Influence of Specific Gravity and Food on Movement of Granules in the Gastrointestinal Tract of Rats

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The suitability of rats as an animal model for estimating the bioavailability of controlled-release granules in humans was investigated. Non-disintegrating granules (diameter of 0.8 mm; specific gravity of 0.9—1.85) were used as a model preparation. Twenty granules were administered to fed rats, fasted rats and rats given soft food, and the number of granules remaining in the gastrointestinal tract was counted at suitable intervals.

Granules with a specific gravity of 1.25 administered to fasted rats were rapidly emptied from the stomach with a 50% gastric emptying time of 1 h as compared with granules with a specific gravity of less than 1.0 or with a high specific gravity such as 1.85. The presence of food in the stomach reduced the emptying rate of granules. The mean transit time of granules through the small intestinal tract was not influenced by the specific gravity or the presence of food. The mean transit time was about 3 h.

It was found that the transit profile of granules through the gastrointestinal tract in rats was similar to that of granules in humans. Accordingly, it is possible to use rats at the preformulation stage for estimating the bioavailability of controlled-release granules in humans.

Keywords granule; specific gravity; food; gastric emptying; intestinal transit time; rat

Introduction

The bioavailability of a drug from an oral dosage form will be influenced by various pharmaceutical and physiological factors. Controlled-release preparations such as enteric-coated and sustained-release preparations are designed so that a drug will be released from a preparation at a limited segment or through the whole region of the gastrointestinal tract.[1-4] Accordingly, gastric residence time and intestinal transit time are very important physiological factors affecting the bioavailability of these preparations.

We have already reported an investigation of the movement of non-disintegrating model preparations (tablets and granules) in the gastrointestinal tract using gastric-emptying-controlled rabbits as a model animal for estimating the bioavailability of controlled-release preparations in humans.[5] The results indicated that tablets were randomly emptied from the stomach, similar to the case of humans. The gastric emptying was restricted by the size of the tablets. On the other hand, granules were gradually emptied from the stomach and the profile was similar to the case of humans under non-fasting conditions. The small intestinal transit time of granules was not influenced by either specific gravity or diameter, similar to the case of humans. But, the small intestinal transit time (1.2 h) was about one-third that of humans. Accordingly, further investigations will be required to evaluate the bioavailability of controlled-release preparations.

The present study was undertaken to investigate the transit of non-disintegrating model granules with different specific gravity through the gastrointestinal tract of rats, instead of rabbits. From the viewpoint of the movement of preparations in the gastrointestinal tract, the feasibility of using rats as a model animal for estimating the bioavailability of controlled-release preparations in humans is discussed.

Experimental

Materials Brilliant blue FCF aluminum lake (Blue 1# Al. lake) and erythrosine aluminum lake (Red 3# Al. lake) were purchased from San-Ei Chemical Industries, Ltd., and glass beads (MK-4GX) and blue spray lacquer were purchased from Shinmaru Enterprises Co., and Rock Paint Co., respectively. Keflex (KX, conventional capsules) and L-Keflex (L-KX, prolonged-action capsules) capsules containing cephalexin (Shionogi & Co., Ltd.) and other materials were commercially available. The dissolution rates of L-KX and KX were measured with the U.S.P. dissolution tester. At pH 1.2 and 4.5, the dissolution rate of L-KX rapidly increased to 30% within 20 min, but the granules were not dissolved thereafter. At pH 6.5, the time required for 100% dissolution was about 30 min. This means that L-KX contains 30% rapid release granules and 70% enteric coated granules. L-KX was 0.1—0.8 mm in diameter with a specific gravity of about 1.1. At pH 1.2, 4.5 and 6.5, the time required for 100% dissolution of KX was about 20 min.

Preparation of Test Granules Granules with Specific Gravity of 1.85: Glass beads (0.7—1.0 mm in diameter) were precoated with blue lacquer, and coated with dichloromethane solution containing 3.8% (w/w) ethylcellulose and 0.2% (w/w) polyethylene glycol 6000 (spray solution) by means of a spray method in a pan, in order to avoid disintegration. The diameter of coated granules was in the range of 0.71—0.84 mm, and a mean diameter of 0.8 mm was adopted in this paper.

Granules with Specific Gravity of 1.25: Plain granules were made by a wet granulating method according to the formula in Table I, using a high speed mixer (Mitsuimiike Seisakusho, HENSHEL 20B). The plain granules were coated by the same method as described above. The mean diameter of coated granules was 0.8 mm.

Granules with Specific Gravity of 0.9: Ethylcellulose powder (0.05—0.3 mm in particle size) and Red 3# Al. lake were mixed and granulated with ethanol using the high speed mixer to form suitable plain granules. The plain granules were coated by the same method as described above. The mean diameter of coated granules was 0.8 mm.

Swelling of Granules The swelling percentage of granules was calculated from the volume change of granules after a disintegration test in water for 7 h using the JP XI equipment. None of the granules showed swelling (Table I) or deformation.

Specific Gravity of Granules The specific gravity of granules was calculated from the volume measured in water at 25 °C and the weight of preparations.

Animals Wistar-strain male rats, weighing about 200 g, were housed singly in cages with a wire net floor in order to prevent coprophagy. Animals were divided into three groups: one group was allowed lab chow (CE-2, Nihon Clea) and water *ad libitum* (group I); another was fasted, but allowed water *ad libitum*, for 24 h prior to this experiment (group II); the third was fasted for 24 h and then intragastrically given 1 ml of soft food, which was prepared by kneading 1 g of lab chow with 4 ml of tap water, through polyethylene tubing (group III). One ml of soft food corresponds to about one-fifth of the amount of food in group I.

TABLE I. Characteristics and Formulae of Granules

Specific gravity	Diameter (mm)	Swelling %[a]	Formulae			
			Plain granules		Coating layer	
1.85	0.8	5>	Glass beads	(99.0)	Ethylcellulose 100	(95)
			Blue lacquer	(1.0)	Polyethylene glycol 6000	(5)
1.25	0.8	5>	Corn starch	(75.9)	Ethylcellulose 100	(95)
			Microcrystalline cellulose	(20.0)	Polyethylene glycol 6000	(5)
			Blue #1 Al lake	(0.1)		
			Hydroxypropylmethyl cellulose	(4.0)		
0.90	0.8	5>	Ethylcellulose 100	(99.8)	Ethylcellulose 100	(95)
			Red #3 Al lake	(0.2)	Polyethylene glycol 6000	(5)

a) Percent volume increase after disintegration test for 7 h.

Administration Studies Administration of Test Granules: The apparatus,[6] consisting of a syringe, sonde and polyethylene tubing (2 mm in diameter, 50 mm in length) for packing solid preparations, was used for oral administration of solid preparations to rats. Twenty granules were packed in the tubing and were intragastrically administered with 0.5 ml of tap water. Animals were sacrificed by decapitation at 0.5, 1.5, 3.5, and 7 h after administration, the stomach and the small intestine were immediately isolated, and the number of granules remaining in the gastrointestinal tract was counted. The number of granules in the region below the cecum was calculated by subtraction of the number remaining in the stomach and the small intestine from the number administered.

Administration of KX and L-KX Granules: Granules contained in KX and L-KX capsules were used for this experiment. Each of KX granules equivalent to a dose of 50 mg/kg and L-KX granules equivalent to a dose of 100 mg/kg was packed in the tubing described above and intragastrically administered with 0.5 ml of tap water to group III. Blood specimens were withdrawn from the jugular vein at predetermined times and the plasma samples were frozen and stored at −20 °C until assay.

Assay The concentration of cephalexin in the plasma was determined by bioassay.[7]

Mean Transit Time[8] The transit of granules in the gastrointestinal tract was expressed in terms of the time required for 50% to leave the stomach (50% gastric emptying time (50% GET)) and for 50% to arrive at the cecum (50% cecal arrival time (50% CAT)). AS a good linear relationship between probit for remaining percent in the stomach or arrival percent at the cecum and log time was obtained, 50% GET and 50% CAT were determined by the probit method. The difference between these two values was taken as representing transit in the small intestine (mean small intestinal transit time).

Results and Discussion

Influence of Specific Gravity on Gastrointestinal Transit of Granules Figure 1 shows the gastrointestinal transit of three kinds of granules (specific gravity of 0.90, 1.25 and 1.85) in fasted rats. Granules with a specific gravity of 1.25 were rapidly emptied from the stomach with a 50% GET of 1 h (Table II) as compared with the other two granules. It has been reported that particles were rapidly emptied from the stomach in humans under fasting with a 50% GET of about 1 h.[9,10] Further, the gastric emptying profiles of granules (diameter, 0.80 mm; specific gravity, 1.25) used in this study agreed very well with those with a 50% GET of about 1 h in humans[8] who had received pellets with a diameter of 0.8—1.1 mm and a specific gravity of 1.2 under fasting.

On the other hand, granules with a specific gravity of less than 1.0 or with a high specific gravity such as 1.85 were emptied slowly with large inter-animal variation. The 50% GET values of granules with specific gravities of 1.85 and 0.90 were 1.7 h and 3.4 h in fasted rats, respectively. These results suggested that gastric emptying of granules with a specific gravity of 0.9 may be delayed because of late arrival at the pylorus owing to the floating behavior. On the other hand, gastric emptying of granules with a specific gravity of 1.85 may be delayed because of movement of the sinking granules to the pylorus was slow. Thus, the gastric emptying of granules in rats was influenced by specific gravity. It is well known that tablets are emptied irregularly from the stomach in humans, but the gastric emptying patterns of granules in rats were relatively smooth regardless of the correspondence of the diameter of tablets administered to that in the case of humans. The fasted gastrointestinal motility pattern is characterized by an interdigestive migratory motor complex in both humans and rats. The former cycle is about 90—120 min, and the latter one is about 25 min.[11] Therefore, the gastric emptying of granules in rats seems to be smooth due to the shorter interdigestive migratory complex cycle.

The amount and rate of distribution of granules in the small intestinal tract increased with increasing gastric emptying rate in the order of granules with a specific gravity of 1.25 > 1.85 > 0.90. These results indicate that the distribution profile of granules in the small intestinal tract is strongly dependent on the gastric emptying rate and suggest that it is possible to regulate drug bioavailability by changing the specific gravity of preparations.

Each kind of granules began to arrive at the cecum within 1.5 h after administration, though the amount was small. The 50% CAT values of granules with specific gravities of 0.90, 1.25 and 1.85 were 6.2, 4.3 and 4.9 h, respectively (Table II), and the cecal arrival time (from the mouth to the cecum) was influenced by specific gravity. The difference seems to be due to the difference in the gastric emptying rate.

On the other hand, the mean small intestinal transit times of granules with specific gravities of 0.90, 1.25 and 1.85 were 2.8, 3.3 and 3.2 h, respectively (Table II). These results indicate that the small intestinal transit time of granules is not particularly influenced by specific gravity, though the gastric emptying time was strongly influenced by specific gravity. The mean small intestinal transit time (about 3 h) of granules in fasted rats agreed well with that (3—4 h) in humans[8,12] who had received pellets with a specific gravity of 1.2 and a diameter of 0.7—1.2 mm.

From the anatomical viewpoint, the length of the small intestine in rats used in this study was about 0.8 m, being about one-eighth of that of humans (6—7 m). Therefore, it was found that the absolute small intestinal transit speed of granules in rats was very slow compared with that of

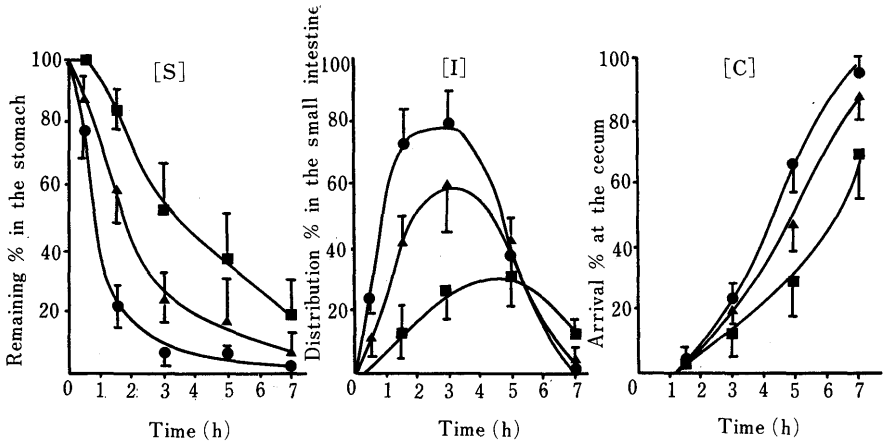

Fig. 1. Influence of the Specific Gravity on Gastrointestinal Transit of Granules with a Mean Diameter of 0.8 mm in Fasted Rats

Each point represents the mean±S.E. (n=4—7). [S], stomach; [I], small intestine; [C], cecum. Specific gravity: ■, 0.90; ●, 1.25; ▲, 1.85.

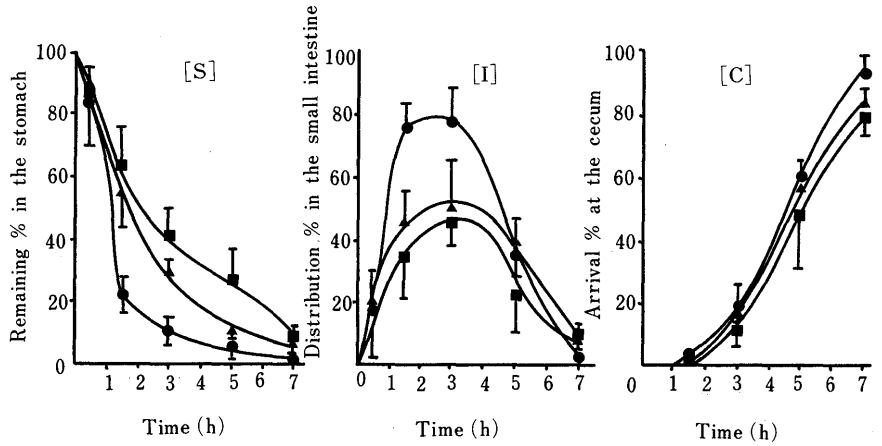

Fig. 2. Influence of Food on Gastrointestinal Transit of Granules with a Specific Gravity of 1.25 and a Mean Diameter of 0.8 mm in Rats

Each point represents the mean±S.E. (n=4—7). [S], stomach; [I], small intestine; [C], cecum. Gastric contents: ●, fasted rats; ■, fed rats; ▲, rats given soft food.

humans. These results indicated that the transit time of granules through the gastrointestinal tract in fasted rats was very similar to that of granules in humans under fasting. Accordingly, this indicates that it is possible to use fasted rats for estimating the bioavailability of controlled-release granules such as enteric-coated and sustained-release granules in humans under fasting.

Influence of Food on Gastrointestinal Transit of Granules Figure 2 shows the gastrointestinal transit of granules with a specific gravity of 1.25 in fed rats (group I), fasted rats (group II) and rats given soft food (group III). In the cases of groups I and III, granules were gradually emptied from the stomach. The 50% GET values of group I, II and III were 1.8, 1.0 and 1.6 h, respectively. Thus, the presence of food in the stomach reduced the gastric emptying rate of granules and an increasing quantity of food somewhat increased the 50% GET (from 1.6 to 1.8 h).

In the case of humans, the presence of food also reduces the gastric emptying rate of small particles.[8] Davis *et al.*[12] have reported that pellets with a specific gravity of 1.2 and a diameter of 0.7—1.2 mm were gradually emptied from the stomach in humans with 50% GET of 2.0±0.3 h (mean± S.E.) after light breakfast intake or 4.8±0.8 h after heavy breakfast intake. The 50% GET values of granules in humans after light meal and usual meal intakes, reported by Maekawa *et al.*,[2] were in the ranges of 1—2 h and 2—4 h, respectively. Thus, it was found that the gastric emptying profiles of granules in groups I and III were similar to those of granules in humans after light meal intake. However, the emptying profiles in humans after usual or heavy meal intake could not be obtained in rats under the experimental conditions used.

Groups I and III with slow gastric emptying exhibited a lower distribution of granules in the small intestinal tract as compared with group II with rapid gastric emptying. The distribution profiles of granules were dependent on the gastric emptying rate.

The 50% CAT values of granules in group I, II and III were 4.9, 4.3 and 4.7 h, respectively (Table II), and the cecal arrival time (from the mouth to the cecum) was increased by the presence of food in the stomach, though the increase was not large. On the other hand, the mean small intestinal transit times of granules in groups I, II and III were 3.1, 3.3 and 3.1 h, respectively (Table II). These results indicate that the mean small intestinal transit time of granules is not influenced by food intake or quantity of food.

In the case of humans, it has been reported that the mean transit time of various preparations through the small intestinal tract was 3—4 h, irrespectively of the dosage forms (tablets-pellets)[8] or the quantity of food (light-heavy

TABLE II. Gastrointestinal Transit Time of Granules

Group[a]	Specific gravity	50% gastric emptying time (h)	50% cecal arrival time (h)	Mean small intestinal time (h)
I	1.25	1.8 (1.5—2.1)[b]	4.9 (4.6—5.3)	3.1
II	0.90	3.4 (3.1—3.9)	6.2 (5.6—7.2)	2.8
	1.25	1.0 (0.9—1.2)	4.3 (4.0—4.7)	3.3
	1.85	1.7 (1.4—2.0)	4.9 (4.5—5.4)	3.2
III	1.25	1.6 (1.3—1.8)	4.7 (4.4—5.1)	3.1

a) Group I, fed rats; group II, fasted rats; group III, rats given soft food. *b*) Figures in parentheses indicate 95% confidence intervals.

breakfast).[12] This agree very well with the results in rats.

From these results, it was found that the transit of granules through the gastrointestinal tract in fed rats (groups I and III) was very similar to that of granules in humans after light meal intake. Accordingly, it appears to be possible to use rats for estimating the bioavailability of controlled-release granules in humans after light meal intake.

Bioavailability of Controlled-Release Preparations Figure 3 shows plasma concentration–time curves after oral administration of KX (conventional type) and L-KX (prolonged action type) granules to rats given soft food (group III). KX granules exhibited a rapid absorption profile while L-KX granules exhibited a prolonged absorption profile. These results were similar to those in humans[7] under usual meal intake, though the time to maximum plasma concentration (T_{max}) was shorter as compared with T_{max} (KX granules, 3 h; L-KX granules, 4 h) in humans because the 50% GET (about 1.6 h) of granules in rats given soft food is faster than that (about 2—4) in humans[2] after usual meal intake. However, these results strongly support the view that rats are useful as an animal model for the preliminary bioavailability test of controlled-release granules.

These results lead to the conclusion that it is possible to use rats at the preformulation stage for estimating the bioavailability of controlled-release granules in humans.

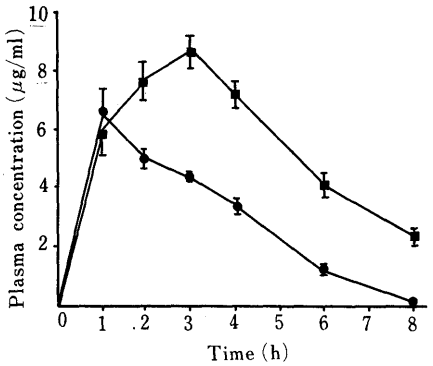

Fig. 3. Mean Plasma Concentration–Time Curves of Cephalexin after Intragastrically Administration of KX Granules and L-KX Granules to Rats Given Soft Food

Each point represents the mean ± S.E. ($n=4$). ●, KX granules, 50 mg/kg; ■, L-KX granules, 100 mg/kg.

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