

Effects of Meals on Gastric Emptying and Small Intestinal Transit Times of a Suspension in the Beagle Dog Assessed Using Acetaminophen and Salicylazosulfapyridine as Markers

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Effects of the amount and the composition of meals on gastric emptying and small intestinal transit times of a suspension were investigated in beagle dogs using acetaminophen and salicylazosulfapyridine as markers.

Gastric emptying time was affected both by the amount and the composition of a meal; it was prolonged proportionally to the amount of a solid meal and varied among the 3 kinds of test meals of the same energy content in the following rank order: lard > skimmed milk > mashed potatoes.

The inter-individual variation of small intestinal transit time in a fed state was smaller than that in a fasted state, whereas the mean transit times in both states were similar. Small intestinal transit time was not affected by the amount of the solid meal. On the other hand, it varied among the 3 kinds of test meals in the following rank order: lard > mashed potatoes > skimmed milk.

It is noteworthy that small intestinal transit time in the beagle dog is approximately 2 h shorter than that in humans both in fasted and fed states.

Keywords gastric emptying rate; small intestinal transit time; beagle dog; fasted state; fed state; meal amount; meal composition; acetaminophen; salicylazosulfapyridine

Introduction

The main site for drug absorption is considered to be the small intestine. Therefore, gastric emptying rate and small intestinal transit time are the main determinants of the rate and extent of drug absorption. It is important to clarify the effects of a meal on gastric emptying rate and small intestinal transit time, as most drugs are received after ingestion of a meal. There are many reports on the effects of a meal on gastric emptying rate and small intestinal transit time in humans.¹⁾ The beagle dog is widely used for the evaluation of drug bioavailability, however, only a few studies on gastric emptying rate and none on small intestinal transit time have been reported.

In our recent paper,²⁾ we described the usefulness of salicylazosulfapyridine (SASP) as a marker compound for measuring small intestinal transit time in the beagle dog. Upon oral administration, SASP is partially absorbed during its transit through the small bowel and the unabsorbed part of the dose is metabolized to form sulfapyridine (SP) in the colon. Timing of the first appearance of SP in plasma (TFA) corresponds to the arrival of the head of SASP in the colon.³⁾

Acetaminophen (AAP) is a well known marker compound for the evaluation of the gastric emptying rate. Although AAP is poorly absorbed from the stomach, it is rapidly absorbed from the small intestine. The rate of absorption reflects the rate of gastric emptying,⁴⁾ and hence the mean absorption time of AAP should reveal the mean gastric emptying time.

Therefore, the combination of AAP and SASP for markers is expected to enable the simultaneous measurement of gastric emptying and small intestinal transit times in the beagle dog.

The present study investigates the effect of the amount and composition of meals on gastric emptying and small intestinal transit times of a suspension in the beagle dog using the above-mentioned double marker technique.

Experimental

Materials The SP and AAP were purchased from Sigma Chemicals

Co. *p*-Anisamide was purchased from Nakarai Chemicals Ltd. Salazopyrin Tablets containing 500 mg of SASP in each tablet were purchased from The Green Cross Co. All other reagents used were of analytical grade available from commercial suppliers.

Preparation of Marker Suspension Salazopyrin Tablets were crushed, added to AAP and suspended in distilled water so that the suspension contained 25 mg of SASP and 10 mg of AAP per 1 ml. The SASP was almost undissolved and AAP was completely dissolved.

Analytical Method To 100 μ l of plasma were added 1 ml of 0.5 M phosphate 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 μ l of mobile phase containing 1 μ g of *p*-anisamide as an internal standard (I.S.), and 100 μ l of this solution was injected into the chromatograph. High performance liquid chromatography (HPLC) was carried out using a Shimadzu LC-6A apparatus equipped with a Nucleosil 7C₁₈ (4 mm i.d. \times 150 mm; M. Nagel) and a Shimadzu SPD-6A ultraviolet monitor (254 nm). Acetonitrile-0.1% AcOH (1:9) was employed as a mobile phase at a flow rate of 1.5 ml/min. Simultaneous measurement of AAP and SP could be achieved. The sensitivity was 0.1 μ g/ml for both compounds with less than 10% of the coefficient of variation. The mean recoveries were 88.0% for AAP and 85.4% for SP. Typical chromatograms obtained from blank plasma and

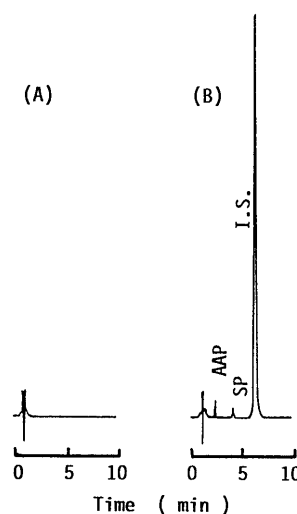


Fig. 1. Chromatograms of Sample Prepared from (A) Control Plasma and from (B) Control Plasma Spiked with AAP 0.1 μ g/ml, SP 0.1 μ g/ml and I.S. (*p*-Anisamide)

from plasma spiked with AAP and SP are shown in Fig. 1.

Animal Experiments Experiments were carried out on 12 beagle dogs weighing 9–13 kg (11 male and 1 female). Each dog was fasted for 20 h and given the marker suspension at a dose of 1 ml/kg followed by 30 ml of water through the catheter either on an empty stomach or 0.5 h after meals which differed in amount and composition. The dogs ate the meals within 10 min. Blood samples were taken with heparinized syringes 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 h after dosing of the marker suspension. Plasma was separated by centrifugation and stored frozen until assay. Each experiment was carried out with 1-week intervals.

The mean absorption time of AAP (MAT) was calculated according to the following equation, $MAT = MRT_{p.o.} - MRT_{i.v.}$, where $MRT_{p.o.}$ and $MRT_{i.v.}$ represent the mean residence times⁵⁾ of oral AAP and 10 mg/kg intravenous AAP as a polyethylene glycol 400–saline (3:1) solution, respectively.

Effect of the Amount of a Meal: Each of 12 beagle dogs was fed 50, 100 and 300 g of a solid meal (DS: Oriental Yeast Co., Ltd.) on 3 different occasions. A 100 g of the solid meal consisted of 9 g fat, 47 g carbohydrate and 27 g protein (total energy: 371 kcal).

Effect of the Composition of a Meal: Nine male beagle dogs weighing 9–13 kg were used. On 3 different occasions, each dog was fed 3 kinds of test meals; 20 g of lard as a high-fat meal, 50 g of mashed potatoes as a high-carbohydrate meal and 50 g of skimmed milk as a high-protein

TABLE I. Composition of Test Meals Ingested

| | Fat (g) | Carbohydrate (g) | Protein (g) | Weight (g) | Energy (kcal) |
|-----------------|---------|------------------|-------------|------------|---------------|
| Lard | 20 | 0 | 0 | 20 | 188 |
| Mashed potatoes | 0 | 41 | 3 | 50 | 180 |
| Skimmed milk | 0 | 26 | 17 | 50 | 179 |

meal. Mashed potatoes and skimmed milk were served after kneading with 150 and 50 ml of hot water (about 80°C), respectively, and cooling to room temperature. The meal compositions are shown in Table I.

Statistical Analysis For comparison between treatments, the values were subjected to analysis of variance according to the randomized block design, and subsequently to Tukey's multiple range test at $p=0.05$. The correlation coefficient between MAT and TFA after the solid meal was calculated by linear regression analysis. The difference in the inter-individual variation of small intestinal transit time between fasted and fed states was examined by Hartley's test at $p=0.05$.

Results and Discussion

Effect of Meals on Gastric Emptying After dosing of the marker suspension, AAP was detected at the initial sampling point (0.25 h) in all cases. This indicates that a portion of the marker suspension was emptied from the stomach soon after the administration, irrespective of the amount or composition of a meal. The value of MAT was rather short in the fasted state and was prolonged after ingestion of the meal proportionally to the amount of the meal, as shown in Fig. 2A. There were no significant differences in the values of MAT among the dogs. The order of MAT after 3 kinds of test meals was as follows; lard > skimmed milk > mashed potatoes, as shown in Fig. 3A. The difference between skimmed milk and mashed potatoes was not statistically significant.

Hunt *et al.*⁶⁾ have reported that the rate of gastric emptying of meals with the same nutritive density in humans was such that the number of calories delivered to the

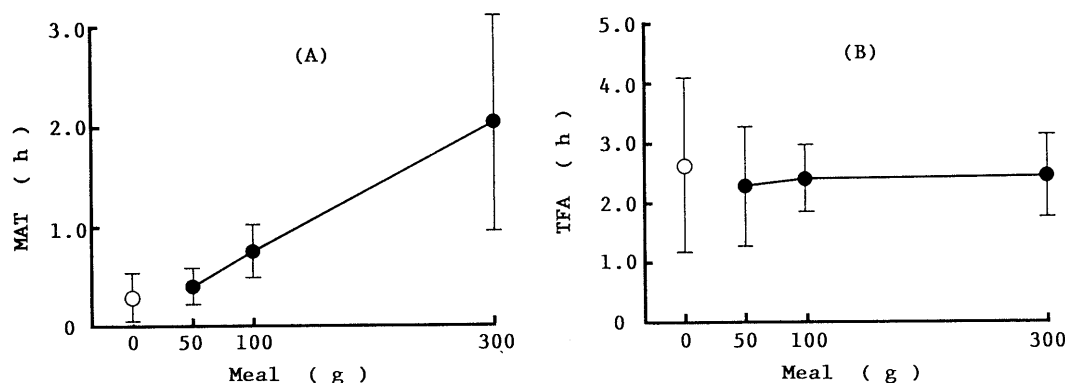


Fig. 2. Effects of the Amount of a Meal on Gastric Emptying and Small Intestinal Transit Times of a Suspension

(A), the mean absorption time of AAP (MAT) in a fasted state and after a solid meal; (B), the time for the first appearance of SP in plasma (TFA) in a fasted state and after a solid meal. Each point represents the mean ± S.D. ($n=12$).

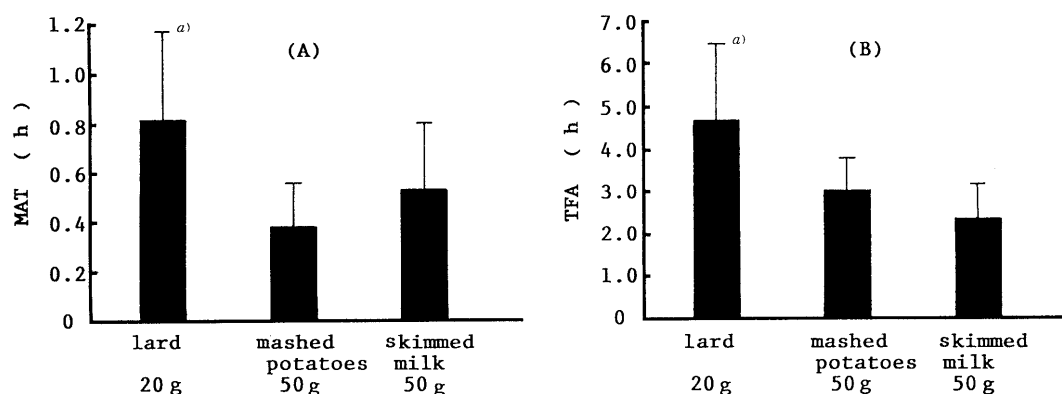


Fig. 3. Effects of the Composition of a Meal on Gastric Emptying and Small Intestinal Transit Times of a Suspension

(A), MAT after test meals; (B), TFA after test meals. Each point represents the mean ± S.D. ($n=9$). ^{a)} Significantly different from the other 2 meals ($p<0.05$).

duodenum tends to be constant with time, independent of the initial volume of meals. Fara⁷⁾ has described that the gastric emptying rates of carbohydrate, protein and fat meals in humans decreased in that order. The values of MAT obtained in the present study agree well with the gastric emptying behavior of meals. Therefore, it seems that marker suspension was well mixed with the meals in the stomach and had good bearing on the emptying of the meals from the stomach.

Effect of Meals on Small Intestinal Transit Time Figure 2B shows the values of TFA after the solid meal compared to those in the fasted state: fasted, 2.6 ± 1.5 h; 50 g, 2.3 ± 1.0 h; 100 g, 2.4 ± 0.6 h; 300 g, 2.5 ± 0.7 h (mean \pm S.D.). The value of TFA after the solid meal was independent of the amount of the meal. Similar to our previous data,²⁾ a remarkable inter-individual variation was observed in the fasted state. On the other hand, inter-individual variation was significantly decreased by feeding. There was no difference in the mean TFA values between fasted and fed states. The significant differences existed in the values of TFA among dogs.

The value of TFA varied among the 3 kinds of test meals, as shown in Fig. 3B: lard, 4.7 ± 1.8 h; mashed potatoes, 3.0 ± 0.8 h; skimmed milk, 2.4 ± 0.8 h (mean \pm S.D.). The rank order was as follows; lard > mashed potatoes > skimmed milk. The difference between mashed potatoes and skimmed milk was not statistically significant. As in the case of the solid meal, inter-individual variation tended to be smaller than that in the fasted state, although a significant difference was not achieved.

Since a portion of the marker suspension seems to leave the stomach immediately after administration, TFA reveals the time taken for the head of the suspension to reach the colon, irrespective of the time taken for the bulk of the suspension to be emptied from the stomach. Therefore, these results indicate that the rate of transit of a suspension through the small bowel in the beagle dog is not affected by the amount of the meal with constant composition, but is affected by varying its composition. It seems that there are differences among dogs in the small bowel motility to transfer the contents through the small intestine in a fed state.

Davis *et al.*^{1c)} have reported that small intestinal transit time of pharmaceutical dosage forms in humans after a standard breakfast was not influenced by doubling the amount of the meal ingested. The present results are consistent with these observations in humans.

Small intestinal transit time in a fasted state in humans is approximately 4.5 h by the SASP method.⁸⁾ On the other hand, no determination of the transit in a fed state by the method has been reported. However, the transit time in a fed state by the SASP method should be comparable to that in a fasted state, since it is well documented that the rate of transit through the small bowel in humans is not affected by feeding.^{1c)} Consequently, except for the case of ingestion of lard, small intestinal transit time in beagle dogs observed in the present study is approximately 2 h shorter than that in humans both in fasted and fed states.

Variations of drug bioavailability are often decreased by the ingestion of meals.⁹⁾ The present results indicate that the difference in inter-individual variation on small intestinal transit time between fasted and fed states may be partly

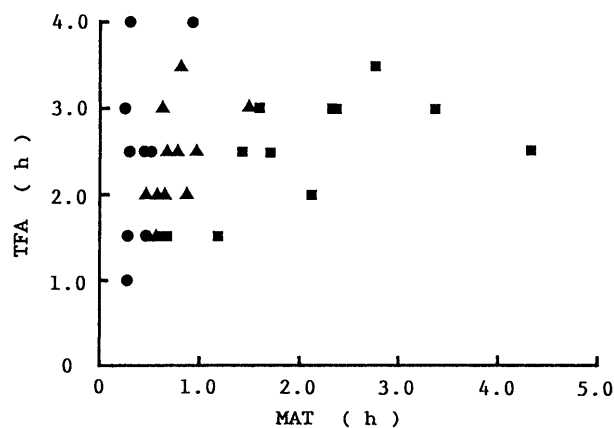


Fig. 4. Correlation between MAT and TFA after a Solid Meal

●, 50 g; ▲, 100 g; ■, 300 g.

responsible for these phenomena.

There are many drugs whose absorption efficiency is known to be increased by the ingestion of high-fat meals. These phenomena have been commonly interpreted based on delayed gastric emptying and/or increased bile output.¹⁰⁾ Our findings suggest that delayed small intestinal transit time may be one of the causes for increased absorption after the ingestion of high-fat meals.

Correlation between MAT and TFA There was a poor, if any, correlation between MAT and TFA after the solid meal (Fig. 4). The correlation coefficients obtained after 50, 100 and 300 g of the solid meal were 0.38 ($p > 0.05$), 0.48 ($p > 0.05$) and 0.61 ($p < 0.05$), respectively. The fact that there were significant differences in TFA among dogs, whereas no significant differences existed in MAT, is consistent with the poor correlation between MAT and TFA.

Read *et al.*^{1a)} have suggested that gastric emptying and small bowel transit may be controlled by their own independent mechanisms in humans. The results from the present study suggest that this is also the case for the beagle dog.

Conclusion

The results about the effects of meals on the gastric emptying and small intestinal transit of a suspension in beagle dogs can be summarized as follows: 1) Gastric emptying time is affected by the amount and the composition of a meal. 2) Small intestinal transit time is independent of the amount of a meal, but is affected by the composition of the meal. It is suggested that ingestion of a high-fat meal reduces the rate of small intestinal transit. 3) There is a remarkable inter-individual variation in small intestinal transit time in a fasted state, however, the variation is decreased by the ingestion of a meal. 4) Small intestinal transit time in the beagle dog is approximately 2 h shorter than that in humans both in fasted and fed states.

The present findings on the small bowel transit of a suspension seems to be applicable for the other dosage forms, since small bowel transit of the dosage form is reported to be independent of its physical state or its size in humans.^{1c)} We would emphasize that small intestinal transit time in the beagle dog is shorter than that in humans both in fasted and fed states. We should pay much attention

to this point on carrying out bioavailability studies on drugs with poor water-solubility and sustained release dosage forms whose absorption efficiency is supposed to be appreciably influenced by small intestinal transit time.

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