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Evaluation of Bioavailability of Cinnarizine Capsules by Use of Gastric-Acidity-Controlled Rabbits

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The bioavailability of cinnarizine (CN) from three capsules was investigated in gastric-acidity-controlled rabbits (GAC-rabbits) with low and high acidities. Two commercial capsules (capsules A, B), which show poor dissolution in a low acidity medium, and one test capsule, whose dissolution in a low acidity medium was improved, were used. Rabbits with low acidity showed significantly lower C_{max} and area under the plasma concentration–time curve (AUC) than rabbits with high acidity after administration of capsules A and B. Capsule A showed higher C_{max} and AUC than capsule B in rabbits with low acidity. The difference of bioavailability between the two capsules appears to be due to the difference of dissolution rate, depending on the gastric pH. The results obtained in this study corresponded very well with the results obtained in humans. Consequently, we conclude that the GAC-rabbit is a promising animal model for testing the bioavailability of a drug preparation showing gastric acidity-dependent bioavailability in humans. On the other hand, the bioavailability of CN from the test capsule was not affected by gastric acidity. Therefore, a small inter-subject variation of CN bioavailability can be expected on oral administration of the test capsule.

Keywords——cinnarizine; commercial capsule; oral bioavailability; gastric acidity; gastric-acidity-controlled rabbit

Cinnarizine (CN), an agent for increasing cerebral blood flow, is widely used orally to treat various problems in cerebral apoplexy, cerebral arteriosclerosis and post traumatic cerebral symptoms. However, it has been reported that the blood levels after oral administration of CN preparations to humans showed a large variation among the subjects.[1] Ogata *et al.*[2] recently reported that the bioavailability of CN from commercial capsules in humans was strongly affected by the gastric acidity, which causes an inter-subject variation of drug bioavailability, and this was ascribed to the pH-dependent dissolution of CN capsules. The gastric acidity of humans varies among the subjects[3,4] and many aged persons, to whom CN preparations are usually administered, are known to show achlorhydria or anacidity.[5] Thus, it is very important to clarify the relationship between gastric acidity and drug bioavailability in humans in order to ensure efficacy. Further, it is also important to develop a suitable animal model for testing the bioavailability of drug preparations showing gastric-acidity-dependent bioavailability in humans and for use in the development of a superior drug preparation, the bioavailability of which is not affected by gastric acidity.

Ogata *et al.*[2] employed beagle dogs as an animal model for testing the bioavailability of CN capsules showing gastric-acidity-dependent bioavailability in humans. However, the beagle is not a suitable animal model, because the effect of gastric acidity on CN bioavailability in the beagle is not as clear as in humans. This may be ascribed to the wide intra-subject variation of the beagle gastric acidity[6] and to rapid gastric emptying of the beagle as compared with humans.[7] We have already reported that gastric-acidity-controlled rabbits (GAC-rabbits) in which gastric acidity was controlled, as well as gastric emptying, were useful for bioavailability studies of drug preparations whose dissolution

and stability were influenced by the pH of the gastric contents.[6,8]

The present study was undertaken to clarify whether the bioavailability of CN capsules is affected by gastric acidity in GAC-rabbits, as has been observed in humans.

Experimental

Materials——Two commercial capsules of CN (capsules A and B) which were used in the human bioavailability test by Ogata *et al.*,[2] and one test capsule,[9] which was prepared with 25 mg of CN and 200 mg of citric acid, were used. The test capsule was generously provided by the National Institute of Hygienic Sciences. All other materials were of analytical reagent grade.

Dissolution Test——Dissolution tests were carried out at 37 °C using media of pH 3.0, 4.0, 5.0 and 6.0 (0.1 M sodium phosphate–1 N (HCl). The dissolution rates from test capsules were determined in 900 ml of the medium by using the JP X paddle method at 100 rpm. The amount of the drug dissolved was determined by high-performance liquid chromatography (HPLC). The dissolution rate is shown as the percent dissolved in 30 min (D_{30}).

Bioavailability Study——Thirty healthy male albino rabbits, weighing 2.5—3.0 kg, were used and were divided into six groups of five rabbits. The rabbit gastric pHs corresponding to low acidity (pH > 5) and high acidity (pH < 3) were controlled according to the method[6,10] previously reported. One capsule from among the capsules described above was inserted into the distal opening of the catheter, and administered orally with 20 ml of water through the catheter inserted into the stomach. This study, however, was not performed according to a cross over design. The rabbits were allowed access to tap water but were not fed until after the final blood sampling following drug administration. Blood samples were taken by cardiac puncture using a heparinized syringe at 1, 2, 3, 4, 6 and 8 h after drug administration. Plasma samples were frozen and stored (−20 °C) until assay.

Asssay——First, 1 ml of 0.1 N NaOH and 6 ml of diethylether were added to 1 ml of the plasma. The mixture was shaken for 10 min, then 5 ml of the organic layer was separated and evaporated under a nitrogen gas stream at 40 °C. The dried residue was dissolved in 50 μl of methanol and a 20 μl aliquot was analyzed by HPLC. In the case of the dissolution test, the sample was analyzed without extraction. HPLC was performed using an ALC/GPC model 204 instrument (Waters Associates) equipped with a μBondapak C_{18} column (300 × 3.9 mm; Waters Associates); 0.05 M ammonium acetate–methanol–acetonitrile (2 : 5 : 3) as the mobile phase; flow rate, 1.0 ml/min; detector, 254 nm. An internal standard was not used in this assay procedure because a good linear relationship (correlation coefficient, 0.999) between peak height and CN plasma concentration (5—100 ng/ml), and good reproducibility (coefficient of variation, 0.9—12.1%) were obtained. The standard curves were prepared for every assay.

Results and Discussion

Dissolution

Figure 1 shows the dissolution rate (D_{30})–pH profile for the test capsule. The dissolution rates of CN from the test capsule, as well as capsules A and B which were used in the human bioavailability test,[2] decreased with increasing medium pH. The dissolution was accompanied by pH change of the medium as indicated by arrows, especially in the medium with initial pH 5.0, and the D_{30} value at initial pH 5.0, which is in the range of pH corresponding to low gastric acidity, was about 80%. Thus, it was found that CN from the test capsule was rapidly dissolved in the medium with pH less than 5. On the other hand, it has been reported that CN from both capsules A and B is rapidly dissolved at pH 1.2 and poorly dissolved at above pH 5 using an oscillating basket method, although capsule A has the largest dissolution rate and capsule B has the smallest dissolution rate at pH 3.9 among 32 commercial capsules tested.[2] When the dissolution tests for capsules A and B, as well as the test capsule, were carried out at pH 5 using the paddle method, no pH change was observed during the dissolution, and the D_{30} values of both capsules were below 5% (not shown in Fig. 1). As the solubility of CN, which is a basic drug, increases with decreasing pH, the improved dissolution of CN from the test capsule in the medium with initial pH 5.0 appears to be ascribable to the effect of decreasing the pH by the use of citric acid in the formulation.

From these results, it was confirmed that the test capsule had dissolution characteristics superior to those of the two commercial capsules. As the buffer capacity of gastric juice is not as strong as might be expected, good bioavailability from the test capsule can probably be

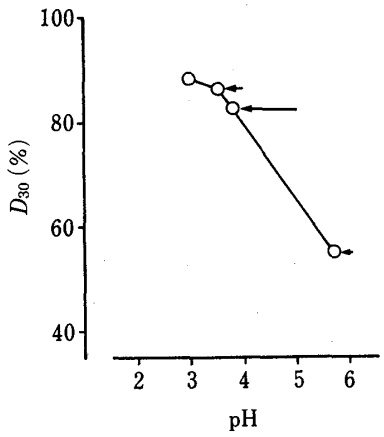

Fig. 1. Dissolution Rate–pH Profile for the Test Capsule

Arrows indicate changes of the medium pH.

obtained in humans with low gastric acidity (pH > 4).

Bioavailability for Capsules A and B

Figure 2 shows the individual plasma concentration–time curves of CN after oral administration of CN capsules A and B to GAC-rabbits with low and high acidities. In the case of capsule A, rabbits with high acidity showed higher plasma levels of CN than rabbits with low acidity, although one of the rabbits with low acidity showed extraordinarily higher plasma levels than the other four rabbits. The time to peak concentration (T_{max}) of both groups was within 3 h except for the one rabbit described above. This absorption profile for capsule A in GAC-rabbits was very similar to that observed in humans.[2] In this study, the differences of the peak concentration (C_{max}) and the area under the plasma concentration–time curve from 0 to 8 h (AUC), calculated by the trapezoidal rule, among treatments were statistically estimated by using a multiple range test on the basis of the results of the analysis of variance test. Data for the rabbit which showed the abnormal absorption profile as described above were omitted from the analysis. Accordingly, Scheffé's[11] test was used for multiple comparison because the numbers of animals per group were not equal. Rabbits with high acidity showed significantly higher C_{max} and AUC than rabbits with low acidity (Fig. 3). CN from capsule A is rapidly dissolved in the medium with pH corresponding to high gastric acidity and poorly dissolved in the medium with pH corresponding to low gastric acidity.[2] Therefore, the difference in bioavailability between the two groups appears to be due to the difference in dissolution rates, depending on the pH of the gastric contents. In spite of the control of gastric acidity, extraordinarily high plasma levels were observed in one of the rabbits with low acidity. This may be ascribed to failure of the control of gastric acidity or to pH change of the gastric contents from low acidity to high acidity owing to the delay of gastric emptying time over the time required to maintain low acidity.

In the case of capsule B, as well as capsule A, rabbits with high acidity showed clearly higher plasma levels of CN than rabbits with low acidity (Fig. 2), and C_{max} and AUC were significantly different between the two groups (Fig. 3). These results, as in the case of capsule A, reflected well the results in dissolution tests.

When the bioavailability was compared for the two capsules, capsule A showed higher C_{max} and AUC than capsule B in rabbits with low acidity (though the differences were not statistically significant), although the difference was not obvious in rabbits with high acidity (Fig. 3). This may be ascribed to the difference of dissolution rate in the gastric contents with low acidity, because CN from capsule A is dissolved in the pH range of low acidity faster than that from capsule B.[2]

A linear relationship existed between C_{max} and AUC, irrespective of the preparation and the gastric acidity, as shown in Fig. 4. This suggests that absorption of CN from capsules after

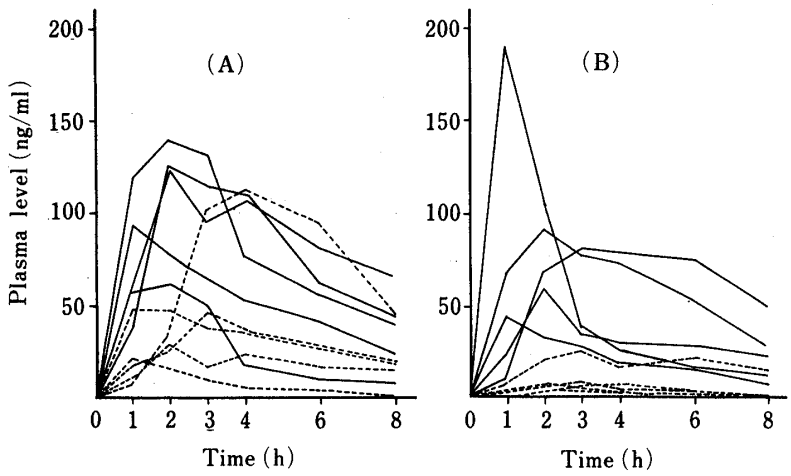

Fig. 2. Individual Plasma Concentrations of Cinnarizine after Oral Administration of Capsules A and B to GAC-Rabbits

(A), capsule A; (B), capsule B; ——, high acidity; – – – –, low acidity.

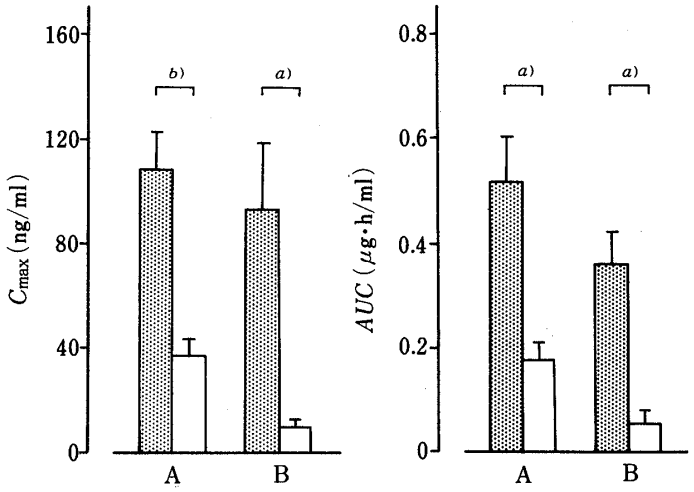

Fig. 3. Comparison of C_{max} and AUC after Oral Administration of Capsules A and B to GAC-Rabbits

A, capsule A; B, capsule B; ▩, high acidity; □, low acidity. *a*) $p<0.01$; *b*) $p<0.05$.
Each bar indicates the mean and S.E. of four or five animals.

oral administration is determined by the same factor, that is, the amount of CN dissolved in the stomach, because CN is hardly dissolved in the small intestinal juice.

From the results obtained with the two commercial capsules, it was found that the bioavailability of CN in rabbits was related to the gastric acidity.

Ogata *et al.*[2] obtained the following results when capsules A and B were administered to humans classified into high and low gastric acidity groups. In both capsules A and B, humans with high acidity showed significantly higher C_{max} and AUC than humans with low acidity. When the bioavailability was compared for the two capsules, capsule A showed significantly higher C_{max} and AUC than capsule B in humans with low acidity, although no significant difference was observed in humans with high acidity. They concluded that the bioavailability of CN from the two capsules in humans was related to the gastric acidity, which influences the dissolution rate of CN from the capsules.

Thus, it was demonstrated that the results obtained in this study corresponded very well with those obtained in humans. Consequently, we conclude that GAC-rabbits can be used in place of beagle dogs as an animal model for testing the bioavailability of a drug preparation showing gastric-acidity-dependent bioavailability in humans.

Bioavailability for the Test Capsule

The bioavailability for commercial capsules of CN is strongly affected by the gastric

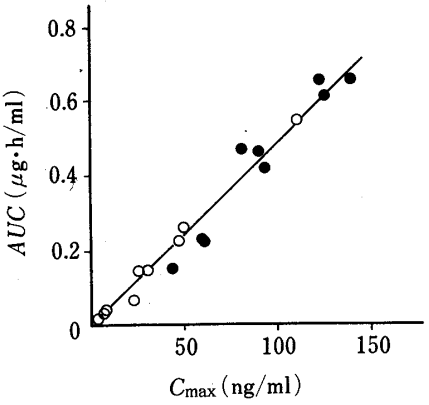

Fig. 4. Relationship between C_{max} and AUC after Oral Administration of Capsules A and B to GAC-Rabbits

●, high acidity; ○, low acidity.

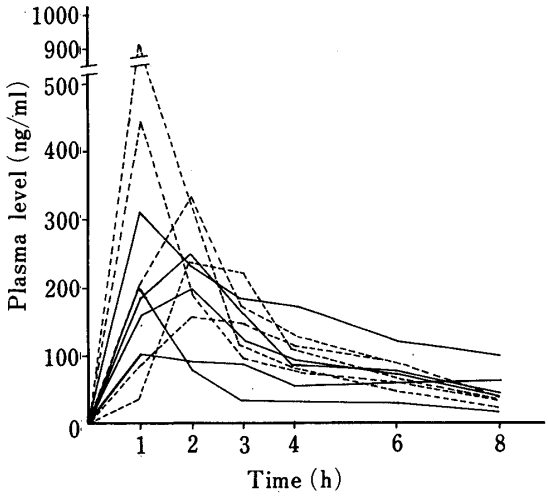

Fig. 5. Individual Plasma Concentration of Cinnarizine after Oral Administration of the Test Capsule to GAC-Rabbit

——, high acidity; – – – –, low acidity.

TABLE I. Statistical Analysis of Differences of C_{max} and AUC after Oral Administration of Three Cinnarizine Capsules to GAC-Rabbits

Gastric acidity		Capsule[a] A	Capsule[a] B	Capsule[a] C	Analysis of variance	Scheffé's[b] test
Low	C_{max} (ng/ml)	37.5 (5.1)[c]	9.6 (2.8)	426.3 (131.2)	$p<0.01$	C>A>B
	AUC (μg·h/ml)	0.174 (0.035)	0.048 (0.025)	1.101 (0.151)	$p<0.01$	C>A>B
High	C_{max} (ng/ml)	108.5 (14.1)	92.8 (25.7)	207.7 (34.8)	$p<0.05$	C>A>B
	AUC (μg·h/ml)	0.516 (0.086)	0.353 (0.064)	0.797 (0.161)	$p<0.05$	C>A>B

a) A, capsule A; B, capsule B; C, test capsule. *b*) Preparations underlined by a common line did not differ significantly ($p<0.05$). *c*) The figures in parentheses indicate the standard errors.

acidity, and the plasma levels of CN in humans with low acidity remain extremely low. Thus, for efficient therapeutics, it is desirable to develop a preparation of CN which can dissolve well in gastric contents with low acidity. Tokumura *et al.*[12] reported that the dissolution of CN at above pH 5 was enhanced by complexation with β-cyclodextrin and thereby the bioavailability of CN was improved at low acidity. We investigated the bioavailability for a test capsule containing citric acid, whose dissolution was improved in a low acidity medium.

Figure 5 shows the individual plasma concentration–time curves of CN. There was no significant difference between the high acidity group and the low acidity group with respect to the absorption profile, although one of the rabbits with low acidity showed extraordinarily high plasma levels. C_{max} and AUC also showed no significant difference between the groups, and the test capsule showed higher C_{max} and AUC than capsules A and B (Table I). From these results, the bioavailability of CN from the test capsule was found not to be affected by the gastric acidity, and it was enhanced by the citric acid. Consequently, we conclude that CN capsule containing citric acid is a promising preparation for clinical treatment in patients with

achlorhydria.

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