Gastrointestinal Absorption of Chlorothiazide: Evaluation of a Method Using Salicylazosulfapyridine and Acetaminophen as the Marker Compounds for Determination of the Gastrointestinal Transit Time in the Dog

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Gastrointestinal absorption properties of chlorothiazide was investigated in dogs by a double-marker method using acetaminophen and salicylazosulfapyridine as the markers.

The mean absorption time of acetaminophen (MAT_{AAP}) and the time for first appearance of sulfapyridine in plasma (TFA_{SP}) were used for the assessment of gastric emptying and oro-colonic transit times, respectively. Chlorothiazide absorption efficiency was increased by pretreatment with atropine sulfate. There was a good correlation between MAT_{AAP} and the extent of bioavailability of chlorothiazide, however, there was no correlation between TFA_{SP} and the extent of bioavailability of the drug. These results indicate that chlorothiazide absorption takes place primarily in a limited segment of the upper small intestine, supporting the assumption reported previously. This double-marker method seems to be a useful tool for the investigation of the relationship between drug absorption and its gastrointestinal transit.

Keywords chlorothiazide; absorption; gastrointestinal transit; dog; gastric emptying rate; small intestinal transit time; upper small intestinal segment; acetaminophen; salicylazosulfapyridine; atropine sulfate

An oral absorption of drugs is said to depend frequently on its gastrointestinal transit time. In this connection, some of drugs which depress or accelerate the gastrointestinal motility have been shown to influence the bioavailability of orally administered drugs.[1] However, the drug absorption in the dog has not been discussed yet under a simultaneous monitoring of its transit through the gastrointestinal tract. One of the reasons for this situation seems to be a difficulty to assess the transit, particularly the small intestinal transit.

In our previous report,[2] salicylazosulfapyridine (SASP) was demonstrated on its usefulness as a marker compound for conveniently measuring the oro-colonic transit time in the dog. This compound is metabolized to form sulfapyridine by the bacterial flora in the large intestine. The time for first appearance of sulfapyridine in plasma (TFA_{SP}) just reflects the arrival of the head of orally administered SASP to the colon. On the other hand, acetaminophen is utilized as a marker compound to measure the gastric emptying rate.[3] Using a couple of these compounds as the markers, it seems possible to assess the bioavailability of appropriate drug substances or granulated dosage forms, by monitoring their small intestinal transit and gastric emptying times in the dog, simultaneously. This study was designed to evaluate the feasibility of the above mentioned double-marker method in the dog.

It is suggested that the absorption of chlorothiazide from the gastrointestinal tract may be site-specific.[4] The absorption of this drug is increased by propantheline, an inhibitor of gastric emptying and intestinal motility.[4a,d] Accordingly, chlorothiazide was chosen as a target drug to estimate an influence of the gastrointestinal transit time on the oral bioavailability.

Experimental

Materials Chlorothiazide, acetaminophen, sulfapyridine and atropine sulfate were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). *p*-Anisamide was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Salazopyrin® tablets containing 500 mg of SASP in each tablet were purchased from The Green Cross Co. (Osaka, Japan). All other reagents used were of analytical grade available from commercial suppliers.

Analytical Method A high performance liquid chromatography (HPLC) capable of simultaneous determination of chlorothiazide, acetaminophen and sulfapyridine was developed. To 100 μl of plasma were added 1 μg of *p*-anisamide (the internal standard), 1 ml of 0.5 M Na_2HPO_4–KH_2PO_4 buffer (pH 7.4) and 2 ml of ethyl acetate. After shaking for 10 min and centrifugation at 3000 rpm for 10 min, 1 ml of supernatant fluid was transferred to a glass tube and evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of the mobile phase and 100 μl of this solution was loaded onto the column. The HPLC was carried out using a Shimadzu LC-6A apparatus equipped with a Nucleosil $7C_{18}$ (150 × 4 mm i.d.) and a Shimadzu SPD-6A ultraviolet (UV) monitor (280 nm). Acetonitrile–1% AcOH (1 : 9, v/v) mixturewas employed as a mobile phase at a flow rate of 1.5 ml/min. The sensitivity was 0.1 μg/ml for chlorothiazide and sulfapyridine and 0.2 μg/ml for acetaminophen, respectively, with less than 10% of the coefficient of variation of the assay. Typical chromatograms obtained from blank plasma and from plasma spiked with chlorothiazide, acetaminophen and sulfapyridine are shown in Fig. 1.

Absorption Studies Seven male beagle dogs weighing 10—13 kg were fasted for 20 h prior to and for 12 h after the drug administration, but were allowed free access to water.

On 5 different occasions each dog received; (treatment 1) a single 10 mg/kg intravenous dose of acetaminophen as a polyethylene glycol 400–saline (1 : 1, v/v) solution, (treatment 2) a single 10 mg/kg intravenous

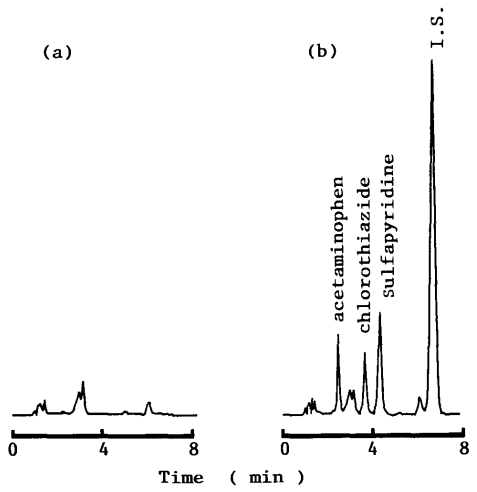

Fig. 1. Chromatograms of (a) Control Plasma and (b) Control Plasma Spiked with Acetaminophen 2 μg/ml, Chlorothiazide 1 μg/ml, Sulfapyridine 1 μg/ml and I.S. (*p*-Anisamide) 10 μg/ml

dose of chlorothiazide as a polyethylene glycol 400–saline (3:1, v/v) solution, (treatment 3) one capsule containing 500 mg chlorothiazide, orally, (treatment 4) one capsule containing 500 mg chlorothiazide, 200 mg acetaminophen and a 324 mg crushed Salazopyrine® tablets (equivalent to 250 mg SASP), orally, (treatment 5) the same capsule as that in treatment 4, orally, with a 0.1 mg/kg intravenous dose of atropine sulfate given 10 min prior to the administration of the capsule. Each dog received 30 ml of water immediately after the administration. Each experiment was carried out with 1-week interval.

Blood samples (0.4 ml) were taken with heparinized syringes 0.25, 0.5, 1, 2, 3, 4 and 5 h after intravenous administration of acetaminophen (treatment 1), 0.5, 1, 2, 4, 6, 9 and 12 h after intravenous administration of chlorothiazide (treatment 2) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 h after oral administration of the capsule (treatment 3, treatment 4 and treatment 5), respectively. The blood samples were centrifuged and the separated plasma samples were kept frozen until assay.

Pharmacokinetic Analysis The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from individual plasma chlorothiazide concentration curves. The area under the plasma concentration–time curve (AUC_{0-12h}) was calculated by the trapezoidal method and the mean residence time (MRT) was calculated by model-independent statistical moment analysis.[5] The extent of bioavailability was calculated as follows:

$$AUC_{0-12\,h,\,capsule} \times dose_{i.v.}/AUC_{0-12\,h,i.v.} \times dose_{capsule}$$

Gastrointestinal Transit Time The mean absorption time of acetaminophen (MAT_{AAP}) was used for an index of the rate of gastric emptying of chlorothiazide. The value was calculated according to the following equation, $MAT_{AAP} = MRT_{p.o.} - MRT_{i.v.}$, where $MRT_{p.o.}$ and $MRT_{i.v.}$ represent the values of oral (treatment 4 and treatment 5) and intravenous acetaminophen (treatment 1), respectively. Oro-colonic transit time was estimated by TFA_{SP}, as mentioned above.

Statistical Analysis Differences in each bioavailability parameter or transit time between treatments were statistically evaluated by paired t-test. Correlation coefficients (r) between MAT_{AAP} and TFA_{SP}, MAT_{AAP} and the extent of bioavailability, and TFA_{SP} and the extent of bioavailability obtained after the capsule without and with atropine sulfate (treatment 4 and treatment 5, respectively) were calculated by linear regression analysis.

Results

Bioavailability of Chlorothiazide There was no difference in bioavailability parameters of chlorothiazide between chlorothiazide alone (treatment 3) and the drug together with the markers (treatment 4); C_{max} (μg/ml): 2.3±0.4 *vs.* 2.9±0.8, T_{max} (h); 2.6±1.6 *vs.* 2.6±1.1, AUC_{0-12h} (μg·h/ml); 9.3±2.2 *vs.* 11.4±2.4, MRT (h); 4.1±0.7 *vs.* 3.9±0.6 (mean±S.D., $n=7$), respectively.

Figure 2 shows the mean plasma levels of chlorothiazide after the oral administration of the capsule with and without pretreatment using atropine sulfate (treatment 5 and treatment 4, respectively). The bioavailability parameters are shown in Table I. The values of each parameter, that is, C_{max}, T_{max}, AUC_{0-12h} and the extent of bioavailability were increased by atropine sulfate.

Gastrointestinal Transit Time The value of MRT for intravenous acetaminophen (treatment 1) was 0.98±0.12 h (mean±S.D.). Following treatments 4 and 5, acetami-

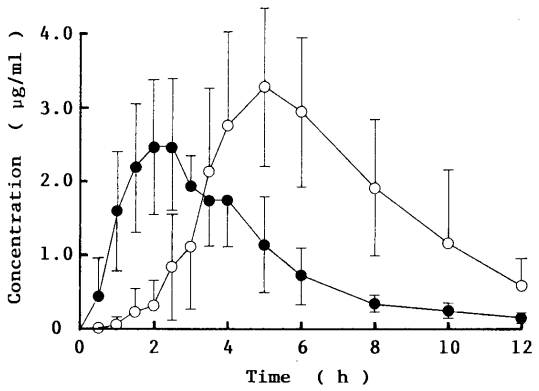

Fig. 2. Plasma Concentrations of Chlorothiazide after Oral Administration with and without Pretreatment Using Atropine Sulfate

●, no treatment; ○, pretreatment with atropine sulfate (0.1 mg/kg, i.v.). The results are expressed as the mean±S.D.

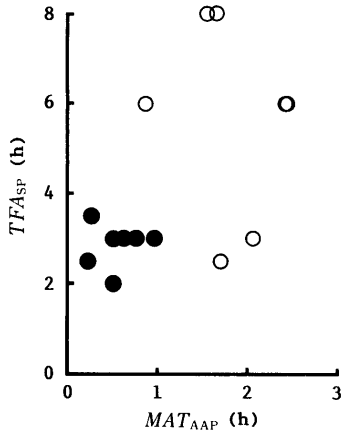

Fig. 3. Correlation between the Mean Absorption Time of Acetaminophen (MAT_{AAP}) and the Time for First Appearance of Sulfapyridine in Plasma (TFA_{SP})

●, no treatment; ○, pretreatment with atropine sulfate (0.1 mg/kg, i.v.).

TABLE I. Bioavailability Parameters of Chlorothiazide after Oral Administration with and without Pretreatment Using Atropine Sulfate

Dog No.	C_{max} (μg/ml)		T_{max} (h)		AUC_{0-12h} (μg·h/ml)		Extent of bioavailability[a] (%)	
	No pretreatment	Pretreatment	No pretreatment	Pretreatment	No pretreatment	Pretreatment	No pretreatment	Pretreatment
1	2.4	4.1	4.0	3.5	12.7	16.4	16.7	21.6
2	1.9	2.1	4.0	5.0	8.6	11.1	12.6	16.3
3	2.4	3.1	2.5	5.0	9.4	15.8	14.8	24.8
4	3.9	5.0	2.5	4.0	15.3	22.3	21.0	30.5
5	3.8	3.5	2.0	5.0	11.9	18.5	21.4	33.3
6	3.7	4.7	1.5	6.0	12.5	25.5	17.5	35.6
7	2.3	3.3	1.5	10.0	9.1	22.8	15.3	38.2
Mean	2.9	3.7	2.6	5.5	11.4	18.9	17.0	28.6
S.D.	0.8	1.0	1.1	2.1	2.4	5.0	3.2	8.0
Significance	$p<0.05$		$p<0.05$		$p<0.01$		$p<0.01$	

a) Calculated as follows: $AUC_{0-12h,\,p.o.} \times dose_{i.v.}/AUC_{0-12h,i.v.} \times dose_{p.o.}$

TABLE II. Gastrointestinal Transit Time with and without Pretreatment Using Atropine Sulfate

Dog No.	MAT_{AAP}[a] (h)		TFA_{SP}[b] (h)	
	No pretreatment	Pretreatment	No pretreatment	Pretreatment
1	0.97	0.87	3.0	6.0
2	0.24	1.64	2.5	8.0
3	0.27	1.54	3.5	8.0
4	0.64	2.42	3.0	6.0
5	0.77	2.06	3.0	3.0
6	0.51	1.71	3.0	2.5
7	0.51	2.44	2.0	6.0
Mean	0.56	1.81	2.9	5.6
S.D.	0.26	0.55	0.5	2.2
Significance	$p<0.01$		$p<0.05$	

a) The mean absorption time of acetaminophen. *b*) The time for first appearance of sulfapyridine in plasma.

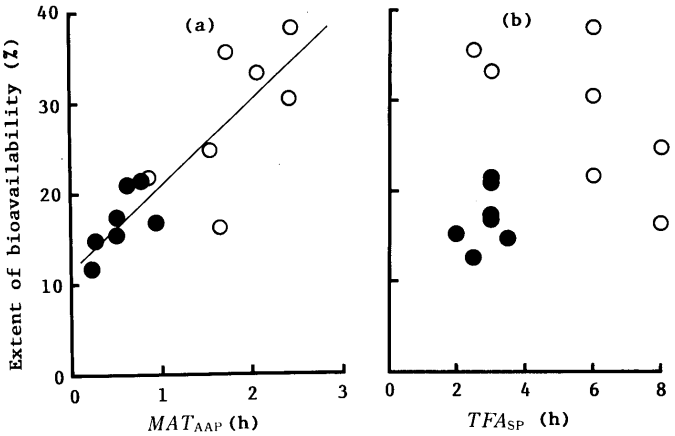

Fig. 4. Correlation between (a) MAP_{AAP} and Extent of Bioavailability, (b) TFA_{SP} and Extent of Bioavailability

●, no treatment; ○, pretreatment with atropine sulfate (0.1 mg/kg, i.v.). The curve in (a) was obtained as follows: $y=11.9+9.2x$ ($r=0.85$, $p<0.01$).

nophen was detected at the initial sampling point (0.5 h) in all cases. Both MAT_{AAP} and TFA_{SP} were significantly prolonged by atropine sulfate, as shown in Table II.

Correlation between MAT_{AAP} and TFA_{SP} There was no correlation between MAT_{AAP} and TFA_{SP} ($r=0.52$, $p>0.05$), as shown in Fig. 3.

Correlation between MAT_{AAP} and the Extent of Bioavailability There was a significant correlation between MAT_{AAP} and the extent of bioavailability ($r=0.85$, $p<0.01$), as shown in Fig. 4a.

Correlation between TFA_{SP} and the Extent of Bioavailability There was no significant correlation between TFA_{SP} and the extent of bioavailability ($r=0.52$, $p>0.05$), as shown in Fig. 4b.

Discussion

Oral absorption efficiency of chlorothiazide has been shown to be poor and dose-dependent. For example, oral chlorothiazide was 56% bioavailable from a 50 mg dose,[4c] but decreased to 16% bioavailable with a 500 mg dose[4b] in humans. Chlorothiazide absorption efficiency was doubled when taken after propantheline[4a] or food.[4d] Similar results have been obtained in dogs.[4e] The above absorption property has been attributed to a saturable or site-specific absorption process and low water solubility.

The gastric emptying of a dosage form is influenced by its physical state or its size.[6] There is a difference in the aqueous solubility between acetaminophen and chlorothiazide, which might lead to a difference in the rate of gastric emptying between these two compounds. However, the gastric motility to empty chlorothiazide should be mirrored by the rate of emptying of acetaminophen, since the canine stomach is considered not to recognize a size less than 1 mm of diameter.[7] Consequently, the rate of gastric emptying of acetaminophen can be presumed to have a correlation with that of chlorothiazide.

It has been reported that the stomach begins to empty powder within several minutes after administration, irrespective of its aqueous solubility.[8] The fact that acetaminophen was detected at the initial sampling point (0.5 h) is consistent with these findings. Therefore it is presumed that a portion of SASP was emptied from the stomach soon after administration. As the timing of the sulfapyridine signal corresponds to the arrival of the head of SASP in the colon,[9] TFA_{SP} should reveal the time taken for the head of SASP to pass through the small intestine. It has been pointed out that once liquid and pellets have left the stomach there is little additional spreading within the small intestine.[10] Consequently, it seems that TFA_{SP} represents not only small intestinal transit time of the head, but also that of the whole bulk of SASP. Davis *et al.*[6] have reported that small intestinal transit of the dosage form is not affected by its physical state or its size. Therefore, chlorothiazide and SASP seem to pass through the small intestine together at the same speed.

The marker compounds, that is, acetaminophen and SASP did not alter the absorption and elimination characteristics of chlorothiazide, since no significant differences in each bioavailability parameter of the drug were observed between the drug alone and the drug with these 2 marker compounds.

The absorption of chlorothiazide was increased by atropine sulfate which is an inhibitor of gastrointestinal motility as well as propantheline. Although MAT_{AAP} and TFA_{SP} were both prolonged by atropine sulfate, there was no significant correlation between these transit times. A good correlation was recognized between the extent of bioavailability and MAT_{AAP}. On the other hand, there was no correlation between the extent of bioavailability and TFA_{SP}. These observations indicate that although the absorption of chlorothiazide is influenced by the gastric emptying rate, it is not influenced by the rate of transit down the whole small intestine. Moreover, the delayed lag time for the appearance of chlorothiazide in plasma and the delayed T_{max} by atropine sulfate indicate that the drug is not absorbed to any extent from the stomach. These facts support the previous assumption that chlorothiazide absorption takes place primarily in a limited segment of the upper small intestine. The increased absorption of chlorothiazide by atropine sulfate is considered to be attributed to slower passage of the drug from the stomach into the small intestine permitting a greater portion of the drug to dissolve in a limited segment of the upper small intestine, leading to the increased absorption.

As shown in the present study, by using acetaminophen

and SASP as double markers we clarified that not the rate of transit down the whole small intestine but the rate of gastric emptying is the main determinant of chlorothiazide absorption efficiency. This double-marker method seems to be a useful tool for the investigation of the relationship between drug absorption and its gastrointestinal transit.

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