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Suitable Size of Preparations to Evaluate the Bioavailability of Enteric-Coated Preparations by the Use of Gastric-Acidity-Controlled Rabbits

TERUTAKA TAKAHASHI,*,[a] MASAAKI MORI,[b] YUKO UEZONO,[a] HIROKAZU MAKITA,[b] YUTAKA NAKANISHI,[a] and YU IMASATO[b]

Pharmaceutical Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka 564, Japan[a] and Ebie 1–5–51, Fukushima-ku, Osaka 553, Japan[b]

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The limiting size of enteric-coated preparations for which it is possible to evaluate the bioavailability by using gastric-acidity-controlled rabbits (GAC-rabbits) was investigated. Enteric-coated preparations with different diameters were administered to GAC-rabbits, and drug bioavailability from each preparation was evaluated. In the case of enteric-coated granules, there was no problem in evaluating the bioavailability. However, in the case of enteric-coated tablets, the maximum size for which it was possible to evaluate the bioavailability was 4.1 mm in diameter. The results indicate that the use of GAC-rabbits in bioavailability studies of enteric-coated preparations is restricted by the size of the preparations.

Keywords——enteric coating; tablet; granules; diameter; gastric-acidity-controlled rabbit; bioavailability

Introduction

In the case of an acid-labile drug or a drug causing gastric lesions by local action, enteric coating may is one possible way to improve drug bioavailability and to minimize gastric side effects. The bioavailability of a drug from an oral controlled-release dosage form (including enteric-coated preparations) will be influenced by various physiological factors such as gastric emptying time, intestinal transit time and variations in pH.[1–4] Thus, it is desirable that an experimental animal should resemble humans with respect to the physiological factors described above in studies to predict the bioavailability of controlled-release preparations in humans by using animals. We have already reported that gastric-acidity-controlled rabbits (GAC-rabbits),[5] in which gastric acidity and gastric emptying are simultaneously controlled, are useful for bioavailability studies of preparations whose dissolution and stability are influenced by the pH of the gastric contents.[5–7] Further, in order to examine the feasibility of using rabbits as a model animal for estimating the bioavailability of controlled-release preparations in humans, the movement of non-disintegrating model preparations in the gastrointestinal tract was investigated.[8] It was found that tablets with a diameter of 7.7 mm were not emptied from the stomach and therefore the use of rabbits in bioavailability studies of controlled-release preparations appeared to be restricted by the size of the preparations.

In order to confirm this finding, the present study was undertaken to investigate the bioavailability of enteric-coated preparations of various sizes in GAC-rabbits. Test preparations were formulated with acid-labile erythromycin and AD-1590,[9] 2-[8-methyl-10, 11-dihydro-11-oxodibenz[*b*,*f*]oxepin-2-yl]propionic acid, which is a new acid non-steroidal anti-inflammatory agent.[10] The usefulness of GAC-rabbits as a model animal for estimating the

bioavailability of enteric-coating preparations in humans is discussed.

Experimental

Materials——AD-1590 (Dainippon Pharmaceutical Co., Ltd.) and erythromycin (Dainippon Pharmaceutical Co., Ltd.), as the free base, were each pulverized and screened to provide fine powder (specific surface diameter, about 10 μm). Lactose, low-substituted hydroxypropylcellulose, hydroxypropylmethylcellulose, magnesium stearate and hydroxypropylmethylcellulose phthalate (HP-55) were of commercial grade. Other materials were of analytical grade.

Test Preparations——Test preparations for oral administration to rabbits are shown in Table I. Each preparation was prepared in a conventional manner, Erythromycin enteric-coated tablets (diameter, 10.1 mm; content, 100 mg in one tablet) used in humans were commercial products.

Disintegration Test——The disintegration time was determined according to the Japanese Pharmacopoeia X (JP X) method. The disintegration time of each plain preparation was within 2 min in water at 37 °C. Enteric-coated preparations also met the requirement of the disintegration test specified in the JP X, as shown in Table II.

Dissolution Test——Dissolution tests were carried out according to the JP X method (paddle method, 100 rpm). Each preparation (containing 150 mg as erythromycin, 15 mg as AD-1590 and 16 mg as AD-1590 in the case of enteric-coated tablets with a diameter of 4.1 mm) was dissolved in 900 ml of the JP X disintegration 1st fluid (pH 1.2) or 2nd fluid (pH 6.8) at 37 °C. The concentrations of AD-1590 and erythromycin in the fluid were determined by ultraviolet spectrophotometry at 314 nm and colorimetry[6] at 482 nm, respectively. Table II shows the dissolution rate for each preparation. In the case of erythromycin, which is a basic drug, erythromycin from the plain tablets dissolved rapidly in the 1st fluid as compared with the 2nd fluid, although no activity of erythromycin was observed in the 1st fluid. On the other hand, erythromycin from the enteric-coated tablets was not dissolved in the 1st fluid but was dissolved in the 2nd fluid at a similar rate to the plain tablets. This indicates that this tablet has enteric characteristics. In the case of AD-1590, which is an acidic drug, AD-1590 from the plain tablets and the plain granules dissolved rapidly in the 2nd fluid, in contrast to erythromycin. AD-1590 from the enteric-coated granules and tablets was not dissolved in the 1st fluid but was completely dissolved within 10 min in the 2nd fluid.

Table I. Test Preparations Used in This Study

Drug	Dosage form	Content	Size or diameter
Erythromycin	Plain tablets	50 mg/tab.	7.6 mm
	Enteric-coated tablets	50 mg/tab.	7.8, 10.1 mm
AD-1590	Plain tablets	15 mg/tab.	5.6 mm
	Enteric-coated tablets	15 mg/tab.	5.9 mm
		2 mg/tab.	4.1 mm
	Plain granules	10%	710—500 μm
	Enteric-coated granules	10%	710—500 μm

Table II. Values of Disintegration Time and Dissolution Rate of Test Preparations

Drug	Preparation		Disintegration time (min)		% dissolved[a]		
					1st[b]		2nd[b]
			1st[b]	2nd[b]	D_{10}	D_{60}	D_{10}
Erythromycin	Plain tablets		<2	<2	100	100	66
	Enteric-coated tablets		>120	<5	0	0	57
AD-1590	Plain tablets		<1	<1	8	16	100
	Enteric-coated tablets	15 mg/tab.	>120	<5	0	0	100
		2 mg/tab.	>120	<5	0	0	100
	Plain granules		<1	<1	15	19	100
	Enteric-coated granules		>60	<2	0	0	100

a) D_{10} and D_{60} indicate % dissolved in 10 and 60 min, respectively. *b*) 1st, JP X 1st fluid; 2nd JP X 2nd fluid. Each datum is the mean of three trials.

Bioavailability Study——Male albino ravbits, weighing 2.5—3.0 kg, were used in the animal test. The rabbit gastric pH values corresponding to low acidity (pH > 5) and high acidity (pH < 3) were controlled with antacid and hydrochloric acid, respectively, according to the method[5] previously reported. The tablets were put on the radix linguae, which was pulled out by using tweezers, and were swallowed and thereafter 20 ml of water was administered into the stomach through a rubber tube. The granules were administered into the stomach with 20 ml of water through a rubber tube. The administration of each preparation was as follows. Three erythromycin plain tablets (dose, 150 mg), one AD-1590 plain tablet (dose, 15 mg) or AD-1590 plain granules (dose, 15 mg) were administered to rabbits with low and high acidities. Three erythromycin enteric-coated tablets with a diameter of 7.8 or 10.1 mm (dose, 150 mg), one AD-1590 enteric-coated tablet with a diameter of 5.9 mm (dose, 15 mg), eight AD-1590 enteric-coated tablets with a diameter of 4.1 mm (dose, 16 mg) or AD-1590 enteric-coated granules (dose, 15 mg) were administered to rabbits with high acidity.

In the human test, after an overnight fast, seven male subjects (age, 34—51 years; weight, 46—75kg) ingested two erythromycin enteric-coated tablets (dose, 200 mg) with 200 ml of water.

Blood samples were collected at predetermined times after administration of each preparation, and the plasma samples were frozen and stored (−20 °C) until assay.

Assay——The concentration of erythromycin in the plasma was determined by bioassay as described previously.[6] The concentration of AD-1590 in the plasma was determined by high-performance liquid chromatography according to the reported method.[11]

Results and Discussion

Enteric-Coated Tablets of Erythromycin

Erythromycin is a representative drug which is enteric-coated in drug formulation to protect it from gastric degradation, thereby improving the bioavailability. Figure 1 shows individual plasma concentration–time curves after oral administration of three plain tablets (7.6 mm in diameter) to GAC-rabbits with low and high acidities. Erythromycin was detected in the plasma of all rabbits with low acidity, whereas three of six rabbits with high acidity showed no detectable plasma level of erythromycin, although the other three showed low levels. This may be ascribed to the pH-dependent stability of erythromycin.[6] Therefore, as the extent of bioavailability of erythromycin is strongly affected by gastric acidity, it is desirable to apply enteric-coated formulations to ensure efficacy.

However, rabbits showed no detectable plasma level of erythromycin until 8 h when three

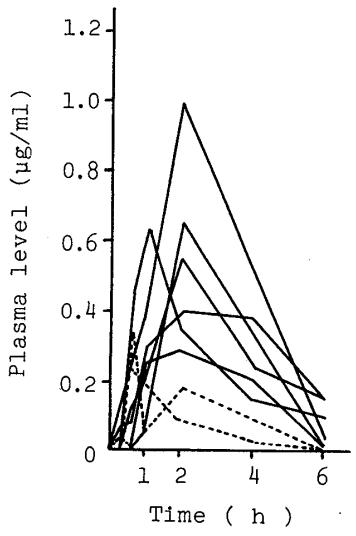

Fig. 1. Individual Plasma Levels of Erythromycin after Oral Administration of Plain Tablets to GAC-Rabbits

Solid lines, low-acidity rabbits ($n=6$); dotted lines, high-acidity rabbits ($n=6$).

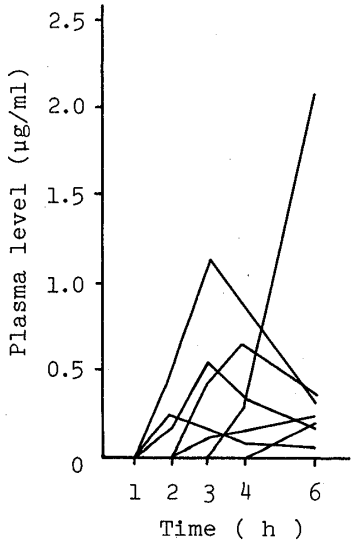

Fig. 2. Individual Plasma Levels of Erythromycin after Oral Administration of Enteric-Coated Tablets to Fasting Humans

enteric-coated tablets with a diameter of 7.8 or 10.1 mm were administered to rabbits with high acidity. The reason why these tablets yielded no detectable plasma level of the drug is probably as follows. Erythromycin may have been dissolved and then degraded in the stomach (an acidic environment) owing to rupture of the coating film, and/or the intact tablets may have been retained without dissolution in the stomach or may have passed through the small intestine (the absorption site) without dissolution. In order to clarify the situation, the inside of the stomach was isolated 8 h after dosing and examined. In all cases, all enteric-coated tablets administered were found intact in the stomach. Consequently, enteric-coated tablets of 7.8 mm or more in diameter were not emptied from the rabbit stomach. On the other hand, Fig. 2 shows individual plasma concentration–time curves after oral administration of one enteric-coated tablet of commercial size (10.1 mm in diameter) to seven fasting humans. All subjects had a detectable plasma level of erythromycin with a variable absorption lag time, although the plasma level varied considerably among the subjects. The inter-subject variation in absorption lag time can probably be ascribed to the inter-subject variation in gastric emptying of the tablets. However, the problem of erratic or incomplete absorption from enteric-coated tablets appears not to be limited to erythromycin; similar problems have been reported in many studies.[1,2,12-17]

From these results, it was clear that rabbits failed to show gastric emptying of tablets with a diameter of less than the size that could be emptied in humans. Therefore, it appears to be impossible to evaluate drug bioavailability from some commercially available enteric-coated tablets by the use of GAC-rabbits.

Enteric-Coated Tablets and Granules of AD-1590

Although AD-1590, as well as other acid non-steroidal anti-inflammatory drugs, causes gastric lesions in rats, the gastric side effects could be reduced by providing AD-1590 with enteric characteristics.[10,11] Thus, the bioavailability of AD-1590 from enteric-coated preparations of different sizes was evaluated in GAC-rabbits. Figure 3 shows individual plasma

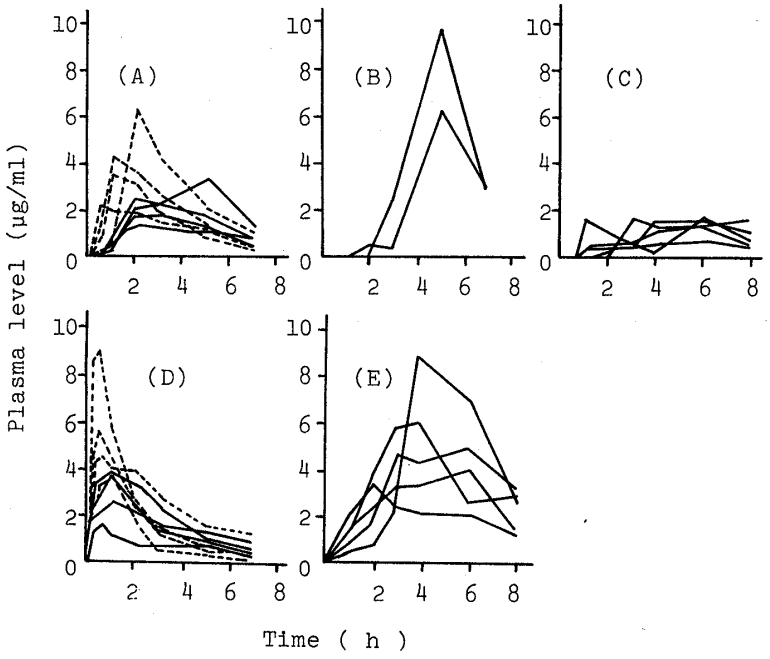

Fig. 3. Individual Plasma Levels of AD-1590 after Oral Administration of Plain or Enteric-Coated Preparations to GAC-Rabbits

Solid lines, high-acidity rabbits; dotted lines, low-acidity rabbits. (A), plain tablets ($n=8$); (B), enteric-coated tablets with a diameter of 5.9 mm ($n=6$); (C), enteric-coated tablets with a diameter of 4.1 mm ($n=5$); (D), plain granules ($n=8$); (E), enteric-coated granules ($n=5$).

concentration–time curves after oral administration of each preparation to GAC-rabbits. When one plain tablet (5.6 mm in diameter) was administered to rabbits with low and high acidities, absorption in the high-acidity group tended to be late as compared with that in the low-acidity group. The difference in absorption rate between two groups can be probably be ascribed to the difference of dissolution rate in the stomach, because AD-1590 from the plain tablets is dissolved faster in the pH region of low acidity (2nd fluid) than in the pH region of high acidity (1st fluid), as shown in Table II. On the other hand, although absorption from the plain granules in the high-acidity group tended to be somewhat late as compared with that in the low-acidity group, the bioavailability was not as much influenced by the gastric acidity as had been predicted from the results obtained in the dissolution test. This seems to be ascribable to relatively rapid gastric emptying of the granules.

Thus, when the gastric lesions and the variation of bioavailability depending on the gastric acidity are taken into consideration, it seems that enteric-coated granules are a desirable dosage form for AD-1590.

When one enteric-coasted tablet with a diameter of 5.9 mm was administered to rabbits with high acidity, four of six rabbits showed no detectable plasma level until 8 h, and as in the case of enteric-coated tablets of erythromycin, the intact tablets remained in the stomach 8 h after dosing. The other two showed steep increases of plasma level after a lag time of 1 or 2 h. This may be considered to be a result of rapid dissolution of AD-1590 from the tablets in the absorption site immediately after the tablets were emptied from the stomach into the duodenum. Thus, the gastric emptying of enteric-coated tablets with a diameter of 5.9 mm showed a large inter-animal variation, and the probability that the tablets would be emptied was small. Consequently, it appears difficult to evaluate the pharmaceutical characteristics of enteric-coated tablets with a diameter of 5.9 mm by the use of rabbits because the evaluation of bioavailability is severely limited by a physiological factor, that is, gastric emptying.

When eight enteric-coated tablets with a diameter of 4.1 mm were administered to rabbits with high acidity, all rabbits showed delayed absorption behavior with a variable lag time, although the plasma levels were fairly low (Fig. 3). Observation of the inside of the stomach showed that, although in one of five rabbits two of eight tablets administered remained in the stomach, all tablets in other four were completely emptied from the stomach 8 h after dosing. As the diameter of the tablets was decreased and further as the number of tablets administered was increased from one to eight, it appeared that the tablets were gradually emptied from the stomach without an "all-or-none" effect. However, this tablet gave lower plasma levels as compared with other preparations. This may be ascribed to insufficient dissolution of AD-1590 from the tablets during passage through the small-intestinal tract. However, if humans ingest this tablet, better bioavailability will probably be obtained as compared to rabbits because the absolute transit time of preparations in the small-intestinal tract of humans is about 3 times that of rabbits.[8] When two enteric-coated tablets (4.1 and 6.1 mm in diameter) were compared, a large difference in plasma level was observed, although no difference in the drug dissolution rate was observed in the JP X 2nd fluid with pH corresponding to that of the intestinal juice (Table II). Therefore we can not explain the difference of bioavailability between the two tablets on the basis of the results in the dissolution test, although the results in dissolution tests do not necessarily correspond to the bioavailability of preparations. In the case of the enteric-coated tablets containing a high drug content (content, 15 mg; diameter, 5.9 mm), it appears that AD-1590 was rapidly dissolved to give a high concentration in the small-intestinal tract immediately after being emptied from the stomach, and higher plasma levels were consequently obtained. On the other hand, in the case of the enteric-coated tablets containing a low drug content (content, 2 mg; diameter, 4.1 mm), the gastric emptying of tablets may be essentially irregular, although all were emptied from the stomach within 8 h after dosing. The irregular gastric emptying, resulting in intermittent administration of AD-

1590 with rapid elimination from the systemic circulation at a low dose (low concentration), might be responsible for the lower plasma levels.

From these results, we can not conclude whether the lower plasma levels for enteric-coasted tablets with a diameter of 4.1 mm are ascribable to pharmaceutical properties or physiological properties including size-dependent gastric emptying and short transit time. However, it is considered possible to evaluate to some extent the bioavailability of enteric-coated tablets with a diameter of less than 4.1 mm by the use of GAC-rabbits, although this size is somewhat smaller than that of usual commercially available enteric-coated tablets, and some problems remain concerning the extent of bioavailabilty.

When enteric-coated granules were administered to rabbits with high acidity, inter-animal variation in the onset of absorption was very small; further, the plasma level of AD-1590 derived from the granules increased gradually and a clear prolongation of the absorption process was observed, as shown in Fig. 3. This prolonged absorption appears to be due to extended emptying of the granules from the stomach into the duodenum and a sufficient dissolution of AD-1590 from the granules during passage through the small intestinal tract. The usefulness of GAC-rabbits as a model animal for estimating the bioavailability of enteric-coated granules in humans has already been demonstrated in the previous study[6] using enteric-coated granules of erythromycin. Accordingly, the results obtained here for the granules strongly support the suitability of GAC-rabbits for bioavailability studies of enteric-coated granules.

Results obtained in this study indicate that the maximum size for which it is possible to evaluate the bioavailability of enteric-coated preparations in GAC-rabbits is 4.1 mm in diameter, and therefore the use of GAC-rabbits in bioavailability studies is severely restricted in terms of the size of preparations. However, a multiple dosage form such as granules is generally applied as an enteric-coated preparation in order to ensure greater efficacy and safety, because enteric-coated tablets show a large variation in the onset of absorption and a marked fluctuation in the plasma level. Thus, we conclude that GAC-rabbits can be used in formulation studies of enteric-coated preparations from a practical point of view, since enteric-coated granules are the preferred formulation.

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