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Gastric acidity dependent bioavailability of cinnarizine from two commercial capsules in healthy volunteers

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Summary

Two commercially available capsules of 25 mg cinnarizine, which were chosen from the results of dissolution tests as the extreme cases out of 32 such capsules available in Japan, were examined in detail to determine their dissolution properties and bioavailabilities. The two capsules had similar dissolution rates at pH 1.2 and at pH 6.0 even though the rate at pH 1.2 was much faster than at pH 6.0. The serum levels of cinnarizine, which were determined by a GC-MS technique after administration of a single cinnarizine capsule, showed a wide variation in human volunteers, and this was ascribable to differences in the gastric acidity of the subjects. The $AUC_{0\text{-}8h}$ values of the two capsules in the subjects having low gastric acidity were only 27 and 14% of those in the high acidity group, and the C_{max} values of the two capsules were also depressed to 32 and 14% of those in the high acidity subject group, respectively. Bioavailability of cinnarizine appears to be determined mainly by the amount dissolved in the stomach.

Introduction

Cinnarizine, 1-benzhydryl-4-cinnamyl-piperizine, is a potent inhibitor of vasoconstrictor responses to various pharmacological stimuli by peripheral blood vessels (van Neuten and Janssen, 1972). Cinnarizine has been extensively tested both pharmacologically and clinically. However, little work has been done on its clinical pharmacokinetics. Morrison et al. (1979) determined plasma levels of cinnarizine using GC with a nitrogen-selective flame ionization detector after a single oral dose of 75 mg cinnarizine as capsules and tablets, and obtained values of 3.04–3.43 h for biological half-life and 160–230 ng/ml for peak concentration. A variable absorption of cinnarizine (19-fold difference of peak concentration and 8.6-fold difference of AUC between the highest and lowest values) was observed in different subjects. Hundt et al. (1980) applied an HPLC technique for cinnarizine assay in plasma, and obtained values of 77–89 ng/ml for peak concentration, and 4.4–5.3 h for plasma half-life after a single oral dose of 50 mg cinnarizine as tablets. A large difference between two tablets was observed in AUC. Cinnarizine capsules commercially available in Japan showed very variable dissolution as determined by the rotating basket method (25 rpm) at pH 2.0 (Akada et al., 1976; Tsuji et al., 1980).

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In our previous papers, the inter-subject differences in bioavailability of diazepam, a weak bases, like cinnarizine, were ascribed to the gastric acidity differences of the subjects (Ogata et al., 1982a and b). The gastric acidity-dependent bioavailability of diazepam was related to the pH-dependent dissolution from the formulations with especially slow dissolution rates at pH 3.9. Thus, the wide variations of cinnarizine absorption among subjects (Morrison et al., 1979) and between formulations (Hundt et al., 1980) may be caused by differences in dissolution properties (Akada et al., 1976; Tsuji et al., 1980) and/or by gastric acidity differences.

The present paper describes a comparison of the bioavailability of two commercial capsules of cinnarizine, the effect of gastric acidity on the bioavailability, and the correlation of the in vivo findings with the in vitro dissolution rates.

Experimental

Materials

A total of 32 different brand preparations of cinnarizine (25 mg) capsules were obtained from manufacturers in Japan. Standard cinnarizine was supplied by Eisai (Tokyo). Other reagents used were of analytical grade.

Solubility

The solubility of cinnarizine at pH values of 1.1 (HCl), 1.3 (0.2 M sodium acetate-HCl), 3.0 (0.2 M sodium phosphate dibasic–0.1 M citric acid), 4.9 (0.2 M sodium acetate–0.2 M acetic acid) and 6.9 (0.1 M sodium phosphate dibasic–0.1 M potassium phosphate monobasic) was determined at 37°C according to the procedure reported previously (Ogata et al., 1979a). The ionic strength of the medium was adjusted to 0.5 by adding potassium chloride. The absorbance of the drug was determined at 253 nm after diluting the sample solution with 4 N HCl.

Cinnarizine content

Ten capsules were analyzed for cinnarizine by weighing the contents, and dissolving the powder corresponding to 10 mg of cinnarizine in methanol. The solution was filtered, the filtrate was diluted with methanol, and the absorbance was determined at 253 nm.

In vitro dissolution study

The dissolution rate of cinnarizine from a capsule was determined at 37°C by the oscillating basket method with a disk (900 ml of medium, OB) (Ogata et al., 1979b) and the paddle method (900 ml of medium, 120 rpm, PD) (Ogata et al., 1984a) at pH values of 1.2 (the 1st fluid for disintegration test, JP X), 2.0 and 3.0 (0.2 M sodium acetate–0.2 N HCl) and 3.9, 5.0 and 6.0 (0.1 M acetate buffer). The procedure for the dissolution rate determination was the same as reported previously (Ogata et al., 1979b).

Bioavailability study

Subjects. Twelve healthy male volunteers (23–55 years old; mean age 37.2 years; 51–68 kg weight, mean 61,9 kg) participated in the study. The gastric fluid acidity of each subject was determined once with a few weeks before the bioavailability study using Gastrotest tablets (Chugai Pharmaceuticals, Tokyo), which contain a protein-bound dye (3-phenylazo-2,6-diaminopyridine) that is released in the stomach by acid at pH 3 or less (Bianchetti and Gerber, 1958). The amount of dye excreted in the urine within 90 min after oral administration of the tablets gives an indication of the gastric acidity. An absorbance of less than 0.170 at 520 nm was interpreted as indicating hypo- or anacidity, whereas an absorbance of more than 0.170 was taken as indicating normal or hyperacidity (Kiyama et al., 1960). The details of procedures were given previously (Ogata et al., 1982b). The subjects evaluated as hypo- or anacidic and as normal or hyperacidic by means of the Gastrotest were designated as low ($n = 8$) and high ($n = 4$) acidity subjects, respectively.

Treatment schedule. The bioavailability of cinnarizine from the two preparations was studied by using a randomized block design. Each subject received a capsule of cinnarizine (25 mg) orally with 200 ml of water with a two-week interval between doses. The subjects fasted from at least 10 h before and until 4 h after taking the drug. Blood samples (6 ml) were collected at 1, 2, 3, 4, 6 and 8

h after drug administration, and the serum samples were stored in a freezer (−15°C) until assayed.

Assay of cinnarizine in serum

Cinnarizine in serum was assayed by GC-MS. First, 2.0 ml of pH 6.5 phosphate buffer (0.5 M), 0.2 ml of papaverine-HCl (2.5 μg/ml) aqueous solution as an internal standard and 6.0 ml of carbon tetrachloride were added to 1.0 ml of serum. The mixture was shaken for 15 min and centrifuged, then 5 ml of the organic layer was taken and shaken with 5 ml of 1 N HCl for 10 min. After centrifugation, 4 ml of the aqueous phase was alkalized by addition of 1 ml of 5 N NaOH, and shaken with 5 ml of diethyl ether for 10 min. Then 4 ml of the organic layer was taken and evaporated to dryness under a nitrogen stream at 35°C. The residue was dissolved with 100 μl of ethanol. A sample of 5 μl of the ethanol solution was subjected to GC. A standard calibration curve was constructed by analysis of samples containing known amounts of cinnarizine (0–100 ng/ml of the drug in serum).

The samples were analyzed on a GC-MS instrument equipped for mass fragmentography (MID) (Hitachi M-80 mass spectrometer) with a data processing system (M-003 Hitachi data processing system). The gas chromatograph was equipped with a 3 mm × 1 m glass column packed with 3% OV-17 on Gas Chrom Q (100-120 mesh). The oven and injection temperatures were maintained at 280°C and 300°C, respectively. The flow rate of helium carrier gas was 50 ml/min. Electron impact energy was 20 eV.

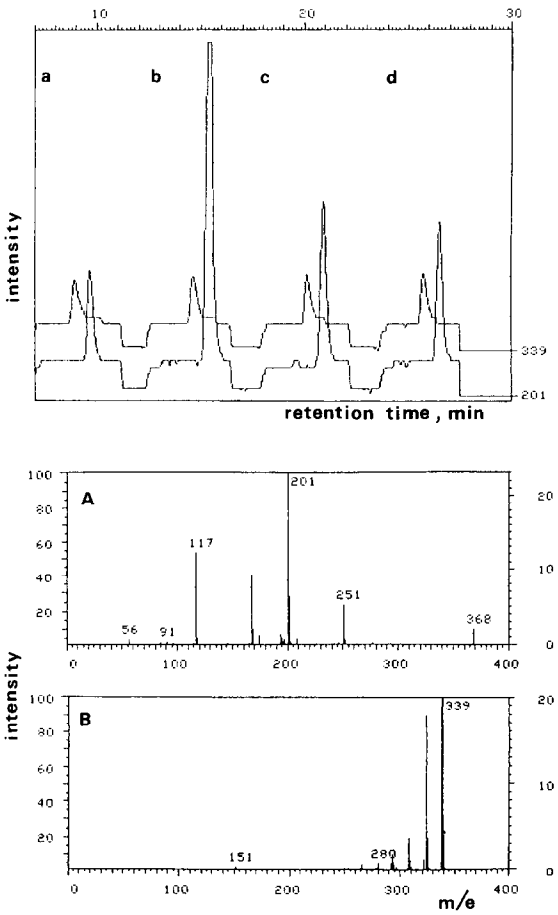

Fig. 1. A: chromatograms of serum samples. Cinnarizine was detected by mass fragmentography at m/e 201 and papaverine (internal standard) at m/e 339. a, serum spiked with 60 ng/ml cinnarizine; b, serum spiked with 250 ng/ml cinnarizine; c and d, serum samples at different times after oral administration of 25 mg cinnarizine. B: mass spectra of cinnarizine (A) and papaverine (B).

Results

Cinnarizine assay in serum

Fig. 1A shows a chromatogram obtained with a serum sample containing cinnarizine together with papaverine as an internal standard (500 ng/ml as papaverine-HCl); detection was carried out by mass fragmentography using the ions at m/e 201 and 339, respectively (Fig. 1B). Blank serum gave no interfering peaks on the chromatogram. The intensity ratio of the ions at m/e 201 and 339 gave a linear relationship when plotted against the concentration of cinnarizine (5–500 ng/ml, using the same pool of serum). The mean absolute recoveries of cinnarizine were 54.3% (n = 5; CV% = 7.0) from 25 ng/ml serum samples and 51.9% (n = 5; CV% = 4.8) from 100 ng/ml serum samples, although papaverine (internal standard) gave 42.8% mean recovery (n = 5; CV% = 3.6) from 500 ng/ml serum samples in the standard extraction procedure. The loss of cinnarizine was mainly due to the transfer of only a part of the volume of fluid; the

TABLE 1

SOLUBILITY OF CINNARIZINE AT 37°C

pH	Solubility (μg/ml)
1.1	1549.5
1.3	853.0
3.0	52.3
4.9	8.8
6.7	2.2

extraction of cinnarizine was almost complete. The lower limit of the assay was 1 ng/ml of cinnarizine in the serum.

Solubility of cinnarizine

Cinnarizine is a weak base which dissolves very easily at pH 1, but has a solubility of less than 10 μg/ml at more than pH 5, as shown in Table 1.

In vitro dissolution rate

We tested 32 commercial capsules of cinnarizine (25 mg) at pH 1.2 by the OB method and at pH 3.9 by the OB and PD methods (Fig. 2). At pH 1.2 cinnarizine dissolved very rapidly from all of the preparations tested, and the range of T_{50} (time required for 50% dissolution) values was very narrow, 1.6–3.7 min. On the other hand, a wide variation in cinnarizine dissolution rates was observed at pH 3.9 in both test methods. Capsules A and B represent the extremes of the dissolution rates among the preparations tested. Therefore, capsules A and B were selected as test preparations for further detailed studies on the relation

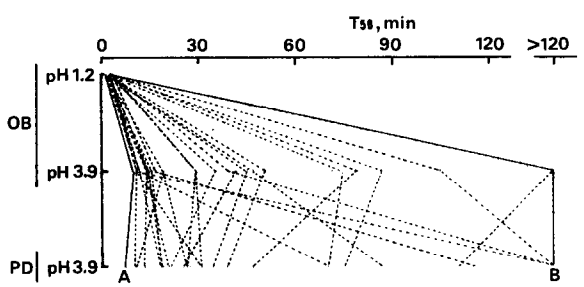

Fig. 2. Cinnarizine dissolution rates from 32 commercially available capsules determined by two methods in two media. Solid lines show dissolution rates of capsules used for the bioavailability test.

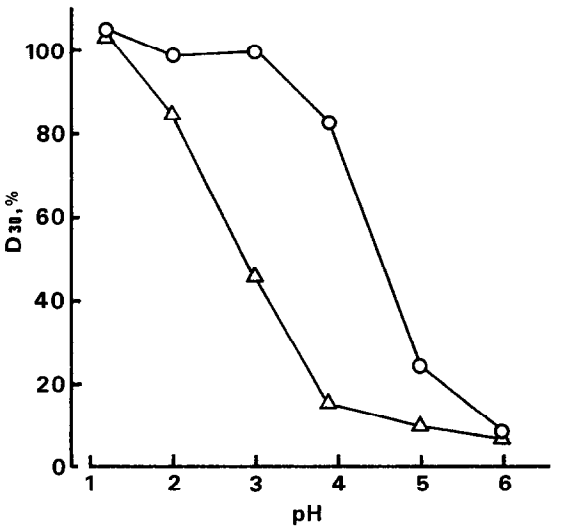

Fig. 3. Dissolution rate–pH profile for capsules A (○) and B (△).

between in vitro dissolution and in vivo bioavailability.

The dissolution study was repeated on capsules A and B at various pH values by using the OB method. Fig. 3 shows the effect of pH on the dissolution rates, represented by D_{30} (% dissolved in 30 min). The two preparations had similar dissolution rates at pH 1.2 and at pH 6.0 but at the different pHs the rates were markedly different. The high solubility of cinnarizine at pH 1.2 (1.5 mg/ml) and the very low solubility (2.2 μg/ml) at pH 6.9 may be a major reason for the similar dissolution rates of capsules A and B in spite of the large difference of dissolution characteristics between the two capsules as shown over pH 2–5.

Drug content

Cinnarizine contents were 96.7 and 103.8% of the labeled amount in capsules A and B, respectively.

Bioavailability

The individual serum levels obtained after oral administration of two cinnarizine capsules were very variable (2.4–110.7 ng/ml for peak concentration, C_{max} and 4.7–573.6 ng · h/ml for area under serum concentration–time curve from 0 to 8

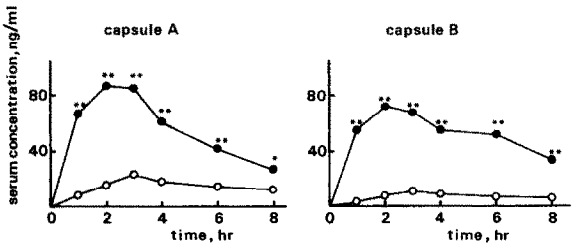

Fig. 4. Mean serum levels of cinnarizine administered as a capsule to subject groups having high gastric acidity (●) and low gastric acidity (○). * and ** represent significant differences from the serum concentration of the low gastric acidity group at the same sampling time with $P < 0.05$ and $P < 0.01$, respectively.

h calculated according to the trapezoidal rule, AUC_{0-8h}) just the same as reported by Morrison et al. (1979). However, the quite remarkable different levels were observed between subjects groups having high and low gastric acidities.

Fig. 4 shows mean concentrations of cinnarizine after oral administration of two capsules to each subject group, and Table 2 shows the mean C_{max}, the time to the peak concentration (T_{max}) and AUC_{0-8h} in each subject group. As the serum levels showed very low values during the experiment in most of the subjects having low gastric acidity, the extrapolated value of AUC could not be calculated with reasonable accuracy. We therefore preferred the AUC_{0-8h} values for estimating the extent of bioavailability, which would have introduced error in estimating the extent of bioavailability with a possibly slight underestimation of AUC in the subjects having low gastric acidity.

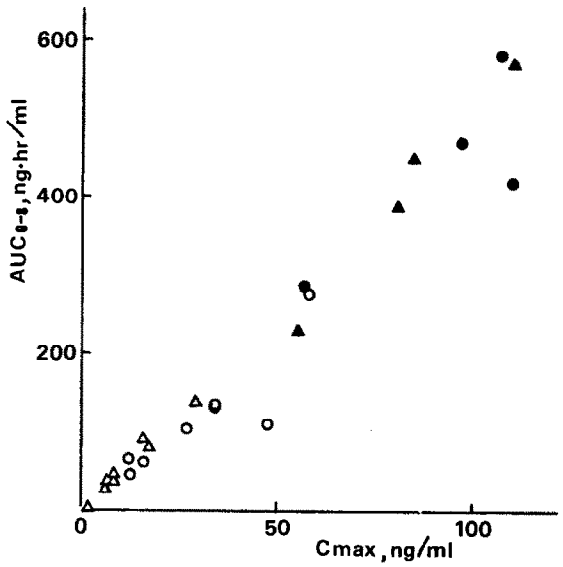

Fig. 5. Correlation of C_{max} and AUC_{0-8h} after oral administration of capsules A (●,○) and B (▲,△) to subject groups having high gastric acidity (filled symbols) and low gastric acidity (open symbols).

Results of ANOVA (analysis of variance) in the high and low gastric acidity groups, are also shown in Table 2. There was no significant difference between capsules A and B in any pharmacokinetic parameter in the high acidity group. However, capsule B showed significantly lower C_{max} and AUC than capsule A in the low gastric acidity group. As shown in Table 2 and Fig. 4, the two capsules showed significant differences in the pharmacokinetic parameters C_{max}, AUC_{0-8h} and

TABLE 2

PHARMACOKINETIC PARAMETERS OF CINNARIZINE AFTER ORAL ADMINISTRATION OF A CINNARIZINE (25 mg) CAPSULE TO SUBJECTS HAVING HIGH AND LOW GASTRIC ACIDITIES

Parameter	High acidity			Low acidity		
	Mean ± S.E.		ANOVA (formulation)	Mean ± S.E.		ANOVA (formulation)
	Capsule A	Capsule B		Capsule A	Capsule B	
C_{max} [a]	93.0 ± 12.3	82.9 ± 11.3	N.S. [d]	30.4 ± 5.9 [e]	12.0 ± 3.1 [e]	$P < 0.01$
T_{max} [b]	2.3 ± 0.3	2.3 ± 0.3	N.S.	3.6 ± 0.6	4.5 ± 0.7	N.S.
AUC_{0-8h} [c]	440.1 ± 61.4	411.9 ± 71.1	N.S.	117.7 ± 25.7 [e]	58.8 ± 15.4 [e]	$P < 0.01$

[a] ng/ml. [b] h. [c] ng · h/ml. [d] Not significant ($P < 0.05$).
[e] Significantly different from the high acidity group ($P < 0.01$) given the same capsule.

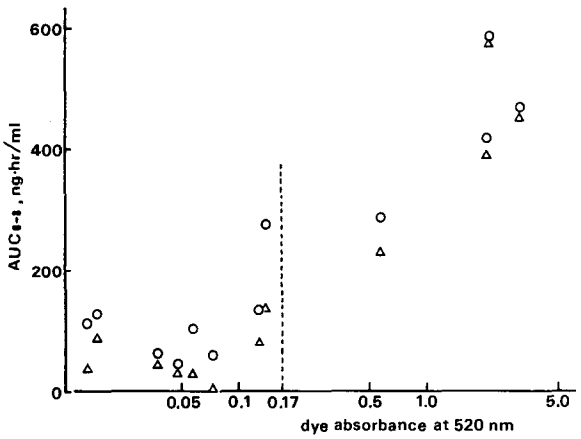

Fig. 6. Relationship between $AUC_{0\text{-}8h}$ after oral administration of capsules A (○) and B (△) and the logarithm of absorbance of dye excreted in urine after administration of Gastrotest tablets. A dye absorbance of 0.170 is the critical value for dividing subjects into high acidity and low acidity groups.

serum levels at each sampling time when the results in the subject groups having high and low gastric acidities were compared by means of the Student's t-test ($P < 0.05$).

Relationship between C_{max} and AUC and between AUC and gastric acidity

A linear relationship was found between individual values of C_{max} and $AUC_{0\text{-}8h}$ irrespective of gastric acidity or the preparation tested (Fig. 5) $AUC_{0\text{-}8h}$ was linearly correlated with the logarithm of absorbance due to dye excreted in urine after administration of Gastrotest tablets (used for evaluating gastric acidity) to the subjects having high gastric acidity (Fig. 6). However, the $AUC_{0\text{-}8h}$ values were small and not related to the logarithm of dye absorbance in the subjects having low gastric acidity. The relation between $AUC_{0\text{-}8h}$ and dye absorbance in urine was similar for both capsules A and B.

Discussion

The assay of cinnarizine in biological fluids has been performed by using GC with a nitrogen-selective flame ionization detector (Akada et al., 1976; Morrison et al., 1979), HPLC (Hundt et al., 1980; Nitsche and Mascher, 1982) and spectrophotometry (Dell and Fiedler, 1977). As cinnarizine undergoes extensive metabolism in humans (Morrison et al., 1979), a microassay for cinnarizine should cover the concentration range up to at most 100 ng/ml in serum for bioavailability studies. The GC-MS technique was successfully applied for cinnarizine assay. For complete extraction of cinnarizine from serum with carbon tetrachloride, the pH had to be adjusted to pH 5 or more, which is different from that used by Nitcher and Mascher (1982).

The gastric acidity of each subject was determined once with a few weeks before the bioavailability study using Gastrotest tablets. The gastric acidity evaluated by Gastrotest tablets was found to be very stable with few fluctuations of the evaluation for weeks and/or for years (unpublished data), which was very similar of the results obtained from the gastric acidity evaluation using GA-Test capsule (Ogata et al., 1984b).

Cinnarizine is a weak base and is very lipophilic in its unionized form as is clear from the solubility–pH profile (Table 1). Since 900 ml of medium was used for the dissolution test, the dissolution rate determination had to be carried out under non-sink conditions at pH 3 or more, and the content of a capsule (25 mg) was in excess of the solubility at pH 4 or more.

The serum levels of cinnarizine after a single oral dose of a 25 mg cinnarizine capsule showed enormous variations as reported by Morrison et al. (1979). It was clarified that the large variation of serum levels of cinnarizine is mainly ascribable to the gastric acidity variations of subjects. The bioavailabilities of capsules A and B, which showed remarkable differences of dissolution rates in the pH range of 3–4, were strongly dependent on the gastric acidity of the subjects. The $AUC_{0\text{-}8h}$ values of capsules A and B in the subjects having low gastric acidity were only 27 and 14% of those in the subjects having high gastric acidity, respectively. The C_{max} values of capsules A and B in the low acidity subjects were also depressed to 32 and 14% of those in high acidity subject group, respectively (Table 2).

As shown in Fig. 5, the relation between C_{max} and $AUC_{0\text{-}8h}$ for both the high and low gastric acidity groups was the same, suggesting that ab-

sorption of cinnarizine was determined by a common factor in both groups. The linear relationship between C_{max} and $AUC_{0\text{-}8h}$ shown in Fig. 5 suggests that the extent of cinnarizine bioavailability is determined simultaneously with C_{max}. $AUC_{0\text{-}8h}$ of the subjects having high gastric acidity was also related to absorbance of the dye excreted in urine after oral administration of Gastrotest tablets; this absorbance has been shown to be proportional to the acidity in the stomach (Hayashi et al., 1961). On the other hand, $AUC_{0\text{-}8h}$ of the subjects having low gastric acidity was very small and was independent of the dye absorbance (Fig. 6).

From these observations, we may speculate that the bioavailability of cinnarizine, C_{max} and $AUC_{0\text{-}8h}$ is determined by the amount dissolved in the stomach after oral administration, which is directly dependent on the gastric acidity of the subjects, and the drug is essentially unavailable after drug particles undissolved in the stomach are transferred to the small intestine because of the very low solubility under the conditions in the intestine.

The situation appears to be different from that in the case of diazepam, which is a weak base and showed similar gastric acidity-dependent bioavailability from plain tablets (Ogata et al., 1982b). A difference of diazepam bioavailability between the high and low gastric acidity groups had been found in terms of C_{max}, but not AUC, suggesting that diazepam is still available even after the undissolved drug particles are transferred to the small intestine. The discrepancy between the two drugs is probably ascribable to the relatively higher solubility, 50 μg/ml at pH 7, and lower dosage, 5 mg, of diazepam in comparison with cinnarizine. Furthermore, it is possible that cinnarizine is absorbed only from a restricted part of the intestine, though the absorption mechanism is unclear as yet.

From the speculation that the bioavailability of cinnarizine is determined by the amount dissolved in the stomach, it is desirable to develop a pharmaceutical means of increasing the amount of cinnarizine dissolved in the stomach in the subjects having low gastric acidity in order to overcome the gastric acidity-dependent bioavailability of cinnarizine and the consequent very poor bioavailability in such subjects.

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