Drug Absorption Profiles after Oral Administration in Goats

(ヤギにおける経口投与後の薬物吸収特性に関する研究)

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1. Abbreviations

α: first-order rate constant associated with the distribution phase

β: first-order rate constant associated with the elimination phase

AAP: acetaminophen

$AUC_{i.v.}$: area under the plasma concentration–time curve after i.v. injection

$AUC_{p.o.}$: area under the plasma concentration–time curve after oral administration

CL: total body clearance

C_{max}: maximum plasma concentration

DF: diclofenac

Eq: equation

F: bioavailability calculated by compartmental analysis

F*: bioavailability calculated by non-compartmental analysis

f_i: fraction ionized

f_u: fraction unionized

k_a: absorption rate constant

k_{el}: elimination rate constant

MAT: apparent mean absorption time

MAT*: real mean absorption time

$MRT_{i.v.}$: mean residence time after i.v. injection

$MRT_{p.o.}$: mean residence time after p.o administration

P: apparent partition coefficient

P^*: intrinsic partition coefficient

SA: sulfanilamide

SMM: sulfamonomethoxine

SMZ: sulfamethazine

SDZ: sulfadiazine

$t_{1/2\beta}$: elimination half-life

$t_{1/2ka}$: half-life of absorption

$t_{1/2kel}$: half-life of elimination

T_{max}: time to maximum plasma concentration

V_{dss}: volume of distribution at a steady state.

GENERAL INTRODUCTION

2.1. Preface

One of the main routes of drug administration is oral ingestion of drugs. Veterinary drugs may be administered to food-producing animals (poultry, cattle, sheep, goats and pigs) either individually or, more often, at a herd or flock level. The oral route is chosen because of enabling large numbers of animals (sometime several thousands) to be treated easily and cheaply at the same time. Administration of drugs in drinking water (*e.g.* in poultry) or as medicated feed (*e.g.* for pigs) ensures that all animals can be treated with minimum efforts. Another advantage of the oral route is the absence of stress that may occur with individual treatments that require first catching and then restraining and injecting animals individually. In addition, it is important for food producing animals to avoid both tissue damage and the presence of local residues, as is often the case for drugs administered individually by intramuscular and subcutaneous injection, especially for the long-acting or depot formulations.

Drugs can be administered orally in many forms including solutions, suspensions, pills, tablets, boluses, pellets, capsules and sustained release chemical devices for ruminants. The major obstacle encountered in veterinary medicine is the enormous interspecies diversity in comparative gastrointestinal anatomy and physiology, which result in the differences in efficiency of oral drug administration (54).

Oral absorption is the movement of the drug from the gastrointestinal tract into blood. The major steps occurring during oral drug absorption are starting by the dissolution of the drug from its form, the solubility as a function of its physicochemical

properties, the effective permeability to the intestinal mucosa and the presystemic metabolism (33).

Many factors may affect the above processes, and finally influence the rate and extent of drug absorption after oral administration. These factors can be divided into three categories (22, 37), namely ① physicochemical properties of a drug, including acid dissociation constant (pKa), lipophilicity, solubility, stability in the gastrointestinal fluids, intestinal permeability and molecular size, ② physiological and anatomical factors, such as stomach structure, gastrointestinal pH, gastric emptying, small intestinal transit time and absorption mechanism and ③ the dosage form factors, such as solution, suspension, capsule and tablet.

2.2. Gastrointestinal absorption and physicochemical considerations

Drug absorption after oral administration is influenced by many physiological factors, but it also depends on the pKa, solubility, lipophylicity and other physiocochemical properties of drugs. Clinically significant differences in the absorption of closely related drugs such as ampicillin and pivampicillin, lincomycin and clindamycin, or secobarbital and sodium secoparbital are the results of differences in physicochemical properties.

The pKa, lipophilicity and solubility of a drug, as well as the pH at absorption site, influence the absorption profile of a drug from solution. The interrelation among these parameters is known as the pH-partition theory of drug absorption. This theory provides

a basic framework for the understanding of drug absorption for gastrointestinal tract and drug transport across the biological membranes in the body.

The pH-partition theory of drug absorption is based on the assumption that the gastrointestinal tract and other biological membranes act like a simple lipid barriers to transport drugs and chemicals. Accordingly, the unionized form of drugs, if sufficiently lipophilic, is preferentially absorbed but the ionized form is not. Therefore, the rate and extent of drug absorption are related to the drug's oil water partition coefficient, the more lipophilic the drug, the faster is its absorption. Most of drugs are absorbed by passive diffusion. Weak acidic and neutral drugs are absorbed from the stomach, but basic drugs are not.

2.2.1. Drug pKa and gastrointestinal pH

The fractions of unionized and ionized forms of drugs in a solution depend on the pKa of the drug and the pH of the solution. The relationship between pH and pKa and the extent of ionization is given by the Henderson-Hasselbalch equation:

for acidic drugs

$$pKa - pH = \log (f_u / f_i)$$

for basic drugs

$$pKa - pH = \log (f_i / f_u)$$

Where f_u and f_i are the fractions of the drugs exist in the unionized and ionized forms, respectively. Accordingly, most weak acidic drugs are predominantly in the unionized form at the low pH of the gastric fluid and therefore, may be absorbed from the stomach as well as from the intestines. Some very weak acidic drugs (pKa > 8) such as phenytoin and theophylline exist as unionized form throughout the gastrointestinal tract. Therefore, their transport across the gut membrane is more rapid and independent of pH, assumed that the unionized from is lipophilic. Furthermore, the unionization of weak acidic drugs changes dramatically with pKa values between 2.5 and 7.5 and therefore, the rate of transport is pH dependent (56).

Most weak bases are poorly absorbed in the stomach, because they are present largely in the ionized form at low pH 1 to 2. Codeine, a weak base with a pKa of approximately 8, will have about 1 in every 1 million molecules in its unionized form at gastric pH 1. Weakly basic drugs with a pKa of less than 5, such as dapsone, diazepam, and chlordiazepoxide, are essentially unionized through the intestine. Basic drugs, which are those with pKa values between 5 and 11, show pH-dependent absorption. Stronger bases, such as guanethidine (pKa > 11) are ionized throughout the gastrointestinal tract and tend to be poorly absorbed.

The main site of drug absorption is the small intestines even though the drug exists mainly in the unionized form in the stomach and ionized form in the small intestines. More than 99% of the weak acid aspirin (pKa = 3.5) exist as unionized form in the gastric fluid at pH 1. On the other hand, only about 0.1% of aspirin is unionized at pH 6.5 in the lumen of the small intestines. Despite this unfavorable ratio of unionized and ionized

forms, aspirin and most of other weak acids are well absorbed from the small intestines. This is due to a relatively large surface area and a long residence time in the small intestines.

According to the Henderson-Hasselbalch equations, an increase in the pH of the stomach should retard the absorption of weak acidic drugs (pKa 2.5 to7.5) but promote the absorption of weak bases (58). The gastric absorption of aspirin is considerably reduced from 41% at pH 4 to 27% at pH 5 (59).

2.2.2. Drug's lipid solubility

Some drugs are poorly absorbed after oral administration even though they are available predominantly in the unionized form in the gastrointestinal tract. This is attributed to the low lipid solubility of the unionized molecule and so little molecules only can cross the absorptive membrane which is lipohilic in nature. Therefore, lipid solubility is important for drug absorption. The effects of lipid solubility on the extent of absorption of a series of barbiturate derivatives (where the dissociation constants (pKa) were almost same but the partition coefficients were different) were studied and the obtained results showed a direct relationship between the extent of absorption and the value of partition coefficient (57).

Polar or hydrophilic drugs such as gentamicin and ceftriaxone are poorly absorbed following oral administration due to low lipid solubility and, therefore, must be administered parenterally.

It must be clearly understood that even though drugs with greater lipid solubility are better absorbed, it is essential that drugs exhibit some degree of aqueous solubility because the biological fluids at the site of absorption are aqueous in nature and so the drug can be dissolved easily to be available in a solution form which is necessary for drug absorption. Therefore, from a practical viewpoint, drugs must exhibit a balance between hydrophilicity and lipophilicity. This factor is always taken into account while a chemical modification is being considered as a way of improving the efficacy of a therapeutic agent.

2.2.3. Drug stability and hydrolysis in the gastrointestinal tract

The incomplete or poor oral bioavailability of some drugs may be due to hydrolysis by acids or enzymes or microbes in the gastrointestinal tract. Hydrolysis of penicillin G and methicillin by gastric acid is an example. Moreover, ruminal microorganisms may inactivate some drugs through metabolic or chemical reactions (6) such as trimethoprim, chloramphenicol, and metronidazole (30, 43).

2.3. Physiological factors affecting drug absorption

Drugs are most commonly administered orally and the differences in the gastrointestinal tract anatomy and physiology among animals play a major role in determining the rate and extent of drug absorption. The major components of the gastrointestinal tract are the stomach, small intestines and large intestines or colon. In

simple stomach animals, the stomach is a pouch-like structure lined with a relatively smooth epithelial surface and the pH of the gastric contents ranged between 1 and 3.

However in ruminants the stomach is compound consists of four distinct chambers. The forestomach (rumen, reticulum and omasum) is a large volume compartment with a capacity ranging between 100 and 225 *l* in cattle, and 10~24 *l* in sheep and goats and a pH values range from 5.5 to 6.5. The reticulo-rumenal fluid can be a trapping compartment for circulating weak bases and thus influence their systemic disposition through the classical Henderson–Hasselbalch mechanism. In addition, the inner structure of the forestomach is lined by a keratinized stratified squamous epithelium, which may also contribute to slow drug absorption. Moreover, ruminal microorganisms may inactivate some drugs through metabolic or chemical reactions.

For gastrointestinal tract, absorption of drugs is due to passive diffusion of the unionized fraction of the drug. Passage will proceed until there is equilibrium on either side of the gastrointestinal barrier. The extent of absorption will be affected by the degree of ionization which is dictated by the pH on either side of the barrier.

Absorption of some weakly acidic or unionized drugs can be demonstrated in the stomach of dogs and rats under experimental conditions. Ethanol is rapidly and completely absorbed from the ligated stomach of dogs. Also similar results with sulfaethidole and barbital have been reported in rats under surgical conditions (11).

The earliest experiment (63) demonstrated the effects of atropine and pilocarpine on the eye after these had been added to a tied-off rumen. Since then, there has been a considerable amount of data to demonstrate that for many drugs passive diffusion occurs

in the ruminal epithelium. This has been demonstrated also for sulfonamides (4), salicylate, pentobarbitone, quinine (28) and thiabendazole (38), using tied-off rumens in *in vivo* situations.

The rate of drug diffusion across the rumino-reticular epitheliumis is slow compared with other parts of the gastrointestinal tract. The first reason is due to poor mixing of the aqueous phase in the rumen resulting in low concentration gradient of unionized drug between rumen and plasma and so low equilibrium pressure. The second reason is the relatively low surface area to volume ratio of the reticulo-rumen. A third reason is the relatively low blood supply, about 20-40% of the portal blood compared with the high blood supply of the small intestines. Adsorption to rumen contents is another reason. Cellulose has a high capacity for drug binding. In the horse, for example, feeding reduces the plasma bioavailability of trimethoprim and phenylbutazone (7). It is probable that drug binding occurs in the rumen and this will also serve to delay the absorptive process, although the drug may be released on cellulose digestion.

Maximum plasma concentration requires long time to be achieved after oral administration of drugs to ruminants. Weak acids, such as the sulfamethazine, whose pKa and lipid solubility should favor rapid absorption from the rumen, do not reach maximum concentrations in plasma for 6-8 h after oral dosage (65). Stronger acids also take some time to reach maximum concentrations in plasma of ruminants; 8 h for phenylbutazone (pKa 4.5) in cattle (14) and 4~8 h for salicylic acid (pKa 3.8) given orally to goats (12) and 5~6 h for meclofenamic acid (pKa 3.8) given intraruminally to sheep (35).

The small intestines are the major site for drug absorption due to a relatively large surface area, a long residence time and a high blood supply. The large epithelial surface area is due do presence of villi and microvilli which form folds in the intestinal mucosa.

The large intestines, like the stomach, has less irregular mucosa than that of small intestine. Drugs which are not absorbed from stomach or small intestines such as enteric-coated tablets are absorbed from large intestines.

2.3.1. Gastrointestinal blood flow

Once the drug is absorbed from the small intestine, it enters via the mesenteric vessels to the hepatic portal vein and the liver prior to reaching the systemic circulation. Any decrease in mesenteric blood flow, as in the case of congestive heart failure, will decrease the rate of drug removal from the intestinal tract, thereby reducing the rate of drug bioavailability. The blood supplying the gastrointestinal tract is important in maintaining the concentration gradient across the epithelial membrane. Highly permeable or lipophylic drugs or drugs that are small enough to be absorbed through the aqueous pores of the membrane are highly depending on the rate of blood flow while the drugs with poor permeability gastrointestinal perfusion is not important.

2.3.2. Gastrointestinal pH

The pH at absorption site is an important factor in drug absorption because many drugs are either weak acids or bases. Big differences exist in the pH between stomach, small intestines and large intestines. Ten thousand-fold difference in the hydrogen ion concentration exists between the stomach and the duodenum. The amount of unionized and ionized forms of drugs in a solution depends on the pKa of the drug and the pH of the solution. Since the gastrointestinal barriers are more permeable to the unionized, lipid soluble solutes, a drug may be absorbed from one segment of the gastrointestinal tract with a favorable pH and vice versa. Weakly basic drugs such as antihistamines are well absorbed in the small intestines where they exist mainly in the unionized form. On the other hand, the acidity of the gastric contents promotes the absorption of weakly acidic drugs such as sulfonamides and NSAIDS.

2.3.3. Gastric emptying and gastrointestinal motility

Generally drugs are better absorbed in the small intestine than in the stomach. Therefore, gastric emptying and gastrointestinal transit time are important factors for the rate and the extent of drug absorption. It is well known that the gastric emptying rate influences the plasma concentration profile of orally administered drugs. Delay in the gastric emptying time significantly decreased the rate of absorption of paracetamole (acetaminophen) and aspirin, while stimulating the gastric emptying accelerated the absorption of these drugs (44, 45). The intestinal transit rate determines the residence time of the drug in the absorption site. Long intestinal transit time is desirable for complete

absorption of drug such as enteric-coated tablets and drugs that absorbed from specific sites in the intestine. Peristaltic contraction promotes drug absorption by enhancing dissolution especially of poorly soluble drugs and by increasing the contact of drug to the absorption membrane.

As in monogastric species, the main site of drug absorption in ruminants may be the proximal part of the gut requiring that a drug transits from the rumen through the omasum and abomasum and the pylorus. Between the reticulo-rumen and the omasum, the reticulo-omasal orifice has a sieving function that can be viewed as the "pylorus" of the reticulo-rumen. It allows only the passage of small particles and of solution. When the drug is in solution, the transit of the ruminal liquid phase becomes the limiting factor with a relatively slow turnover rate in the range of 6~15 h (62).

2.3.4. First-pass metabolism

After oral administration, a drug must pass sequentially from the gut lumen through the gut wall, then through the liver, before reaching the systemic circulation. Metabolism may occur in the lumen before absorption, in the gut wall during absorption and/or in the liver after absorption but before reaching the systemic circulation. The entire blood supply draining most of the gastrointestinal tract returns to the systemic circulation by way of the liver. Therefore, the entire dose of the orally administered drugs that are completely absorbed is exposed to the liver before entering the blood stream. Since the liver is the main site of drug biotransformation because of its high level of drug metabolizing

enzymes, there is a possibility that large fraction of the dose will never reach the systemic circulation because of hepatic metabolism after absorption. This phenomenon is called first-pass effect and the extent of bioavailability depends on the extent of hepatic catabolism.

2.4. Scope of the thesis

Oral drug absorption in ruminants is generally more complex, unpredictable and may exhibit a markedly different kinetics, compared with that in monogastric species. Oral dosing is generally considered to be inappropriate for ruminants because of slow drug absorption and/or loss in the rumen. Therefore, intramuscular and subcutaneous injections are frequently used in cattle, sheep, and goats resulting in tissue irritation and local residues. The absorption of certain drugs from the forestomach of ruminants may be markedly high if they have appropriate physicochemical properties. Therefore, the main purpose of the present thesis is to clarify the correlations between drugs absorption profiles after their oral administration to goats and their physicochemical properties. To achieve this, the author performed the following steps to meet the final goal.

First of all, oral pharmacokinetic profiles of two weak acidic drugs, diclofenac and sulfamonomethoxine in Shiba goats, is examined in Chapter one. Second, the gastric emptying profile of Shiba goats is evaluated by oral pharmacokinetics of acetaminophen in Chapter two. Finally, Oral absorption profiles among sulfamethazine, sulfadiazine, and sulfanilamide in Shiba goatsae compared in Chapter three. All results obtained have been summarized and a general discussion and conclusion has been depicted. Clinical

applications and further perspectives of oral administration to ruminants have been also discussed.

CHAPTER ONE

Oral Pharmacokinetics of the Acidic Drugs, Diclofenac and Sulfamonomethoxine in Shiba Goats

3.1. ABSTRACT

Oral pharmacokinetics of the acidic drugs, diclofenac (DF) and sulfamonomethoxine (SMM), which have different physicochemical properties, were examined in Shiba goats. DF or SMM was intravenously and orally administered to 5 male goats at a dose of 1 and 10 mg/kg bodyweight, respectively using a crossover design with at least a 3-week wash out period. The T_{max} of DF and SMM were reached 1.5 and 5.6 h after they have been orally administered, respectively, and this was followed by their slow elimination. The elimination of both drugs was markedly faster after being intravenously rather than orally administered, which indicated flip-flop phenomena after the oral administration. The mean absorption times (MATs) of DF and SMM were 6 and 15 h, respectively. This slow absorption may have been due to slow gastric emptying in goats. The large difference observed in MATs between DF and SMM may have been because DF, which is more lipophilic than SMM, was partly absorbed from the forestomach. Therefore, these results suggest that the absorption of highly lipophilic drugs from the forestomach may be markedly high in Shiba goats. In case of drugs whose elimination is quite fast, their efficacies may appear from the early stage after oral administration even in ruminants, because elimination rate is the determinant factor of T_{max} in flip-flop phenomena. Such drugs may be used orally even in ruminants.

3.2. INTRODUCTION

Oral dosing is generally considered to be inappropriate for ruminants because of slow drug absorption and/or drug loss in the rumen. Therefore, intramuscular and subcutaneous injections are frequently used in cattle, sheep, and goats. The slow drug absorption reported after the oral administration of drugs to ruminants may be due to the unique anatomical and physiological properties of the gastrointestinal tract. The forestomach (rumen, reticulum, and omasum) is a large volume compartment with a capacity ranging between 100 and 225 *l* in cattle, and 10~24 *l* in sheep and goats. This may result in the dilution of drugs and a long gastric emptying time (5). Therefore, orally administered drugs may have a long residence time in the forestomach. The inner structure of the forestomach may also contribute to slow drug absorption; it is lined by a keratinized stratified squamous epithelium, which limits the absorption of drugs. Moreover, microflora in the rumen may inactivate some drugs through metabolic or chemical reactions (6).

Although it is well-known that drugs are mainly absorbed from the small intestine after oral dosing, the absorption of some drugs from the stomach may also be markedly high. This has been demonstrated for salicylic acid (17), sulfaethidole and barbital (11), and metoprolol (18) in rats. In ruminants, this has been demonstrated also for sulfonamides (4), salicylate, pentobarbitone, quinine (28) and thiabendazole (38), using tied-off rumens in *in vivo* situations.

Since the effective surface area of the stomach that actually contributes to drug absorption is small, the physicochemical properties of drugs may be important factors for

their absorption from the stomach (75). I also previously found the rapid antipyretic effects of DF in dairy cows with infectious disease following its oral administration in a preliminary trial. Moreover, sulfamethoxazole had a rapid appearance in the plasma of goats ($T_{max} = 0.8 \pm 0.2$ h) after its intraruminal administration (51). These findings suggest that the absorption of some drugs from the forestomach of ruminants may be markedly high if they have appropriate lipid solubility and unionization in the rumen fluid. The main purpose of this study was to clarify the relationship between drug absorption profiles after their oral administration to ruminants and their physicochemical properties. To achieve this, the oral pharmacokinetic profiles of two weak acidic drugs, DF and sulfamonomethoxine, were examined in male Shiba goats.

3.3. MATERILAS AND METHODS

3.3.1. Animals

All animals were maintained in accordance with the recommendations of the 'Guide for the Care and Use of Laboratory Animals' approved by the Faculty of Agriculture, Tokyo University of Agriculture and Technology (approval number 76/25). Five clinically healthy male Shiba goats, weighing 25~43 kg and aged 2~3 years were used in this study. These goats were housed in pens at an ambient temperature and with good ventilation. Animals were fed hey cubes (#1A Cubes ®, Eckenberg farms Inc.,

Mattawa, WA, USA) at 800 g/goat twice a day with water and mineralized salt licks were available *ad libitum*.

3.3.2. Reagents and chemicals

The sodium salt of DF and flufenamic acid (FA) were obtained from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). SMM and sulfadimethoxine were obtained as a sodium salt from Daiichi Pharmaceutical Company (Tokyo, Japan). All other reagents and chemicals used in this study were of HPLC or analytical grade.

3.3.3. Experimental design

3.3.3.1. Pharmacokinetic study

DF or SMM were dissolved in sterilized distilled water for injection and administered either into the left jugular vein or orally to five male Shiba goats at doses of 1 and 10 mg/kg, respectively, using a crossover design with at least a 3-week washout period. In case of the oral administration of these drugs, drug solutions were given with three hay cubes. The SMM study was started 3 weeks after the DF study. Blood (3 m*l*) was collected from the right jugular vein immediately prior to the treatment and 0.5, 1, 2, 3, 4, 6, 9, 12, and 24 h after dosing. Plasma was separated by the centrifugation of blood at 1,600 ***g*** for 10 min and stored at –20°C until analysis.

3.3.3.2. Stability of DF and SMM in the rumen juice

Two male Shiba goats were restrained and nasal catheters were passed into the rumen. Thereafter, 40 m*l* of rumen fluid was aspirated through the catheter and processed for incubation immediately after its collection. Fifty microliters of DF or SMM solutions (200 μg/m*l*) was added to 950 μl of the rumen fluid to give a final concentration of 10 μg/m*l* of the incubation mixture. Five samples of each drug were prepared and incubated in a thermostatic shaking water bath at 39°C for 24 h under anaerobic conditions. After the incubation, the concentrations of DF and SMM were measured by HPLC.

3.3.3.3. Octanol-buffer (pH 6.5) partitioning experiments

Octanol-buffer partitioning studies were performed using a shake flask method as recommended by the Organization for Economic Cooperation and Development (47). Before partitioning, the two solvents are mutually saturated at 25°C for 24 h. briefly, two large stock bottles, one containing 1-octanol and a sufficient quantity of sodium phosphate buffer (50 mM/*l*, pH 6.5), and the other containing the buffer and a sufficient quantity of octanol, were shaken using magnetic stirring at 500 rpm for 24 h at 25°C, and then to let them stand long enough to allow the two phases to separate. Solutions of the DF or SMM (10 μg/m*l*) were prepared in the octanol saturated buffer. 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. Equilibration was done by hand shaking of the funnels (by rotation of the funnels through 180 degree about its transverse axis, approximately a hundred time during five minutes) allowing the trapped air to rise through the two phases. The funnels were then fixed vertically by rack

until complete separation of the two phases. The buffer phase was collected and centrifuged at 1,600 ***g*** for 10 min at 25°C and the supernatant octanol phase was discarded. The total drug concentration in the buffer phase was then determined by HPLC and the total drug concentration in the octanol phase was calculated from the difference between initial and final concentrations in the buffer phase.

3.3.4. Drug assays

3.3.4.1. Diclofenac

DF concentrations in the plasma and rumen juice were determined by HPLC with UV-detection, as described previously (1) with some modifications. Briefly, 100 μl of FA solution (10 μg/m*l*) was added as an internal standard to 500 μl of the plasma or rumen juice sample, followed by the addition of 200 μl of phosphoric acid (0.15 M). Subsequently, 4 m*l* of diethyl ether was added to the mixture and shaken for 3 min. The sample was centrifuged at 3,000 ***g*** for 10 min at 5°C. The obtained supernatant (organic layer) was transferred into a pear shaped flask and evaporated to dryness by an evaporator (Rotavapor® R-114, Shibata Scientific Technology, Ltd., Tokyo, Japan) at 30°C. The residue was reconstituted in 200 μl of the mobile phase and filtered using a 0.45-μm HPLC filter (Chromatodisc®, 4 P, 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), a UV detector (SPD-6A), an integrator (Chromatopac C-R7A plus), and a loop injector (Model 7125). The mobile phase was a mixture of 0.1 M acetate buffer (pH

6.3) and acetonitrile (65:35, v/v). Analytical separation was accomplished by 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 278 nm. The recovery from plasma samples was 106.1 ± 2.8% at 1 μg/m*l* (n = 5), while that from rumen juice samples was 110.3 ± 8.5% at 10 μg/m*l* (n = 5). The inter-day CV values ranged from 0.83 to 3.72% for plasma samples and from 3.11 to 14.1% for rumen juice samples (n = 5, 3 times).

3.3.4.2. Sulfamonomethoxine

SMM concentrations were determined in the plasma and rumen juice samples by HPLC with UV-detection. Two hundred microliters of perchloric acid (0.5 M) were added to 200 μl of the plasma sample. The mixture was vortexed for 30 s and then centrifuged at 20,000 ***g*** for 5 min at 5°C. The obtained supernatant was filtered using the 0.45-μm HPLC filter. Fifty microliters of the filtrate was injected into the HPLC column.

In the case of rumen juice samples, SMM concentrations were determined after extraction with ethyl acetate. After being incubated for 24 h, 100 μl of sulfadimethoxine solution (10 μg/m*l*) was added as an internal standard to the rumen juice samples. Subsequently, 5 m*l* of ethyl acetate was added. The mixture was vortexed for 30 s then centrifuged at 3,000 ***g*** for 10 min at 5°C. The obtained supernatant was transferred into a pear shaped flask and evaporated to dryness at 30°C. The residue was reconstituted in 200 μl of the mobile phase and filtered using the 0.45-μm HPLC filter. Fifty microliters of the filtrate was injected into the HPLC column.

The mobile phase was a mixture of 50 mM acetate buffer (pH 5) and acetonitrile (75:25, v/v). Analytical separation was accomplished using a reversed-phase C_8 column (Mightysil RP-8 GP®, 4.6 μm×250 mm, Kanto Chemical Co., Tokyo, Japan). The flow rate was 1 m*l*/min. The wavelength of the detector was 270 nm. The recovery from plasma samples was 101.7 ± 4.34% at 1 μg/m*l* (n = 5), while that from rumen juice samples was 99.4 ± 4.2% at 10 μg/m*l*. The inter-day CV values ranged from 3.23 to 5.82% for plasma samples and from 3.39 to 4.67% for rumen juice samples (n = 5, 3 times).

3.3.5. Pharmacokinetic analysis

The plasma concentration-time curves of DF 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.

$$Cp_{iv}(t) = \frac{Dose}{V}\left\{\frac{\alpha - k_{21}}{\alpha - \beta} \cdot e^{-\alpha \cdot t} + \frac{k_{21} - \beta}{\alpha - \beta} \cdot e^{-\beta \cdot t}\right\} \qquad \text{(Eq. 1)}$$

$$Cp_{po}(t) = \frac{Dose \cdot F \cdot k_a}{V}\left\{\begin{array}{l} \frac{k_{21}-\alpha}{(k_a-\alpha)(\beta-\alpha)} \cdot e^{-\alpha \cdot t} \\ + \frac{k_{21}-\beta}{(k_a-\beta)(\alpha-\beta)} \cdot e^{-\beta \cdot t} \\ + \frac{k_{21}-k_a}{(\alpha-k_a)(\beta-k_a)} \cdot e^{-k_a \cdot t} \end{array}\right\} \qquad \text{(Eq. 2)}$$

In Eq. 2, F is bioavailability. Equation 1 and 2 were simultaneously fit to the plasma concentration-time curves of DF 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 (72).

On the other hand, the plasma concentration-time curves of SMM after it was intravenously administered fit well with the one compartment model. Therefore, the curves obtained after the intravenous injection (Cp_{iv} (t)) and those after the oral administration (Cp_{po} (t)) were described by Eq. 3 and 4, respectively.

$$Cp_{iv}(t) = \frac{Dose}{V} e^{-kel \cdot t} \qquad \text{(Eq. 3)}$$

$$Cp_{po}(t) = \frac{Dose \cdot F}{V} \cdot \frac{k_a}{k_a - k_{el}} (e^{-k_{el} \cdot t} - e^{-k_a \cdot t}) \qquad \text{(Eq. 4)}$$

Equation 3 and 4 were simultaneously fit to the plasma concentration-time curves after the intravenous injection and oral administration to the same goats, respectively.

Several pharmacokinetic parameters were calculated by non-compartmental analysis. The area under the concentration versus time curve (AUC) was calculated by 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, mean residence time (MRT), mean absorption time (MAT), and the distribution volume at a steady state (V_{dss}) were calculated by conventional methods.

3.4. RESULTS

The plasma concentrations of DF and SMM rapidly increased and peaked 1~2 h and 5~6 h after being orally administered, respectively, followed by their slow elimination.

On the other hand, plasma concentrations decreased rapidly after the intravenous injection with relatively short half-lives (3.05 ± 1.13 h for DF and 1.00 ± 0.11 h for SMM), indicating flip-flop phenomena after the oral administration of both drugs (Figs. 1-1 and 1-2). As shown in Tables 1-1 and 1-2, a pharmacokinetic analysis indicated the slow absorption of both drugs in male Shiba goats. The calculated MATs of DF and SMM were approximately 6 and 15 h, respectively. The absorption half-life ($t_{1/2ka}$) of DF was slightly longer than its elimination half-life ($t_{1/2\beta}$). On the other hand, the $t_{1/2ka}$ of SMM was markedly longer than its $t_{1/2kel}$ (approximately 10 times). The bioavailabilities of both drugs were more than 70%, as listed in Tables 1-1 and 1-2.

Since the bioavailabilities (F) of DF and SMM were incomplete, I evaluated the stability of both drugs in the rumen juice collected from male Shiba goats. The recovery from rumen juice samples was completed after a 24-h incubation and was 104.8 ± 11.9% for DF and 99.4 ± 2.85% for SMM.

3.5. DISCUSSION AND CONCLUSION

In the present study, the absorption profiles of DF and SMM after their oral administration to male Shiba goats were examined. The results of a pharmacokinetic analysis revealed the slow absorption of both drugs. The MAT values obtained were long (6 h for DF and 15 h for SMM). The oral pharmacokinetic profiles of DF and SMM have been clarified in several animal species. The absorption rate constant values for DF were previously shown to be 0.5~1.2 h^{-1} in pigs (48), 0.5 h^{-1} in rabbits (2), and 0.38 min^{-1} in rats (49). These values were markedly higher than those obtained from the male Shiba

goats in the present study (0.19 h^{-1}). The absorption of SMM was shown to be fast in pigs (31) as well as horses and humans (9). The obtained k_a values (1.8 h^{-1} in pigs and 1.38 h^{-1} in horses) were markedly higher than those obtained from the male Shiba goats in the present study (0.07 h^{-1}). Since the absorption of drugs from the small intestines is generally fast, gastric emptying is the determining factor for drug absorption after the oral administration of drugs (23, 55). Markedly higher k_a values were obtained for SMM in pigs and DF in rats after their intraduodenal administration than after their oral administration (31, 49). This may also have been the case for the male Shiba goats used in the present study. Therefore, the slow absorption rate of DF and SMM in the male Shiba goats may be due to their long residence time in the forestomach.

Although a pharmacokinetic analysis indicated the slow absorption of DF and SMM after their oral administration to goats, the C_{max} of both drugs achieved rapidly (T_{max} of DF and SMM were 1.5 and 5.6 h, respectively). In addition, the plasma concentration-time curves shown in Figs. 1-1 and 1-2 revealed the flip-flop phenomena. These phenomena occur when the absorption rate constant is smaller than the elimination rate constant (74); therefore, the slope of the terminal log-linear phase after the oral administration of a drug reflects the absorption rate constant. When oral pharmacokinetics exhibits these phenomena, the determining factor of T_{max} is function of the drug elimination rate constant, the faster elimination results in the shorter T_{max}. The elimination half-lives ($t_{1/2\beta}$ or $t_{1/2kel}$) obtained for both DF and SMM were relatively shorter (Tables 1-1 and 1-2). Therefore, the elimination of DF and SMM in male Shiba goats may have been fast enough to achieve C_{max} rapidly after their oral administration. This result

suggests that, even in ruminants, an oral route may be suitable for drugs that have a fast elimination if they are not subjected to an extensive first-pass effect in the liver.

A marked difference was observed in the oral absorption profiles of DF and SMM. The MAT of DF was less than half that of SMM in the present study. This result suggests that absorption of DF from the forestomach of male Shiba goats may have been markedly high. The pH value of the rumen juice in the present study was 6.4, as has been reported previously (19, 27). Furthermore, the pKa of DF is 4 (53), suggesting that negligible DF molecules exist as an unionized form (0.1~1%) in the contents of the rumen. On the other hand, the pKa of SMM is 6 (32, 46, 66), which suggests that 10~50% of SMM molecules exist as an unionized form. These findings indicate that SMM is more suitable for absorption from the forestomach of goats. However, the partition coefficient between octanol and buffer (pH 6.5) in the present study is different. That of DF is 91.78 ± 9.45, whereas that of SMM is 1.72 ± 0.174. Therefore, DF may have been absorbed from the forestomach because of its extremely high lipid solubility.

In the present study, Eq. 1 and 2 or Eq. 3 and 4 were simultaneously fit to intravenous and oral plasma concentration-time data from the same goats, respectively, in order to calculate pharmacokinetic parameters. Data obtained from intravenous and oral administration routes are typically independently analyzed. Therefore, it is not uncommon to obtain different values for the same parameter, such as the elimination rate constant, even though both data are obtained from the same individuals. This difference may result in inaccuracies in the absorption rate constants obtained. In order to avoid this problem, I adopted a simultaneous analysis. As a result, a good fit between the observed

points and theoretical curves in the cases of DF and SMM, was obtained as shown in Figs. 1-1 and 1-2. Therefore, I considered the absorption rate constants obtained to be reliable.

Although the oral bioavailabilities of DF and SMM were incomplete (Tables 1-1 and 1-2), both drugs were stable in the rumen juice in the *in vitro* spiked test, which indicated that both drugs were subjected to the first-pass effect in the liver. Previous studies demonstrated that DF had good gastrointestinal tolerability (39) and underwent first-pass metabolism (13, 29, 61).

Most sulphonamides are unlikely to undergo degradation in the rumen juice. Sulfamethoxydiazine, sulfathiazole, sulfadimidine, and sulfamoxole were stable in the rumen juice of dwarf goats during anaerobic incubation at 39°C (68). A previous study also suggested that the low bioavailability of sulfamethoxazole after its oral administration to goats was most likely due to the first-pass effect in the liver (51).

The present study was done using five male Shiba goats. It was suggested that female dwarf goats have higher hydroxylation capacity for sulfamethazine than males (69). They found that CL values of the sulfonamide in females were 3.5 times higher than males after intravenous injection. They also indicated that this higher capacity is due to lower testosterone levels in females. These facts may suggest that female Shiba goats have higher hydroxylation capacity for SMM than males. Since acetylated metabolites of SMM were not found in plasma, SMM may be biotransformed mainly into hydroxylated metabolites in Shiba goats, like sulfamethazine in dwarf goats. Female Shiba goats, therefore, may show lower bioavailability due to higher first-pass effect in the liver and

shorter T_{max} due to faster elimination after its oral administration, compared with males in the present study.

In conclusion, gastric emptying may be the determining factor for drug absorption after the oral administration of drugs to Shiba goats. The absorption of highly lipophilic drugs from the forestomach may be markedly high in ruminants. As elimination of both DF and SMM is fast, their efficacies may appear from the early stage after oral administration in ruminants, because the elimination rate from the body is the determining factor for T_{max} in flip-flop phenomena.

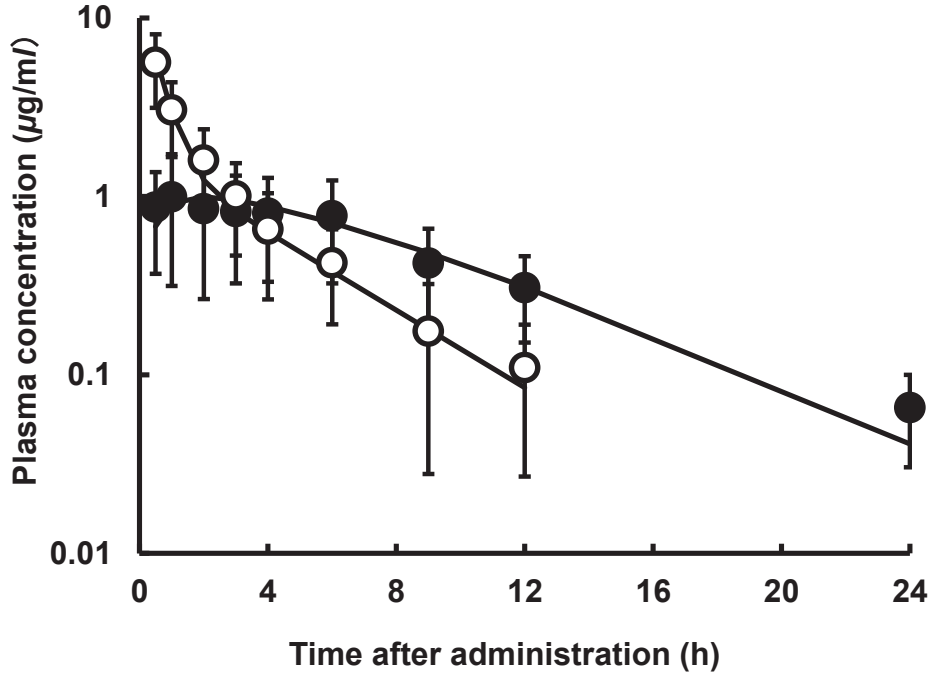

Fig. 1-1.

Plasma concentration-time curves of DF (1 mg/kg bodyweight) after its single intravenous (open circles) and oral administration (closed circles) to male Shiba goats. Each point and vertical bar represents the mean and standard deviation, respectively (n = 5). Each line is calculated by Eq. 1 or 2 using pharmacokinetic parameters in Table 1-1.

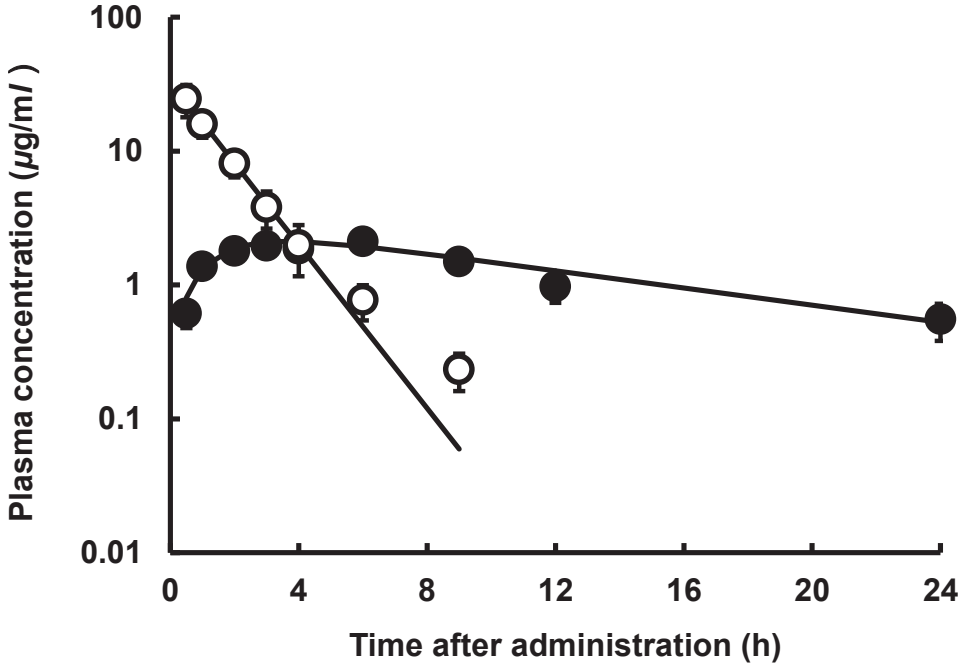

Fig. 1-2.

Plasma concentration-time curves of SMM (10 mg/kg bodyweight) after its single intravenous (open circles) and oral administration (closed circles) to male Shiba goats. Each point and vertical bar represents the mean and standard deviation, respectively (n = 5). Each line is calculated by Eq. 3 or 4 using pharmacokinetic parameters in Table 1-2.

Table 1-1.

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

Parameter		Mean ± SD (n = 5)
k_a	(h^{-1})	0.194 ± 0.073
C_{max}	(μg/m*l*)	1.12 ± 0.58
T_{max}	(h)	1.51 ± 1.41
α	(h^{-1})	2.09 ± 0.97
β	(h^{-1})	0.250 ± 0.078
$t_{1/2ka}$	(h)	4.13 ± 1.94
$t_{1/2\beta}$	(h)	3.05 ± 1.13
$AUC_{i.v.}$	(μg·h/m*l*)	14.7 ± 6.2
$AUC_{p.o.}$	(μg·h/m*l*)	10.4 ± 4.0
CL	(*l*/h/kg)	0.0784 ± 0.0309
F	(%)	75.4 ± 24.0
F*	(%)	73.9 ± 20.2
$MRT_{i.v.}$	(h)	2.38 ± 1.01
$MRT_{p.o.}$	(h)	8.42 ± 2.15
MAT	(h)	6.05 ± 2.74
V_{dss}	(*l*/kg)	0.181 ± 0.102

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

Table 1-2.

Pharmacokinetic parameters of SMM in male Shiba goats determined after a single intravenous and oral administration of 10 mg/kg bodyweight.

Parameter		Mean ± SD (n = 5)
k_a	(h^{-1})	0.0737 ± 0.0296
C_{max}	(μg/m*l*)	2.15 ± 0.29
T_{max}	(h)	5.60 ± 2.30
k_{el}	(h^{-1})	0.703 ± 0.084
$t_{1/2ka}$	(h)	10.5 ± 3.6
$t_{1/2kel}$	(h)	1.00 ± 0.11
$AUC_{i.v.}$	(μg·h/m*l*)	49.9 ± 11.3
$AUC_{p.o.}$	(μg·h/m*l*)	37.5 ± 6.7
CL	(*l*/h/kg)	0.212 ± 0.067
F	(%)	79.3 ± 16.5
F*	(%)	77.1 ± 14.8
$MRT_{i.v.}$	(h)	1.49 ± 0.19
$MRT_{p.o.}$	(h)	16.6 ± 4.6
MAT	(h)	15.1 ± 4.72
V_{dss}	(*l*/kg)	0.321 ± 0.134

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

CHAPTER TWO

Evaluation of Gastric Emptying Profiles of Shiba Goats by Oral Pharmacokinetics of Acetaminophen

4.1. ABSTRACT

The pharmacokinetics of oral acetaminophen (AAP) in Shiba goats were examined in order to evaluate the property of amomasal emptying. AAP was intravenously and orally administered at 30 mg/kg bodyweight to five male Shiba goats using a crossover design with at least a 3-week washout period. In addition, the stability of AAP in rumen juice was evaluated by an *in vitro* experiment.

Concentrations of AAP in plasma and rumen juice were measured by HPLC. The obtained mean absorption time (MAT) and absorption half-life ($t_{1/2ka}$) were short (4.93 and 3.35 h, respectively). Oral bioavailability was extremely low (16%). Since AAP was stable in rumen juice for 24 h, it is suggested that the low bioavailability is mainly due to its extensive first-pass effect in the liver. The short $t_{1/2ka}$ and MAT of the AAP indicates a marked absorption from the forestomach of goats probably due to its smaller molecular weight and its extreme unionization. Therefore, AAP was considered not suitable for the evaluation of the gastric emptying in Shiba goats although it is generally considered as a good indicator of the gastric emptying in several animal species.

4.2. INTRODUCTION

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 can influence the absorption of drugs from the gastrointestinal tract. Among these factors, the gastric emptying rate is important (21, 23, 60, 62). The rate of gastric emptying determines the time taken to reach the absorption site, and thus affects significantly the rate and extent of drug absorption. Delay in the gastric emptying time significantly decreased the rate of absorption of paracetamol (AAP) and aspirin, while stimulating the gastric emptying accelerated the absorption of these drugs (44, 45).

As in monogastric species, the main site of drug absorption in ruminants may be the proximal part of the small intestines requiring that a drug transits from the rumen and reticulum through the omasum and abomasum and the pylorus. Between the reticulo-rumen and the omasum, the reticulo-omasal orifice has a sieving function that can be viewed as the “pylorus” of the reticulo-rumen. It allows only the passage of small and dense particles and of solution. This processes leads to a long residence of the orally administered drugs in the stomach of ruminants. When the drug is in solution, the transit of the ruminal liquid phase becomes the limiting factor with a relatively slow turnover rate in the range of 6~15 h. If a drug is strongly bound to cellulose, the transit to the distal part of the gut will be associated with that of small particles that require cellulose breakdown and the delay will be longer as the turnover time for the solid phase is approximately 50~60 h. 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 (6). This explains why drugs showing a very short half-life by the

intravenous route, such as salicylic acid (1 h), may nevertheless give sustained plasma concentrations in ruminants when administered by the oral route because gastric emptying controls the overall rate of drug absorption (21, 62).

Because of these facts, it is well considered that oral route is inappropriate for ruminants. However, I demonstrated the effectiveness of this route in case of DF and SMM in Shiba goats (20). In that study, the mean absorption times of DF and SMM were 6 and 15 h, respectively. MAT of DF was less than half, compared with SMM. This may indicate that DF is markedly absorbed from the forestomach of Shiba goats because DF is more lipid soluble than SMM. Also, it was suggested that, the gastric emptying time is long. Therefore, the estimation of the gastric emptying rate is necessary for detecting the rate of drug absorption, especially for less lipid soluble drugs which can't be absorbed from the rumen.

AAP is an analgesic and antipyretic agent that is mainly absorbed from the small intestine of humans, dogs and most animal species, but not in the stomach (10, 24, 50, 52, 71). The AAP absorption test, which involves measurement of plasma AAP concentrations in short intervals following oral administration, is considered a reliable method to evaluate the gastric emptying rates of the stomach in humans (10), ponies, horses (16, 34) and other species and of the abomasum in calves (36) because it was not absorbed from the abomasum. Therefore, the present study was undertaken to examine the pharmacokinetics of AAP after oral dosing to evaluate the property of gastric emptying in Shiba goats.

4.3. MATERIAL AND METHODS

4.3.1. Animals

All animals 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). Five clinically healthy male Shiba goats, weighing 21~44 kg and aged 2~3 years were used in this study. These goats were housed in pens at an ambient temperature and with good ventilation. Animals were fed hey cubes (#1A Cubes, Eckenberg farms Inc., Mattawa, WA, USA) at 800 g/goat twice a day with water and mineralized salt licks were available *ad libitum*.

4.3.2. Reagents and chemicals

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

4.3.3. Experimental design

4.3.3.1. Pharmacokinetic study

AAP was administered into left jugular vein or orally at a dose of 30 mg/kg to the five male goats using a crossover design with at least 3-week washout period. Blood (3 m*l*) was collected from the right jugular vein immediately prior to the administration and 0.5, 1, 2, 3, 4, 6, 9 and 12 h following intravenous injection and 0.5, 1, 2, 4, 6, 9, 12 and 16 h after oral administration of the AAP. Plasma samples were separated by the centrifugation of blood at 1,600 ***g*** for 10 min and stored at -20°C until analysis.

4.3.3.2. Stability of AAP in rumen juice

Two goats were restrained and nasal catheters were passed into the rumen. Thereafter, 40 m*l* of rumen fluid was aspirated through these catheters and processed for incubation immediately after collection. Procedure of the stability test was just same as that described in Chapter one, except 100 μg/m*l* of acetaminophen concentration was used. Concentrations of AAP were measured by HPLC.

4.3.3.3. Octanol-buffer (pH 6.5) partitioning experiments

Octanol-buffer partitioning studies were performed using a shake flask method as recommended by the Organization for Economic Cooperation and Development (47) as described in Chapter one. The total drug concentration in the buffer phase was then determined by HPLC.

4.3.4. Acetaminophen assay

Determination of the AAP in plasma, rumen juice and buffer samples was performed using an HPLC system, as described previously (25) with some modifications. Briefly, to 200 μl of the plasma or rumen juice sample, 200 μl of perchloric acid (0.15 M) was added and stirred. The mixture was centrifuged at 20,000 ***g*** for 10 min. Fifty μl of the supernatant was injected into the HPLC system after filtration using 0.45 μm HPLC filter (Chromatodisc 4P, Kurabo Biomedical Industries, Ltd, Osaka, Japan).

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of a pump (LC-10AD), a UV detector (SPD-6A), an Integrator (Chromatopac C-R7A plus) and an injector loop (model 7125). The mobile phase was a mixture of 0.1 M acetate buffer (pH 4) and acetonitrile (90:10, v/v). Triethylamine 150 $\mu l/l$ mobile was added. Analytical separation was accomplished by using a reversed-phase ODS column (TSK-gel ODS-120T®, 4.6 μm×250 mm, TOSOH Co., Tokyo, Japan). The flow rate was 1m*l*/min. The wavelength of the detector was 248 nm. Sample preparation and analysis were conducted at room temperature. The recovery of AAP from plasma samples was 100.1 ± 2.65% at 1 μg/m*l* (n=5), while that from rumen juice samples was 97.0 ± 2.03% at 25 μg/m*l* (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).

4.3.5. Pharmacokinetic analysis

The plasma concentration-time curves of AAP after 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.

$$Cp_{iv}(t) = \frac{\text{Dose}}{V_1}\left\{\frac{\propto - k_{21}}{\alpha - \beta} \times e^{-\alpha \cdot t} + \frac{k_{21} - \beta}{\alpha - \beta} \times e^{-\beta \cdot t}\right\} \qquad \text{Eq. (1)}$$

$$Cp_{po}(t) = \frac{\text{Dose} \cdot F \cdot k_a}{V_1}\left\{\begin{array}{l} \dfrac{k_{21} - \alpha}{(k_a - \propto)(\beta - \alpha)} \times e^{-\alpha \cdot t} \\ + \dfrac{k_{21} - \beta}{(k_a - \beta)(\alpha - \beta)} \times e^{-\beta \cdot t} \\ + \dfrac{k_{21} - ka}{(\alpha - k_a)(\beta - k_a)} \times e^{-k_a \cdot t} \end{array}\right\} \qquad \text{Eq. (2)}$$

Equation 1 and 2 were simultaneously fit to the plasma concentration-time curves of AAP after 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 (72).

Several pharmacokinetic parameters were calculated by 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, mean residence time (MRT), mean absorption time (MAT), and the distribution volume at a steady state (V_{dss}) were calculated by conventional methods.

4.4. RESULTS

The plasma concentrations of AAP rapidly increased and peaked 0.90 ± 0.22 h after being orally administered, and followed by its slow elimination. On the other hand, the plasma concentrations eliminated rapidly after the intravenous injection with the short half-lives (1.14 ± 0.46 h) as shown in Fig. 2-1. As shown in Table 2-1, a pharmacokinetic analysis indicated the slow absorption and fast elimination of AAP in Shiba goats. The calculated mean absorption time (MAT) of AAP was 4.93 ± 0.87 h. The AAP absorption half-life ($t_{1/2ka}$) was 3.35 ± 0.50 h, is three times its elimination half-life ($t_{1/2\beta}$) which was 1.14 ± 0.46 h. Non-compartmental and compartmental bioavailability (F and F*) of AAP was 16.0 ± 8.52 and 17.0 ± 8.28%, respectively. Since the bioavailability of AAP was markedly very low, the stability of AAP in the rumen juice collected from Shiba goats was evaluated. The recovery of AAP from rumen juice samples at 100μg/m*l* (n = 5) after a 12 and 24 h incubation at 39°C was 90.50 ± 1.45 and 88.67 ± 0.84%, respectively.

4.5. DISCUSSION AND CONCLUSION

The pharmacokinetic analysis indicated that, the calculated MAT was approximately 5 h and the calculated absorption half-life ($t_{1/2ka}$) was approximately 3 times the elimination half-life ($t_{1/2kel}$). Therefore, it was suggested that, AAP showed slower absorption and faster elimination. The MAT of AAP is the shortest (4.93 ± 0.87 h) when compared with that of DF (6.75 ± 2.74 h) and SMM (15.1 ± 4.71 h) in the previous study in Shiba goats (20). The absorption rate constant of AAP (0.21 ± 0.032 h) is larger than that of DF (0.19 ± 0.07 h) and SMM (0.074 ± 0.03 h). These facts suggest

that AAP was absorbed from the forestomach to a greater extent, compared with DF and SMM. The partition coefficient of AAP (2.07 ± 0.170) is extremely lower than that of DF (91.78 ± 9.45) at pH 6.5 as listed in (Table 2-2). This fact suggests that factors other than fraction of unionized form and lipophilicity predominantly influenced the AAP absorption from forestmach. As such factors, molecular size might be considered, because molecular weight of AAP (151.2) is much smaller than that (318.1) of DF (Table 2-2). The faster absorption rate of sulfanilamide from the gastrointestinal tract of rats has been reported (42), compared with another sulfonamides. Since its partition coefficient was smallest, they referred the fast absorption to the smaller molecular weight of the sulfanilamide.

Since the AAP is stable in the rumen juice, its low bioavailability after oral administration may be due to its extensive first-pass effect in the liver. This may be due to the larger hepatic capacity of herbivores which result in greater metabolism of lipophilic compounds (6). First-pass metabolism in rats decreased the AAP systemic exposure during absorption by 41.2%, based on AUC from 0~20 min, and decreased the overall oral bioavailability by 27%, based on AUC from 0~240 min (15).

In conclusion, AAP was much more absorbed from the forestomach of Shiba goats, possibly due to its smaller molecular weight. Therefore, AAP is considered not suitable for the evaluation of the gastric emptying in goats. In addition oral AAP was found to be greatly affected by first-pass effect in the liver. This fact may suggest that AAP should not be used orally as an analgesic and/or antipyretic in Shiba goats because of its markedly low oral bioavailability.

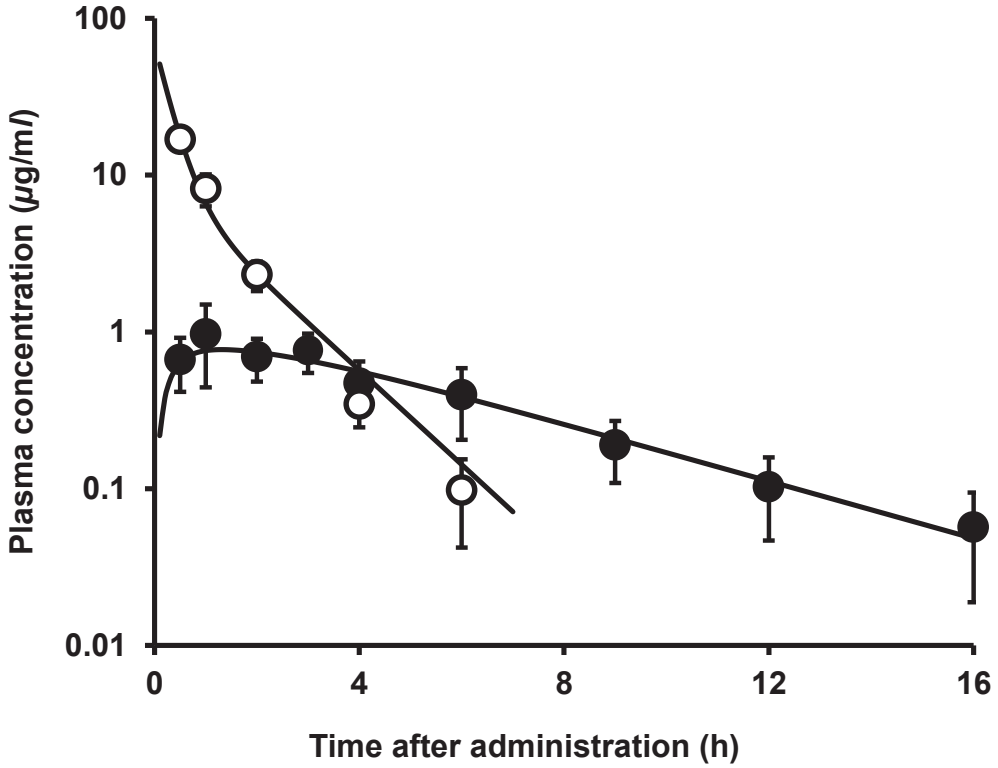

Fig. 2-1.

Plasma concentration time curve of AAP (30 mg/kg bodyweight) after its single intravenous (open circles) and oral administration (closed circles) to male Shiba goats. Each point and vertical bar represents the mean and standard deviation, respectively (n = 5). Each line is calculated by Eq. 1 or 2 using pharmacokinetic parameters in Table 2-1.

Table 2-1.

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

Parameter	Mean ± SD (n = 5)
k_a (h^{-1})	0.210 ± 0.032
C_{max} ($\mu g/ml$)	0.986 ± 0.507
T_{max} (h)	0.900 ± 0.220
α (h^{-1})	3.33 ± 2.10
β (h^{-1})	0.695 ± 0.267
$t_{1/2ka}$ (h)	3.35 ± 0.50
$t_{1/2\beta}$ (h)	1.14 ± 0.46
$AUC_{i.v.}$ ($\mu g \cdot h/ml$)	35.2 ± 8.0
$AUC_{p.o.}$ ($\mu g \cdot h/ml$)	5.19 ± 2.17
CL (l/h/kg)	0.869 ± 0.163
F (%)	17.0 ± 8.3
F* (%)	16.0 ± 8.5
$MRT_{i.v.}$ (h)	0.617 ± 0.148
$MRT_{p.o.}$ (h)	5.46 ± 0.86
MAT (h)	4.93 ± 0.87
V_{dss} (l/kg)	0.546 ± 0.192

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

Table 2-2.

Absorption profile and some physicochemical properties of SMM, DF and AAP. Data are presented as Mean ± SD

Drug	SMM	DF	AAP
pKa	6 [46]	4 [53]	9.56 [40]
f_u%	30	0.3	100
P	1.72 ± 0.17	91.8 ± 9.5	2.07± 0.17
*P**	7.15 ± 0.86	29118.7 ± 2735.8	2.07 ± 0.17
Molecular weight	303.3	318.1	151.2
MAT (h)	15.1 ± 4.7	6.05 ± 2.74	4.93 ± 0.87
k_a (h^{-1})	0.074 ± 0.030	0.19 ± 0.07	0.210 ± 0.032

f_u%: Unionized fractions (calculated at pH 6.5).

P: octano/phosphate buffer (50 mM, pH 6.5) apparent partition coefficient in the present study at 25°C.

*P**: octano/phosphate buffer (50 mM, pH 6.5) intrinsic partition coefficient in the present study at 25°C.

MAT: mean absorption time in the present study.

k_a: absorption rate constant.

CHAPTER THREE

Oral Absorption Profiles of Sulfonamides in Shiba Goats: a Comparison among Sulfamethazine, Sulfadiazine, and Sulfanilamide.

5.1. ABSTRACT

The pharmacokinetics of sulfamethazine (SMZ, pKa 7.5), sulfadiazine (SDZ, pKa 6.5), and sulfanilamide (SA, pKa 10.5) were investigated in Shiba goats (n = 5) after intravenous and intraruminal administration of 10 mg/kg bodyweight using a crossover design with at least a 3-week washout period. In addition, the stability of these sulfonamides in rumen juice was evaluated by an *in vitro* experiment. The octanol/buffer partition coefficient was also measured at rumen pH (6.5).

After intravenous injection, the mean half-lives were 1.09 ± 0.38 h, 1.56 ± 0.27 h, and 3.71 ± 0.34 h, for SMZ, SDZ, and SA, respectively. The T_{max} of SMZ, SDZ, and SA were reached 2.0 ± 1.23 h, 6.0 ± 0.00 h and 7.8 ± 1.64 h, after they have been intraruminally administered, respectively, and this was followed by their slow elimination due to a slow rate of drug absorption from the gastrointestinal tract. The mean oral bioavailability varied from 44.9 ± 16.4% for SMZ to 83.9 ± 17.0% for SDZ, and 49.2 ± 2.11% for SA. The low bioavailability of SMZ and SA was most likely due to an extensive first-pass effect because they were stable in rumen juice. The mean absorption times of SMZ, SDZ, and SA were 7.52 ± 0.85, 13.17 ± 2.02 and 9.09 ± 1.67 h, respectively. The differences in MAT partially attribute to fraction of unionized form in rumen juice and lipid solubility. It is,therefore, suggested a possibility that oral route may be suitable for drugs having high lipid solubility and pKa that results in high unionization in rumen juice, even in ruminants.

5.2. INTRODUCTION

Oral dosing is generally considered to be inappropriate for ruminants because of slow drug absorption. Therefore, intramuscular and subcutaneous injections are frequently used in cattle, sheep, and goats. However, I previously found a rapid antipyretic effect of DF in dairy cows with infectious disease following oral administration in a preliminary trial. This finding suggests a rapid absorption of DF from the gastrointestinal tract. Therefore, oral administration can be used for some drugs in ruminants and therefore the problem of both tissue damage and local residues, as is often the case for drugs administered by intramuscular and subcutaneous injection, can be avoided.

In Chapter one, the oral pharmacokinetics of DF and SMM which have different physicochemical properties were examined in Shiba goats in order to clarify which property is important for oral absorption of drug. I found a large difference in the mean absorption time. That of DF was 6 h, while that of SMM was 15 h although SMM molecules are more unionized than DF molecules in the rumen. However, the partition coefficient between octanol and water (pH 7) is different. That of DF is approximately 8, whereas that of SMM is less than 1. Therefore, DF may have been absorbed from the forestomach because of its extremely high lipid solubility. In turn, this fact suggests that drugs which have appropriate lipophylicity may be substantially absorbed from the forestomach of ruminants.

It is generally recognized that, most drugs are absorbed from the gastrointestinal tract by a process of passive diffusion of the unionized fraction across a lipid membrane (26). Therefore, in addition to the lipid solubility, unionization or pKa of drugs is also an

important factor. Other factors can affect the rate of drug absorption. These factors include drug solubility and pH of rumen fluid (8).

The main purpose of this study was to clarify the relationship between drug absorption profiles after their oral administration to ruminants and their physicochemical properties. To achieve this, the oral pharmacokinetic profiles of three sulphonamides; SMZ, SDZ and SA with different physicochemical properties were studied in Shiba goats.

5.3. MATERIALS AND METHODS

5.3.1. *Animals*

All animals 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). Five clinically healthy male Shiba goats, weighing 25~60 kg and aged 2~3 years were used in this study. These goats were housed in pens at an ambient temperature and with good ventilation. Animals were fed hey cubes (#1A Cubes, Eckenberg farms Inc., Mattawa, WA, USA) at 800 g/goat twice a day with water and mineralized salt licks were available *ad libitum*.

5.3.2. *Reagents and chemicals*

SMZ was obtained from MP Biomedicals, LLC (Rue Geiler de Kaysersberg Illkirch Cedex, France). SDZ was obtained as a sodium salt from Sigma-Aldrich Corporation (St. Louis, MO, USA). SA was obtained Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sulfadimethoxine was obtained as a sodium salt from Daiichi Sankyo Pharmaceutical Company (Tokyo, Japan). All other reagents and chemicals used in the present study were of HPLC or analytical grade and obtained commercially. Sulfonamides solutions (100 mg/m*l*) were prepared in distilled water. For SDZ this was done by dissolving the sodium salt; the other two sulfonamides (SMZ and SA) were dissolved by adding a few drops of diluted (1N) sodium hydroxide solution.

5.3.3. Experimental design

5.3.3.1. Pharmacokinetic study

SMZ or SDZ or SA were administered either into the left jugular vein or intraruminally to five male goats at doses of 10 mg/kg bodyweight, using a crossover design with at least a 3-week washout period. Intraruminal administration was carried out by nasogastric catheter, which was flushed with 60 m*l* tap water after dosing. The interval between each study was at least three weeks. Blood (3 m*l*) was collected from the right jugular vein immediately prior to the treatment and at 1, 2, 4, 6, 9, 12 and 24 h after intravenous injection and at 1, 2, 4, 6, 9, 12, 24, 32 and 48 h after intraruminal administration. Plasma was separated by the centrifugation of blood at 1,600 ***g*** for 10 min and stored at –20°C until analysis.

5.3.3.2. Stability of sulfonamides in the rumen juice

Two goats were restrained and nasal catheters were passed into the rumen. Thereafter, 40 m*l* of rumen fluid was aspirated through the catheter and processed for incubation immediately its collection. Fifty microliters of SMZ or SDZ or SA solutions (200 μg/m*l*) was added to 950 µ*l* of the rumen fluid to give a final concentration of 10 μg/m*l* of the incubation mixture. Five samples of each drug were prepared and incubated in a thermostatic shaking water bath at 39°C for 24 h under anaerobic conditions. After incubation, the concentrations of sulfonamides were measured by HPLC.

5.3.3.3. Octanol-buffer (pH 6.5) partitioning experiments

Octanol-buffer partitioning studies were performed using a shake flask method as recommended by the Organization for Economic Cooperation and Development (47). Before partitioning, the two solvents are mutually saturated at 25°C for 24 h as has been described before in Chapter one. Solutions of the three sulfonamides (10 μg/m*l*) were prepared in the octanol saturated buffer. 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. Equilibration was done by hand shaking of the funnels (by rotation of the funnels through 180 degree about its transverse axis, approximately a hundred time during five minutes) allowing the trapped air to rise through the two phases. The funnels were then fixed vertically by rack until complete separation of the two phases. The buffer phase was collected and centrifuged at 1,600 ***g*** for 10 min at 25°C and the supernatant octanol phase was discarded. The total drug concentration in the buffer phase was then determined by HPLC and the total drug concentration in the octanol phase was calculated from the difference between initial and final concentrations in the buffer phase.

5.3.4. Assays of the three sulfonamides

SMZ or SDZ or SA concentrations were determined in the plasma, rumen juice and buffer samples by HPLC with UV-detection. Two hundred microliters of perchloric acid (0.5 M) were added to 200 μl of the plasma sample. The mixture was vortexed for 30 s and then centrifuged at 20,000 ***g*** for 10 min at 5°C. The obtained supernatant was filtered using the 0.45-μm HPLC filter. Fifty microliters of the filtrate was injected into the HPLC column.

In the case of rumen juice samples, SMZ or SDZ or SA concentrations were determined after extraction with ethyl acetate. After being incubated for 24 h, 50 μl of the internal standard (200 μg/m*l*) was added to the rumen juice samples. The internal standards used in the present study were sulfadimethoxine, SA and SDZ for SMZ, SDZ and SA, respectively. Subsequently, 5 m*l* of ethyl acetate was added. The mixtures were vortexed for 30 s then centrifuged at 3,000 ***g*** for 10 min at 5°C. The obtained supernatants were transferred into a pear shaped flasks and evaporated to dryness at 30°C. The residue was reconstituted in 500 μl of the mobile phase and filtered using the 0.45-μm HPLC filter. Fifty microliters of the filtrate was injected into the HPLC column.

The mobile phases used were a mixture of 50 mM/*l* acetate buffer (pH 5) and acetonitrile (75:25, v/v) for SMZ, a mixture of 50 mM/*l* acetate buffer (pH 4) and acetonitrile (75:25, v/v) for SDZ and a mixture of 50 mM/*l* acetate buffer (pH 5) and acetonitrile (80:20, v/v) for SA. Analytical separation was accomplished using a reversed-phase C_8 column (Mightysil RP-8 GP, 4.6 μm×250 mm, Kanto Chemical Co., Tokyo, Japan). The flow rates were 1, 0.8 and 0.8 m*l*/min for SMZ, SDZ and SA, respectively. The wavelength of the detector was 270 nm.

The recoveries of SMZ, SDZ and SA from plasma samples at 1 μg/m*l* (n = 5) were 109.2 ± 2.00%, 87.9 ± 1.52% and 95.0 ± 1.75% while those from rumen juice samples at 10 μg/m*l* (n = 5) were 83.5 ± 2.06%, 84.3 ± 2.09% and 88.1 ± 2.35%, respectively. The inter-day CV values for plasma samples ranged from 1.67 to 2.14% for SMZ, 0.63 to 3.84% for SDZ and from 1.21 to 2.29% for SA while those for rumen juice samples ranged from 1.86 to 2.79% for SMZ, 1.96 to 5.24% for SDZ and from 1.61 to 3.57% for SA, (n = 5, 3 times).

5.3.5. Statistical analysis

Data were expressed as mean ± standard deviation. Pharmacokinetic parameters relating to oral drug absorption were statistically analyzed. Differences in the mean values between groups were analyzed by Scheffe's multiple comparison test after one-way ANOVA single factor test. Equal variances among the groups were confirmed by Bartlett test. The differences were considered significant when $P < 0.05$.

5.3.6. Pharmacokinetic analysis

The plasma concentration-time curves of SMZ, SDZ and SA after they were intravenously administered fit well with the one compartment model. Therefore, the curves obtained after the intravenous injection (Cp_{iv} (t)) and those after the oral administration (Cp_{po} (t)) were described by Eq. 1 and 2, respectively.

$$Cp_{iv}(t) = \frac{Dose}{V} e^{-k_{el} \cdot t} \qquad \text{(Eq.1)}$$

$$Cp_{po}(t) = \frac{Dose \cdot F}{V} \cdot \frac{k_a}{k_a - k_{el}} (e^{-k_{el} \cdot t} - e^{-k_a \cdot t}) \qquad \text{(Eq. 2)}$$

In Eq. 2, F is bioavailability. Equation 1and 2 were simultaneously fit to the plasma concentration-time curves after the intravenous injection and oral administration to the same goats, respectively, in order to calculate pharmacokinetic parameters by the nonlinear least squares method using the curve fitting program, MULTI (72).

Several pharmacokinetic parameters were calculated by non-compartmental analysis. The area under the concentration versus time curve (AUC) was calculated by 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, mean residence time (MRT), mean absorption time (MAT), elimination half-life ($t_{1/2kel}$), peak plasma drug concentration (C_{max}), time of occurrence of C_{max} (T_{max}), and the distribution volume at a steady state (V_{dss}) were calculated by conventional methods.

5.4. RESULTS

The plasma concentration versus time curves obtained for a single intravenous or intraruminal dose of SMZ, SDZ, or SA are shown in Figs. 3-1, 3-2, and 3-3, respectively. The plasma concentrations of SMZ, SDZ and SA rapidly increased and peaked at 2.0, 6.0 and 7.8 h after being orally administered, respectively, followed by their slow elimination. On the other hand, plasma concentrations decreased rapidly after the intravenous injection with much shorter half-lives (Table 3-1), indicating flip-flop phenomena after the intraruminal administration of the three drugs.

As shown in Table 3-1, the pharmacokinetic analysis indicated the slow absorption of the three sulfonamides in Shiba goats after intraruminal administration. The calculated MAT and absorption half-life ($t_{1/2ka}$) of the three sulfonamides were long. The MAT of SDZ was significantly longer than that of SMZ and SA. The $t_{1/2ka}$ of SDZ was also significantly longer than that of SMZ and SA. The order of MAT values was different from that of pKa and therefore that of unionized fraction pH 6.5 (SA > S MZ > SDZ, see Table 3-2). It was also different from that of partition coefficient values at pH 6.5 (SMZ > SDZ > SA, see Table 3-2). Oral bioavailabilities of SMZ and SA were found to be significantly lower than that of SDZ.

The recovery of sulfonamides from rumen juice samples after a 24-h incubation was 88.6 ± 4.61% for SMZ, 89.9 ± 3.61% for SDZ and 76.5 ± 4.85% for SA. These values were quite higher than bioavailability, suggesting that the low bioavailability of SMZ and SA was mainly due to the extensive first-pass effect in liver.

5.5. DISSCUSSION AND CONCLUSION

The oral drug absorption in ruminants is generally more complex, unpredictable and may exhibit a markedly different kinetics when compared with those in monogastric species. This may be due to the unique anatomical and physiological features of the gastrointestinal tract. The forestomach (rumen, reticulum, and omasum) is a large volume compartment (100~225 *l* in cattle, and 10~24 *l* in sheep and goats). This may result in the dilution of drugs and a long residence time in the forestomach (5). In addition, the inner structure of the forestomach is lined by a keratinized stratified squamous epithelium,

which may also contribute to slow drug absorption. In Chapter one, however, I indicated substantial absorption of diclofenac from forestmach after oral administration to Shiba goats. I also suggested that this may be due to high lipid solubility of the drug. In the present study, therefore, the absorption profiles of SMZ, SDZ and SA which have different lipophylicity and pKa (Table 3-2) were examined after their intraruminal administration to Shiba goats.

Marked differences were observed in the oral absorption profiles of the 3 sulfonamides. The MAT of SDZ (13.2 ± 2.02 h) was significantly longer than that of SA (9.09 ± 1.67 h) and SMZ (7.52 ± 0.850 h). In addition, the $t_{1/2ka}$ of SDZ (10.9 ± 1.08 h) was significantly longer than those of SA (7.46 ± 1.70 h) and SMZ (5.17 ± 0.663 h). These results suggest that absorption of SDZ from the forestomach of goats may have been markedly slower than that of SMZ and SA. The pH value of the rumen juice in the present study was 6.5, as has been reported previously (18, 27). Considering rumen physiology versus the physicochemical properties of SMZ, SDZ and SA, it is possible that more absorption for SMZ did occur within this gastric compartment compared to SDZ and SA. The pKa values of SMZ, SDZ and SA are 7.5, 6.5 and 10.5, respectively (41, 66) suggesting that the SMZ molecules exist mainly as an unionized form (90%), SDZ molecules exist as 50% unionized and SA molecules exist mainly as unionized form (more than 99.9%), in the contents of the rumen. Therefore, SA is more suitable for absorption from the forestomach of goats compared to SMZ and SDZ because of its extreme unionization. However, the obtained partition coefficient between octanol and buffer (pH 6.5) in the present study was different. That of SMZ is 1.96, approximately four times like that of SDZ (0.47) and eight times like that of SA (0.26). Therefore, SMZ

may have been more absorbed from the forestomach than SDZ and SA because of its relatively higher lipid solubility and high unionization. The absorption rate of SA is larger than that of SDZ although the partition coefficient of SDZ is relatively higher than that of SA. A smaller molecular weight of SA (Table 3-2) may be considered to be one of the reasons reflecting the small lipophylicity and a rapid diffusion of SA through the gastrointestinal membranes (42). A similar unusually rapid urinary excretion was reported (73). Since the drugs are absorbed mainly and rapidly from the small intestines and gastric emptying is the determining factor for drug absorption after the oral administration of drugs (23, 27). Therefore, the slow absorption of three sulfonamides in goats in the present study may be due to their long residence time in the forestomach. Markedly higher k_a values were obtained for SMM in pigs after its intraduodenal administration than after their oral administration (31).

In Chapter one, I have suggested the absorption of DF from the forestomach of Shiba goats (20). The MAT, $t_{1/2ka}$ and k_a of DF was 6 h, 4.13 h and 0.19^{-h}, respectively. This indicates that DF was substantially absorbed from the forestomach because of its extremely higher lipid solubility although it exists mainly in the ionized form. The logarithm of the partition coefficient of the unionized form of DF at pH 6.6 is 4.34 (64), extremely higher than those of the three sulfonamides in the present study. Therefore, lipid solubility together with unionization may be an important factors for absorption of drugs from the forestomach of ruminants.

In the result section I suggested that, the lower bioavailabilities of SMZ and SA after intraruminal administration are mainly due to a considerable first-pass effect in the

liver. This is further supported the stability of both drugs in the rumen juice in the *in vitro* spiked test in the present study. Based on their chemical structures, most sulphonamides are unlikely to undergo biodegradation in the rumen juice. Negligible biodegradations of sulphamethoxydiazine, sulphathiazole and sulphamoxole in ruminal fluid of the dwarf goats during anaerobic incubation at 39°C were found (68). A previous study also suggested that the low bioavailability of sulphamethoxazole after its oral administration to goats was most likely due to the first-pass effect in the liver (51). In goats, the systemic bioavailability was 20, 11.4 and 23.3% after oral administration of sulphamethoxazole, sulphadimethyloxazole and sulphadimethoxine, respectively (3). In dwarf goats, the oral bioavailability of SMZ is low (26.4%), probably as a consequence of a marked first-pass effect in the liver (67, 70). These findings support our suggestion about the incomplete bioavailability of the three sulfonamides in the present study.

In conclusion, the results of the present study suggest that, drugs that have appropriate physicochemical properties such as high lipid solubility, good unionization and a small molecular weight may be markedly absorbed from the forestmach of goats. A possibility raises that oral route may be suitable for such drugs, even in goats.

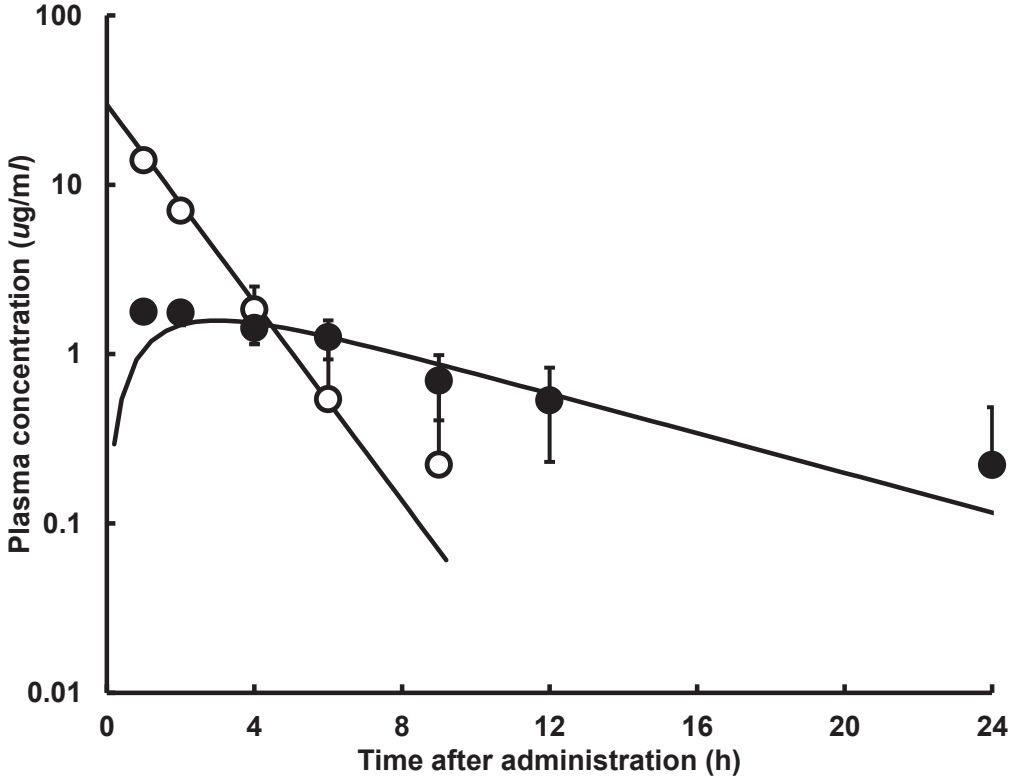

Fig. 3-1.

Plasma concentration-time curves of SMZ (10 mg/kg bodyweight) after its single intravenous (open circles) and intraruminal administration (closed circles) to male Shiba goats. Each point and vertical bar represents the mean and standard deviation, respectively (n = 5). Each line is calculated by Eq. 1 or 2 using pharmacokinetic parameters in Table 3-1.

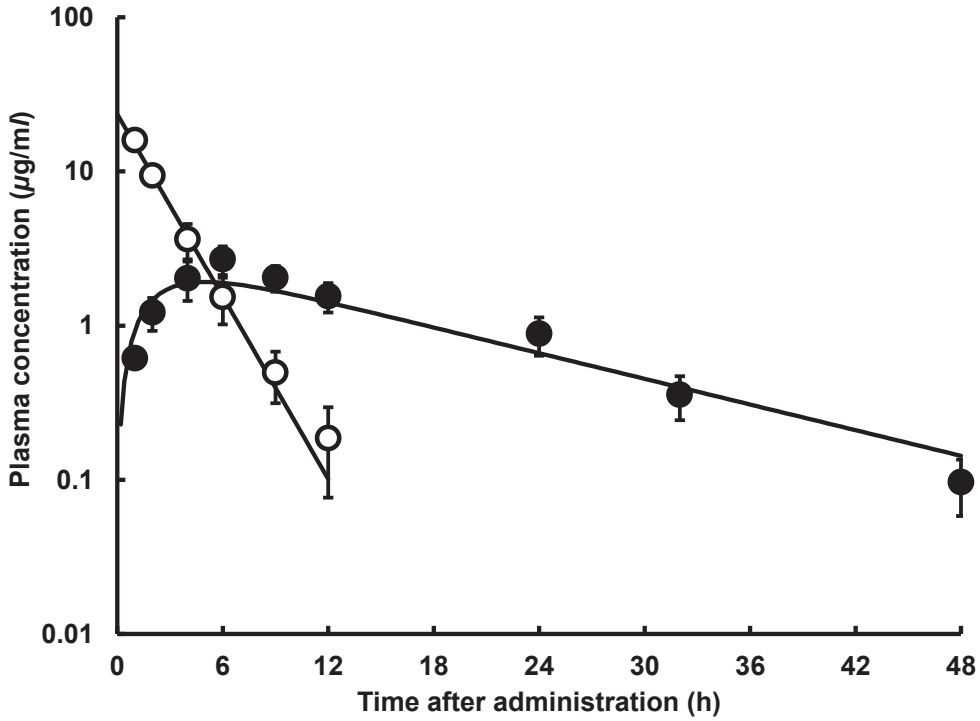

Fig. 3-2.

Plasma concentration-time curves of SDZ (10 mg/kg bodyweight) after its single intravenous (open circles) and intraruminal administration (closed circles) to male Shiba goats. Each point and vertical bar represents the mean and standard deviation, respectively (n = 5). Each line is calculated by Eq. 1 or 2 using pharmacokinetic parameters in Table 3-1.

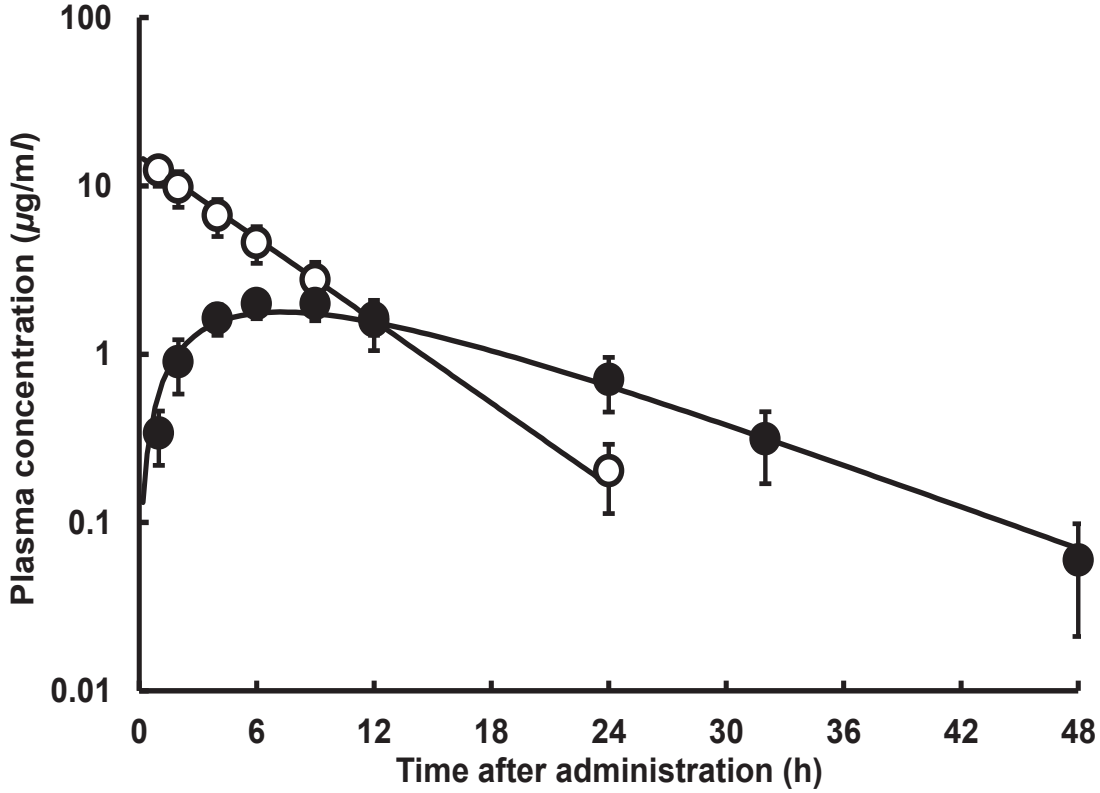

Fig. 3-3.

Plasma concentration-time curves of SA (10 mg/kg bodyweight) after its single intravenous (open circles) and intraruminal administration (closed circles) to male Shiba goats. Each point and vertical bar represents the mean and standard deviation, respectively (n = 5). Each line is calculated by Eq. 1 or 2 using pharmacokinetic parameters in Table 3-1.

Table 3-1.

Pharmacokinetic parameters of SMZ, SDZ and SA in male Shiba goats (n = 5) determined after a single intravenous and intraruminal administration of 10 mg/kg bodyweight.

		SMZ	SDZ	SA
Parameter	Units	Mean ± SD	Mean ± SD	Mean ± SD
k_a	h^{-1}	0.136 ± 0.017[bc]	0.0639 ± 0.0062[a]	0.0971 ± 0.0229[a]
C_{max}	μg/m*l*	2.14 ± 1.05	2.70 ± 0.57	2.08 ± 0.38
T_{max}	h	2.00 ± 1.23	6.00 ± 0.00	7.80 ± 1.64
k_{el}	h^{-1}	0.728 ± 0.357	0.454 ± 0.073	0.188 ± 0.016
$t_{1/2ka}$	h	5.17 ± 0.66[b]	10.9 ± 1.1[ac]	7.46 ± 1.70[b]
$t_{1/2kel}$	h	1.09 ± 0.38	1.56 ± 0.27	3.71 ± 0.34
$AUC_{i.v.}$	μg·h/m*l*	55.2 ± 31.3	55.0 ± 4.7	81.3 ± 19.9
$AUC_{p.o.}$	μg·h/m*l*	22.5 ± 13.3	46.0 ± 9.2	39.8 ± 9.0
CL	*l*/h/kg	0.311 ± 0.329	0.183 ± 0.016	0.129 ± 0.031
F	%	41.6 ± 14.9	79.8 ± 13.0	48.1 ± 1.8
F*	%	44.9 ± 16.4	83.9 ± 17.0	49.2 ± 2.1
$MRT_{i.v.}$	h	1.61 ± 0.56	2.13 ± 0.34	5.33 ± 0.40
$MRT_{p.o.}$	h	9.13 ± 1.02	15.3 ± 1.9	14.4 ± 2.0
MAT	h	7.52 ± 0.85[b]	13.2 ± 2.0[ac]	9.09 ± 1.67[b]
V_{dss}	*l*/kg	0.374 ± 0.207	0.386 ± 0.033	0.683 ± 0.144

[a]: means presence of a significant difference from SMZ.

[b]:means presence of a significant difference from SDZ.

[c]:means presence of a significant difference from SA.

k_a = absorption rate constant; C_{max} = maximum plasma concentration; T_{max} = time to maximum plasma concentration; k_{el} = elimination rate constant; $t_{1/2ka}$ = half-life of absorption; $t_{1/2kel}$ = half-life of elimination; $AUC_{i.v.}$ = area under the plasma concentration–time curve from time zero to infinity after i.v. injection; $AUC_{p.o.}$ = area under the plasma concentration–time curve from time zero to infinity after intraruminal administration; CL = total body clearance; F = bioavailability calculated by compartmental analysis; F* = bioavailability calculated by non-compartmental analysis; MAT* = real mean absorption time; $MRT_{i.v.}$ = mean residence time after i.v. injection; $MRT_{p.o.}$ = mean residence time after p.o administration; MAT = apparent mean absorption time; V_{dss} = volume of distribution at a steady state.

Table 3-2.

Some physicochemical parameters and MAT of SMZ, SDZ and SA.

Sulfonamides	Chemical structure	pKa (f_u%)	*P*	*P**	Molecular weight	MAT (h)
SMZ	$C_{12}H_{14}N_4O_2S$	7.5 [66] (90)	1.96 ± 0.16	2.16 ± 0.18	278.3	7.52 ± 0.85
SDZ	$C_{10}H_{10}N_4O_2S$	6.5 [66] (50)	0.468 ± 0.049	0.935 ± 0.098	272.3	13.2 ± 2.0
SA	$C_6H_8N_2O_2S$	10.5 [41] (100)	0.257 ± 0.047	0.257 ± 0.047	172.2	9.09 ± 1.67

f_u: Unionized fractions (calculated at pH 6.5).

P: octano/phosphate buffer (50 mM, pH 6.5) apparent partition coefficient in the present study at 25°C.

*P**: octano/phosphate buffer (50 mM, pH 6.5) intrinsic partition coefficient in the present study at 25°C.

MAT: mean absorption time in the present study.

General Discussion and Conclusion

Oral ingestion of drugs is considered one of the main routes of drug administration due to convenience, easy treatment of large number of animals, absence of stress and avoiding both tissues damage and local residues after injection.

Differences in the anatomy and physiology of the gastrointestinal tract between human and animals also among animals results in major species differences in strategies for and efficiency of oral drug administration (54). In ruminants, the forestomach (rumen, reticulum, and omasum) is a large volume compartment (100~225 *l* in cattle, and 10~24 *l* in sheep and goats) resulting in dilution of drugs and a long residence time in the forestomach (5). In addition, the keratinized stratified squamous epithelium lining the forestomach may also contribute to slow drug absorption. Moreover, microflora in the rumen may inactivate some drugs through metabolic or chemical reactions (6). All of these makes the oral drug absorption in ruminants more complex and unpredictable and exhibiting markedly different kinetics when compared with those of simple stomach animals.

Although the main absorption site of drugs after oral dosing is the small intestine, the absorption of some drugs from the stomach may also be markedly high. This has been demonstrated for salicylic acid (17), sulfaethidole and barbital (11) and metoprolol (18) in rats. This has been demonstrated also for sulfonamides (4), salicylate, pentobarbitone, quinine (28) and thiabendazole (38) in ruminants. Absorption of drugs from stomach shortens the MAT of drugs.

Since the effective surface area of the stomach that actually contributes to drug absorption is small, the physicochemical properties of drugs such as pKa, lipophilicity, solubility, stability in the gastrointestinal fluids and molecular size may be important factors for their absorption from the stomach (75).

In this thesis, I aimed to clarify the correlations between drugs absorption profiles after oral administration to ruminants and their physicochemical properties. To achieve this, several drugs with different physicochemical properties (Table 4-2) were chosen. Followings are the investigations and major outcomes of the present research.

6.1. Oral pharmacokinetics of the acidic drugs, diclofenac and sulfamonomethoxine in Shiba goats.

This study is presented in Chapter one, in which the oral absorption profiles of DF and SMM were investigated in Shiba goats, a small ruminant animal to evaluate the correlation of their absorption parameters with their physicochemical properties. The results of a pharmacokinetic analysis revealed the slow absorption of both drugs. A marked difference was observed in the oral absorption profiles of DF and SMM. The MAT of DF (6.05 ± 2.74 h) was less than half that of SMM (15.1 ± 4.70 h) in the present study. The $t_{1/2ka}$ of DF (4.13 ± 1.94 h) is also less than half that of SMM (10.5 ± 3.60 h) as shown in Table 4-1. These results suggests that absorption rate of DF from the forestomach of male Shiba goats may have been markedly higher than that of SMM because of its extremely higher lipophylicity (Table 4-2). The $t_{1/2ka}$ values were also longer than that of those reported in human and simple stomach animals like horses, pigs, rabbits and rats suggesting the long residence time in the forestomach. These results may indicate that absorption of highly lipophilic drugs from the forestomach may be markedly high in ruminants and the gastric emptying may be the determining factor for drug absorption after the oral administration of drugs to Shiba goats.

6.2. Evaluation of gastric emptying profiles of Shiba goats by oral pharmacokinetics of acetaminophen.

This study is presented in Chapter two, in which the pharmacokinetics of acetaminophen after oral dosing to Shiba goats were examined in order to evaluate the property of gastric emptying. The obtained MAT and $t_{1/2ka}$ were unexpectedly short (4.93 ± 0.867 and 3.35 ± 0.501 h, respectively) as shown in Table 4-1 due to its relatively low lipophylicity when compared with DF (Table 4-2). These results suggests that AAP was markedly absorbed from the forestomach of male Shiba goats and this may be due to its smaller molecular weight and extreme unionization throughout the gastrointestinal tract (Table 4-2). This result may indicate that AAP was considered not suitable for the evaluation of the gastric emptying in in Shiba goats although it is generally considered as a good indicator of the gastric emptying in several animal species. These results may also indicate that acidic drugs having small molecular weight and high pKa (more than 8) may be markedly absorbed from the forestomach of ruminants, like AAP, even if they have relatively low lipid solubility. It was observed that oral bioavailability of AAP was extremely low (16.0 ± 8.52%) while the drug was stable in rumen juice for 24 h at 39°C suggesting its extensive first-pass effect in the liver. This result may indicate that AAP cannot be used as analgesic antipyretic in Shiba goats.

6.3. Oral absorption profiles of sulfonamides in Shiba goats: a comparison among sulfamethazine, sulfadiazine, and sulfanilamide.

This study is presented in Chapter three, in which the pharmacokinetics of sulfamethazine, sulfadiazine, and sulfanilamide after intraruminal administration to Shiba goats were examined in order to clarify the relationship between drug absorption profiles after their oral administration to ruminants and their physicochemical properties. As shown in Table 4-1, a pharmacokinetic analysis indicated the slow absorption of the three sulfonamides after intraruminal administration. The obtained MAT and $t_{1/2ka}$ of the three sulfonamides were long. Marked differences were observed in the oral absorption profiles of the 3 sulfonamides. The MAT of SDZ (13.2 ± 2.02 h) was significantly longer than that of SA (9.09 ± 1.67 h) and SMZ (7.52 ± 0.850 h). In addition, the $t_{1/2ka}$ of SDZ (10.9 ± 1.08 h) was significantly longer than those of SA (7.46 ± 1.70 h) and SMZ (5.17 ± 0.663 h). These results suggest that absorption of SDZ from the forestomach of goats may have been markedly slower than that of SMZ and SA. This may have been due to difference of the partition coefficient between octanol and buffer (pH 6.5) of the three sulfonamides. That of SMZ is 1.96 ± 0.126, approximately four times like that of SDZ (0.468 ± 0.049) and eight times like that of SA (0.257 ± 0.047). Therefore, SMZ may have been more absorbed from the forestomach than SDZ and SA because of its relatively higher lipid solubility and high unionization. These results indicate that the absorption rate of sulfonamides from forestomach of ruminants depend mainly on their degree of lipid solubility. Comparing the absorption profiles of SA and SDZ, SA had unexpectedly shorter MAT and $t_{1/2ka}$ than SDZ although the partition coefficient of SDZ is nearly twice that of SA. A smaller molecular weight of SA (Table 4-2) may be considered to be one of the reasons reflecting the small

lipophylicity and a rapid diffusion of SA through the gastrointestinal membranes. The extreme unionization of SA throughout the gastrointestinal tract due to high pKa may be another reason. Therefore, it is indicated that drugs with small molecular weight and high unionization may be markedly absorbed from the forestomach of ruminants, even though they have a low degree of lipid solubility. Comparing the absorption profiles of SA and AAP it was found that AAP was more absorbed from the forestomach of Shiba goats than SA. MAT and $t_{1/2ka}$ of AAP were shorter than those of SA (Table 4-1). This may have been due to the higher lipid solubility and small molecular weight of AAP. The partition coefficient of AAP at pH 6.5 was approximately 8 times like that of SA. Also the smaller molecular weight of the AAP may be considered also as another reason (Table 4-2).

As shown in Table 4-2, SA and AAP have high pKa, extremely unionized at pH 6.5 and small molecular weights and the apparent and intrinsic partition coefficient of each drug are same.

In conclusion, the appropriate physicochemical properties of drugs such as high lipid solubility, good unionization and a small molecular weight may be an important factors for drug absorption from the forestmach of goats. It is, therefore, suggested a possibility that oral route may be suitable for such drugs, even in ruminants.

Table 4-1.

Mean pharmacokinetic parameters parameters ± SD (n = 5) of sulfamethazine (SMZ), sulfadiazine (SDZ), sulfanilamide (SA), sulfamonomethoxine (SMM), diclofenac (DF) and acetaminophen (AAP).

Drug		**SMZ**		**SDZ**		**SA**		**SMM**		**DF**		**AAP**	
D/V	(mg/*l*)	31.2	10.1	23.5	2.8	14.5	2.9	33.3	8.33	14.9	7.4	68.8	32.0
V	(*l*/kg)	0.355	0.139	0.430	0.052	0.711	0.133	0.307	0.112	-	-	-	-
k_{el}	(h^{-1})	0.728	0.357	0.454	0.073	0.188	0.016	0.703	0.084	-	-	-	-
F_{comp}	(%)	0.416	0.149	0.798	0.130	0.481	0.018	79.3	16.5	75.4	24.0	0.176	0.083
$F'_{non\ comp}$	(%)	0.449	0.164	0.839	0.170	0.492	0.021	77.1	14.8	73.9	20.2	0.160	0.085
AUC_{iv}	(μg·h/m*l*)	55.2	31.3	55.0	4.7	81.3	19. 9	49.9	11.3	14.7	6.2	35.7	7.6
AUC_{po}	(μg·h/m*l*)	22.5	13.3	46.0	9.2	39.8	9.0	37.5	6.7	10.4	4.0	5.34	2.16
$t_{1/2kel}$ or $t_{1/2\beta}$	(h)	1.09	0.38	1.56	0.27	3.71	0.34	0.997	0.112	3.05	1.13	1.14	0.46
$t_{1/2ka}$	(h)	5.17	0.66	10.9	1.1	7.46	1.70	10.5	3.6	4.13	1.94	3.35	0.50
CL	(*l*/h/kg)	0.311	0.329	0.183	0.016	0.129	0.031	0.212	0.067	0.0748	0.0309	0.869	0.163
MRT_{iv}	(h)	1.61	0.56	2.13	0.34	5.33	0.40	1.49	0.19	2.38	1.01	0.617	0.148
MRT_{PO}	(h)	9.13	1.02	15.3	1.9	14.4	2.0	16.6	4.6	8.42	2.15	5.46	0.86
V_{dss}	(*l*/kg)	0.374	0.207	0.386	0.033	0.683	0.144	0.321	0.134	0.181	0.102	0.546	0.192
k_a	(h^{-1})	0.136	0.017	0.0639	0.0062	0.0971	0.0229	0.0737	0.0296	0.194	0.073	0.210	0.032
MAT	(h)	7.52	0.85	13.2	2.0	9.09	1.67	15.1	4.7	6.05	2.74	4.93	0.87
C_{max}	(μg/m*l*)	2.14	1.05	2.70	0.57	2.08	0.38	2.15	0.29	1.12	0.58	0.985	0.453
t_{max}	(h)	2.00	1.23	6.00	0.00	7.80	1.64	5.60	2.30	1.51	1.41	0.900	0.224
α	(h^{-1})	-	-	-	-	-	-	-	-	2.09	0.97	3.37	2.06
β	(h^{-1})	-	-	-	-	-	-	-	-	0.250	0.078	0.695	0.267
k_{21}	(h^{-1})	-	-	-	-	-	-	-	-	0.460	0.166	1.05	0.64

Table 4-2.
Absorption profile and some physicochemical properties of SMZ, SDZ, SA, SMM, DF and AAP.

Drug	SMZ	SDZ	SA	SMM	DF	AAP
pKa	7.5 [66]	6.5 [66]	10.4 [41]	6 [46]	4 [53]	9.56 [40]
f_u%	90	50	100	30	0.3	100
P	1.96 ± 0.13	0.468 ± 0.049	0.257 ± 0.047	1.72 ± 0.17	91.8 ± 9.5	2.07± 0.17
*P**	2.16 ± 0.18	0.935 ± 0.098	0.257 ± 0.047	7.15 ± 0.86	29118.7 ± 2735.8	2.07 ± 0.17
Molecular weight	278. 3	272.3	172.2	303.3	318.1	151.2
MAT	7.52 ± 0.85	13.2 ± 2.0	9.09 ± 1.67	15.1 ± 4.7	6.05 ± 2.74	4.93 ± 0.87
k_a	0.136 ± 0.017	0.0639 ± 0.0062	0.0971 ± 0.0229	0.0737 ± 0.0296	0.19 ± 0.07	0.210 ± 0.032

f_u%: unionized fractions (calculated at pH 6.5).

pKa: dissociation constants, referred from (40, 41, 46, 53, 66).

P: octano/phosphate buffer (50 mM, pH 6.5) apparent partition coefficient in the present study at 25°C.

*P**: octano/phosphate buffer (50 mM, pH 6.5) intrinsic partition coefficient in the present study at 25°C.

MAT: mean absorption time in the present study.

k_a: absorption rate constant.

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9. ABSTRACT

In this thesis, I aimed to clarify the relationship between oral absorption profiles of several drugs and their physicochemical properties in goats. To achieve this, the oral pharmacokinetic profiles of several drugs with different physicochemical properties were studied in Shiba goats, a small ruminant animal.

In Chapter one, the oral pharmacokinetics of acidic drugs, diclofenac (DF) with pKa 4 and sulfamonomethoxine (SMM) with pKa 6 were investigated. The pharmacokinetic analysis revealed the slow absorption of both drugs. A marked difference was observed in the oral absorption profiles of DF and SMM. The mean absorption time (MAT) of DF (6.05 ± 2.74 h) was less than half that of SMM (15.1 ± 4.70 h). The $t_{1/2ka}$ of DF (4.13 ± 1.94 h) is also less than half that of SMM (10.5 ± 3.60 h). Both drugs were stable in rumen juice. These findings suggests that absorption rate of DF from the forestomach of male Shiba goats may have been markedly higher than that of SMM because of its extremely higher lipophylicity, because partition coefficients (octano/phosphate buffer at pH 6.5) of DF and SMM were 91.8 ± 9.45 and 1.72 ± 0.174, respectively. The $t_{1/2ka}$ values were also longer than those reported in human and simple stomach animals like horses, pigs, rabbits and rats, suggesting the long residence time in the forestomach. These results may indicate that absorption of highly lipophilic drugs from the forestomach may be markedly high in ruminants and the gastric emptying may be the determining factor for drug absorption after the oral administration of drugs to Shiba goats.

In Chapter two, the oral pharmacokinetics of acetaminophen (AAP) were examined to evaluate the property of gastric emptying of Shiba goats. The obtained MAT and $t_{1/2ka}$ were unexpectedly short (4.93 ± 0.867 and 3.35 ± 0.501 h, respectively), even though it has a quite low lipophylicity (partition coefficient = 2.07 ± 0.170) when compared with DF (partition coefficient = 91.8 ± 9.45). These results suggest that AAP was markedly absorbed from the forestomach probably due to its smaller molecular weight (151.2) and extreme unionization throughout the gastrointestinal tract (pKa = 9.56). This result indicates that AAP was not suitable for the evaluation of the gastric emptying in Shiba goats, although it is generally considered as a good indicator of the gastric emptying in several animal species. These results may also indicate that acidic drugs having small molecular weight and high pKa (more than 8) may be markedly absorbed from the forestomach of ruminants, like AAP, even if they have relatively low lipid solubility. The oral bioavailability of AAP was extremely low (16.0 ± 8.52%), while the drug was stable in rumen juice for 24 h at 39°C, suggesting its extensive first-pass effect in the liver. This result may indicate that AAP cannot be used orally as analgesic antipyretic in Shiba goats.

In Chapter three, the oral absorption profiles of sulfamethazine (SMZ, pKa 7.5), sulfadiazine (SDZ, pKa 6.5), and sulfanilamide (SA, pKa 10.5) were examined after intraruminal administration to Shiba goats. The pharmacokinetic analysis indicated the slow absorption of the three sulfonamides after intraruminal administration. The obtained MAT and $t_{1/2ka}$ of the three sulfonamides were long. Oral bioavailabilities of SMZ (44.9 ± 16.4%) and SA (49.2 ± 2.11%) were found to be significantly lower than that of SDZ (83.9 ± 17.0). Marked differences were observed in the oral absorption profiles of the 3

sulfonamides. The MAT of SDZ (13.2 ± 2.02 h) was significantly longer than that of SA (9.09 ± 1.67 h) and SMZ (7.52 ± 0.850 h). In addition, the $t_{1/2ka}$ of SDZ (10.9 ± 1.08 h) was significantly longer than those of SA (7.46 ± 1.70 h) and SMZ (5.17 ± 0.663 h). These results suggest that absorption of SDZ from the forestomach of goats may have been markedly slower than that of SMZ and SA. This may have been due to difference in lipid solubility of the three sulfonamides. The partition coefficient between octanol and buffer (pH 6.5) of SMZ is 1.96 ± 0.126, approximately four times that of SDZ (0.468 ± 0.049) and eight times that of SA (0.257 ± 0.047). Therefore, SMZ may have been more rapidly absorbed from the forestomach than SDZ and SA. These results indicate that the absorption rate of sulfonamides from forestomach of ruminants depend mainly on their degree of lipid solubility. Comparing the absorption profiles of SA and SDZ, SA had unexpectedly shorter MAT and $t_{1/2ka}$ than SDZ, although the partition coefficient of SDZ is nearly twice that of SA. A smaller molecular weight of SA (172.2) than that of SDZ (272.3) may be considered to be one of the reasons for a rapid diffusion of SA through the gastrointestinal membranes. The extreme unionization of SA throughout the gastrointestinal tract due to high pKa may be another reason. Therefore, it is indicated that drugs with small molecular weight and high unionization may be markedly absorbed from the forestomach of ruminants, even though they have a low degree of lipid solubility. Comparing the absorption profiles of SA and AAP, it was found that AAP was more rapidly absorbed from the forestomach of Shiba goats. MAT and $t_{1/2ka}$ of AAP were shorter than those of SA. This may have been due to the higher lipid solubility and smaller molecular weight of AAP. The partition coefficient of AAP at pH 6.5 was approximately 8 times that of SA.

In conclusion, the present study indicates that physicochemical properties of drugs such as high lipid solubility, high unionization and a small molecular weight may be necessary for drug absorption from the forestomach of goats. It is, therefore, suggested that oral route may be suitable for drugs having such properties, even in ruminants.

10. 要　旨

本博士論文では，ヤギにおいて薬物の物理化学的特性と経口投与後の吸収との関係を明らかにすることを目的とし，物理化学的特性の異なる複数の薬物の経口投与後の体内動態を検討した。

第 1 章では，弱酸性薬物であるジクロフェナク（DF，pKa=4）とスルファモノメトキシン（SMM，pKa=6）をシバヤギに経口投与し，投与後の動態を解析して得られた経口吸収の特性を比較した。いずれの薬物も投与後の吸収は緩やかであったが，DF の平均吸収時間（MAT）は 6 時間で，SMM の 15 時間の半分以下であり，吸収速度にかなりの差が認められた。また，これに対応して DF の吸収半減期（$t_{1/2ka}$）が平均で 4.13 時間であったのに対し，SMM の $t_{1/2ka}$ は 10.5 時間とかなり長かった。いずれの薬物も，ルーメン液中では安定であった。以上の結果から，DF と SMM の吸収速度の差は，DF の前胃からの吸収速度が SMM の場合よりもかなり速いことに起因するものと考えられた。また，前胃からの吸収速度が速いのは，DF が極めて高い脂溶性を持つためと考えられた。オクタノール／pH6.5 の緩衝液の分配係数を測定したところ，SMM が 1.72 ± 0.174 であったのに対し，DF は 91.8 ± 9.45 とはるかに高い値を示した。本研究で得られた DF と SMM の $t_{1/2ka}$ を他の動物種と比較すると，かなり長い値であった。これは，前胃での長い滞留時間によるものと推察された。以上の結果から，脂溶性の極めて高い薬物は反芻獣の前胃から比較的速やかに吸収される可能性が示唆された。さらに，胃排出が経口投与後の吸収の支配要因である可能性が示唆された。

第 2 章で胃排出が経口投与後の薬物の支配要因である可能性が示されたことから，第 3 章ではシバヤギの胃排出特性を明らかにする目的で，アセトアミノフェン（AAP）

の経口投与後の動態を検討した。得られた MAT と $t_{1/2ka}$ はそれぞれ 4.93 時間および 3.35 時間と予想に反してかなり短かった。オクタノール／pH6.5 の緩衝液の分配係数で脂溶性を DF と比較すると，AAP の分配係数は 2.07（DF のおよそ 1/50）であったことから，DF よりも脂溶性がかなり低かった。MAT や $t_{1/2ka}$ の値は AAP が前胃から比較的速やかに吸収されていることを示唆するが，これには，脂溶性以外の要因が関与している可能性が考えられた。可能性ある要因として，DF よりも分子量が小さいこと，また pKa が 9.56 の弱酸のため，消化管内ではそのほとんどが非イオン型で存在することなどが考えられた。AAP は多くの動物種で胃排出評価に用いられているが，シバヤギでは AAP の経口投与後の動態からは，胃排出を評価できなかった。また、本研究では AAP の経口投与後の生体内利用率が極めて低いことが示された（16-17%）。AAP はルーメン液中で安定であったことから，この低い利用率は肝初回通過効果によることが示された。このことは，AAP をシバヤギに経口投与しても，十分な薬効が得られない可能性を示すものと考えられた。

第 3 章では，物理化学的特性の異なる 3 つのサルファ剤，スルファメサジン（SMZ, pKa 7.5），スルファダイアジン（SDZ, pKa 6.5）およびスルファニルアミド（SA, pKa 10.5）の経口投与後の吸収特性を比較した。その結果，SDZ の MAT および $t_{1/2ka}$ は 13.2 および 10.9 時間で，SA の 9.09 および 7.46 時間，SMZ の 7.52 および 5.17 時間よりも有意に長かった。これらの結果は，SDZ の前胃からの吸収速度が SMZ や SA よりもかなり緩やかであることを示唆した。この吸収速度の差は，主として脂溶性の差に起因するものと考えられた。pH6.5 での分配係数は，SMZ が 1.96，SDZ が 0.468，SA が 0.257 であった。以上から，本研究に用いたサルファ剤の前胃からの吸収は，その脂溶性に依存するものと考えられた。しかしながら，SA と SDZ を比較すると，SA の方が脂溶性が低い（分配係数が SDZ のおよそ半分）にもかかわらず，短い MAT および $t_{1/2ka}$ を

示した。これは，SA の分子量が SDZ よりもかなり小さいことによるものと推察された。さらに，SA の pKa が高いために，ルーメン液中ではほとんどすべてが非イオン型で存在することも，もうひとつの要因として推察された。したがって，分子量が小さくルーメン液中でほとんどが非イオン型で存在する薬物は，脂溶性が多少低くても前胃から比較的速やかに吸収されるものと考えられた。SA の吸収を AAP と比較すると，SA の方が経口投与後の吸収が遅かった。これは SA の方が AAP よりも分子量が大きく，脂溶性が低いことによるものと考えられた。

本研究の結論として，ヤギの前胃から速やかに吸収されるためには，脂溶性が高い，ルーメン内で非イオン型の比率が高い，さらに分子量の小さいという物理化学的特性が必要であることが示された。さらに，これらの特性を有する薬物は，反芻獣に対して経口投与で用いることができる可能性が示唆された。