a}-\beta)(\alpha-\beta)}\cdot \mathrm{e}^{-\beta\cdot \mathrm{t}} \\ +\dfrac{\mathrm{k}_{21}-\mathrm{k_a}}{(\alpha-\mathrm{k_a})(\beta-\mathrm{k_a})}\cdot \mathrm{e}^{-\mathrm{k_a}\cdot \mathrm{t}}\end{array}\right\} \quad \text{(Eq. 2)}$$

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 non-linear 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 (CL_{tot}), bioavailability (F), mean residence time (MRT), MAT and the distribution volume at a 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 ($t_{1/2ka}$) 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 suggested that AAP was absorbed more from the forestomach, similar to DF. The partition coefficient of

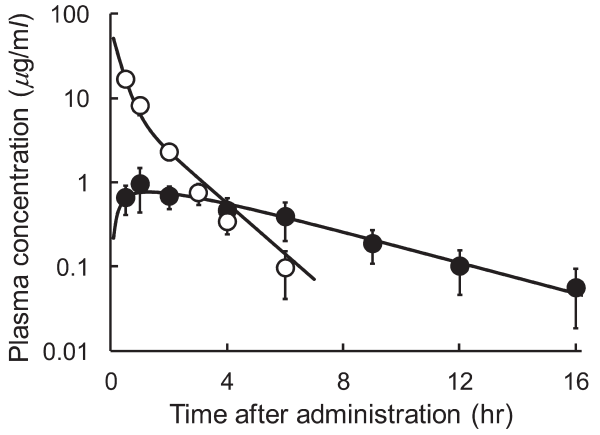

Fig. 1. Plasma concentration time curve of AAP (30 mg/kg body weight) after its single intravenous (open circles) and oral administration (closed circles) to goats. Concentrations are presented as the logarithm of mean and SD (n=5).

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

Parameters	Mean ± SD
C_{max} (μg/m*l*)	0.986 ± 0.507
T_{max} (hr)	0.900 ± 0.224
α (hr^{-1})	3.33 ± 2.10
β (hr^{-1})	0.693 ± 0.267
k_a (hr^{-1})	0.210 ± 0.032
$t_{1/2ka}$ (hr)	3.37 ± 0.48
$t_{1/2\beta}$ (hr)	1.14 ± 0.49
F* (%)	17.0 ± 8.3
F (%)	16.0 ± 8.5
$AUC_{i.v.}$ (μg·hr/m*l*)	35.2 ± 8.04
$AUC_{p.o.}$ (μg·hr/m*l*)	5.19 ± 2.17
CL (*l*/hr/kg)	0.869 ± 0.16
$MRT_{i.v.}$ (hr)	0.617 ± 0.15
$MRT_{p.o.}$ (hr)	5.46 ± 0.86
MAT (hr)	4.93 ± 0.87
V_{dss} (*l*/kg)	0.546 ± 0.19

C_{max}=maximum plasma concentration; T_{max}=time to maximum plasma concentration; α=first-order rate constant associated with the distribution phase; β=first-order rate constant associated with the elimination phase; k_a=absorption rate constant; $t_{1/2ka}$=absorption half-life; $t_{1/2\beta}$=elimination half-life; F=bioavailability calculated by a non-compartmental analysis; F*=bioavailability calculated by a compartmental analysis; $AUC_{i.v.}$=area under the plasma concentration–time curve after an intravenous injection from zero time to the last sampling time; $AUC_{p.o.}$=area under the plasma concentration–time curve after oral administration from zero time to the last sampling time; CL=total body clearance; $MRT_{i.v.}$=mean residence time after an intravenous injection; $MRT_{p.o.}$=mean residence time after oral administration; MAT=mean absorption time; V_{dss}=volume of distribution at a steady state.

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.

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

Parameters	AAP	DF
pk_a	9.56	4
f_u %	100	0.03
P	2.07 ± 0.17	91.8 ± 9.5[a)]
P*	2.07	29,100
Molecular weight	151.2	318.1
MAT (h)	4.93 ± 0.867	6.05 ± 2.74[4]
k_a (h^{-1})	0.210 ± 0.032	0.194 ± 0.073[4]

pk_a: Dissociation constant. Referred from reference [9] (AAP) and [16] (DF). f_u %: 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_a in the table. MAT: Mean absorption time. k_a: Absorption rate constant. a) Measured by the same method used for AAP in the present study.

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/m*l* (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.

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