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Bioavailability of Pyridoxal Phosphate from Enteric-Coated Tablets. II. Effects of Gastric Acidity of Humans

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The relationship between the gastric acidity of subjects and the bioavailability of pyridoxal phosphate from commercial enteric-coated tablets having various values of apparent critical pH for dissolution (above which the coated films disintegrate within 3 h) was studied. The tablet with an apparent critical dissolution pH of 5.5 appeared not to disintegrate in the stomach even in subjects with anacidity, and the bioavailability was not affected by the gastric acidity of the subjects. However, the bioavailability of the tablet with an apparent critical dissolution pH of 6.6 and a short lag for dissolution at that pH did depend on the gastric acidity of the subjects, and the higher the gastric acidity, the more slowly was the drug absorbed. On the other hand, the enteric-coated tablet with an apparent critical dissolution pH of 4.5 might be subject to disintegration in the stomach of subjects with anacidity.

Keywords——bioavailability; human; gastric acidity; apparent critical dissolution pH; pyridoxal phosphate; enteric-coated tablets; correlation

Human gastric acidity has been found to affect the bioavailabilities of some drugs in our laboratory recently.[1-3] Some formulations of diazepam showed significantly low C_{max} in subjects with low gastric acidity compared with that in subjects with normal or high acidity.[1] Some of the sugar-coated tablets of metronidazole, even though the drug is very soluble in water, also showed significantly inferior bioavailabilities in subjects with low gastric acidity as compared to those in subjects with normal gastric acidity, due to poor dissolution of the waterproof film.[2] The tablets mentioned above showed pH-dependent dissolution characteristics, with slow dissolution at pH 4 to 7.[1-3]

In the previous work,[4] we estimated the apparent critical dissolution pH (the lowest pH at which the enteric-coated tablets disintegrated) of commercial enteric-coated tablets of pyridoxal phosphate (I). The enteric-coated tablets with an apparent critical dissolution pH of 6.6 and a long lag in dissolution at that pH showed low bioavailabilities. Since an enteric-coated tablet should not disintegrate in the stomach but should disintegrate rapidly in the intestine, there may be an optimal critical dissolution pH for an enteric-coated tablet. In this study, the effect of the gastric acidity of subjects on the bioavailability of enteric-coated tablets of I was investigated.

Experimental

Formulations——Five lots of enteric-coated tablets of I (A, B, C, D and E), which were the same as those used in the previous study[4] were used. Their apparent critical dissolution pH values[4] were 6.6, 6.6, 6.6, 5.5 and 4.5, respectively by the oscillating basket method.[5] A sugar-coated tablet was also used (tablet F from the previous study).[4]

Lag Times for Dissolution——Dissolution tests of tablets of I were done at pH 1.2, 4.5, 5.5, 6.6 and 7.5 by the oscillating basket method.[5] The concentration of I in the medium was determined at 390 nm. Lag times for

dissolution of tablets C, D, E and F were less than 15 min, while those of tablets A and B were longer than 35 min.[4]

Gastric Acidity of Human Subjects——All subjects were evaluated for gastric acidity once by using a Gastrotest® tablet[1] within a few weeks before the bioavailability study. The urine excreted within 1.5 h after oral administration of a Gastrotest® tablet and the blank urine were diluted with water to 200 ml and aliquots were mixed with an equal volume of 25% hydrochloric acid solution. The absorbance of the acidified urine was measured at 520 nm.

Bioavailability Test——Twelve subjects were divided into six groups, and a 6×6 latin square design was employed. After overnight fasting, subjects received a test tablet orally with 200 ml of water. Urine samples were collected at 1, 2, 3, 4, 5, 6, 7, 9, 11 and 22 h after administration, and stored frozen until assay. The excretion rate of pyridoxic acid (II), one of the metabolites of I, at time t (V_t) was corrected by subtracting the basal excretion rate of II.[4] V_{max} and T_{max} were the peak excretion rate corrected by subtracting the basal excretion rate and the time to reach V_{max}, respectively, and they were observed values. Cumulative urinary excretion of II through 22 h (E_{22}) was also corrected by subtracting the basal excretion of II. T_{lag} was calculated by fitting the urinary data to the apparant two-compartment model using MULTI,[6] a program for the nonlinear least-squares method for a microcomputer. Mean residence time (MRT) was calculated by means of the trapezoidal rule.[7]

Assay——Three ml of water and 0.5 ml of 1.5 N ammonium hydroxide were added to 1 ml of the urine. Four milliliters of the supernatant was applied to a Dowex 1×8 column, then II was eluted with 50 ml of 0.1 N hydrochloride and the effluent was passed directly into an Amberlite CG-120 column. This column was eluted with 2 N hydrochloride, and II in the effluent was lactonized with 4 N hydrochloride in a boiling water bath. After adjustment of the pH to between 9 and 11 using 0.4 M sodium carbonate, fluorescence was measured at 430 nm with excitation at 356 nm.[4]

Results and Discussion

The Gastrotest® tablet contains 3-phenylazo-2,6-diaminopyridine bound to a protein, and the dye is released depending on the amount of hydrochloric acid secreted in the stomach; the released dye is absorbed from the intestine and rapidly excreted in the urine.[8] Consequently, the more hydrochloric acid is secreted in the stomach, the deeper red the urine is. The absorbance values of the acidified urine of the subjects found to secrete little or no acid by a titration method were under 0.17.[9] The subjects who participated in the bioavailability test were divided into two groups according to the urine absorbance, *i.e.*, an absorbance of the acidified urine of less than 0.170 at 520 nm was interpreted as indicating hypoacidity or anacidity (low acidity group) and an absorbance of more than 0.170 as indicating normal acidity or hyperacidity (high acidity group). Table I shows the age, height and weight of the subjects tested, and the absorbance of the acidified urine at 520 nm after administration of a Gastrotest® tablet. Two subjects in the low acidity group were evaluated as anacidic by the use of a color scale provided with the Gastrotest® (Table I). Since the gastric acidity estimated

Table I. Age, Height, Weight and Gastric Acidity of Subjects

Subject	Age (year)	Height (cm)	Weight (kg)	Absorbance at 520 nm	Gastric acidity
1	21	180	69	2.003	High
2	30	163	55	0.137	Low
3	23	170	63	0.969	High
4	51	168	68	0.048	Low[a]
5	22	179	65	0.740	High
6	39	170	55	0.141	Low
7	22	166	57	1.425	High
8	23	175	60	0.402	High
9	23	175	60	0.637	High
10	52	160	54	0.015	Low[a]
11	38	165	69	1.516	High
12	33	172	61	0.155	Low

a) Evaluated as anacid according to a color scale provided with the Gastrotest®

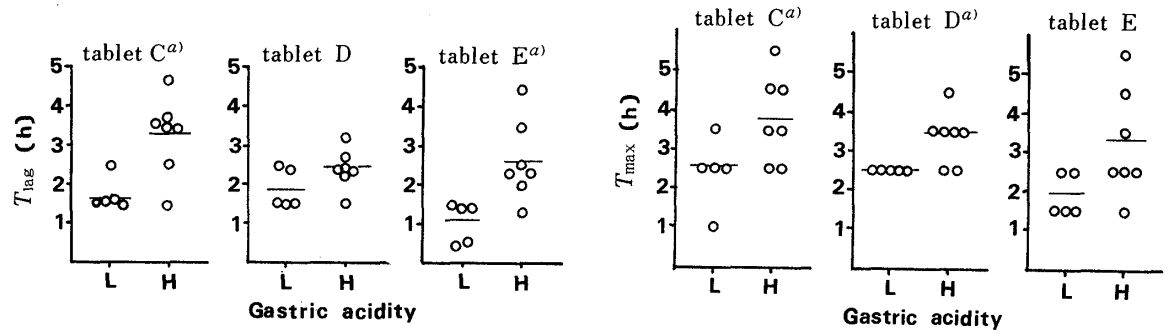

Fig. 1. The Effects of Gastric Acidity on T_{lag} and T_{max}

L, low gastric acidity group; H, normal gastric acidity group; vertical line, mean. *a*) Significant difference between low and high gastric acidity groups.

by using the Gastrotest® method showed good reproducibility in the same subjects in successive tests for four years (unpublished data), the evaluation of the gastric acidity was done once within a few weeks before the bioavailability study.

Bioavailability parameters were compared by using Student's *t*-test between the high and low gastric acidity groups after the administration of tablets C, D and E (Table II and Fig. 1), whose apparent critical dissolution pH values were 6.6, 5.5 and 4.5, respectively. Though absorption of I appeared to be faster in the low gastric acidity group for all tablets, the differences in excretion rates of II at 1.5 and 2.5 h ($V_{1.5}$ and $V_{2.5}$) between the two groups were larger in tablets C and E than in tablet D. The lag time for excretion of II (T_{lag}) was significantly shorter in the low gastric acidity group with tablets C and E. The delayed absorption in the high gastric acidity group after the administration of tablets C and E did not lead to a significant difference in the extent of bioavailability, since no significant difference was detected in cumulative urinary excretion through 22 h (E_{22}).

Correlation coefficients between the logarithm of dye absorbance at 520 nm after Gastrotest® administration and bioavailability parameters of the formulations were calculated and are summarized in Table III, though the absorbance of the acidified urine does not linearly correlate with the amount of acid secreted in the stomach because of the dissociation characteristics of the dye,[8] the variation of transit rate of the free dye in the digestive tract and the clearance of the dye.[10] Significant correlation coefficients were observed in peak II excretion rate, T_{lag}, $V_{2.5}$ and E_{22} for tablet C, which also indicate that the higher the gastric acidity of subjects, the lower the rate and extent of bioavailability of I were in the case of tablet C.

Though the correlations between the gastric acidity and the parameters of bioavailability of tablet E (apparent critical dissolution pH 4.5) were not significant, the correlation coefficients were larger than those obtained for tablet D. The bioavailability of tablet D, however, which had a lower apparent critical dissolution pH (5.5) than tablet C, did not correlate significantly with gastric acidity; the correlation coefficients were very low.

The results of *t*-test and correlation analysis showed that the absorption of I from tablets C and E was faster in the subjects with low gastric acidity than in the subjects with high gastric acidity. The excretion rate–time curves after the administration of enteric-coated tablets C, D and E were compared with those of the sugar-coated tablet F in the anacidity, and low and high gastric acidity groups. Since the excretion rate–time curves of tablet E differed from those of tablet F in the low and high gastric acidity groups, I probably does not dissolve in the stomach of subjects in both groups. On the other hand, the curves were almost superimposed in the anacidity group, suggesting that I in the enteric-coated tablet with the apparent critical dissolution pH of 4.5 dissolved in the stomach of the subjects with anacidity, and was

TABLE II. Mean Bioavailability Parameters of Pyridoxal Phosphate in High and Low Gastric Acidity Groups after Oral Administration of Tablets C, D and E

Parameter	Tablet C		
	Low	High	*t*-test
$V_{1.5}$ (mg/h)	0.470±0.824	0.000±0.000	n.s.
$V_{2.5}$	1.427±0.999	0.478±0.583	$p<0.01$
V_{max} (mg/h)	1.685±0.909	1.222±0.307	n.s.
T_{lag} (h)	1.68 ±0.44	3.20 ±0.99	$p<0.01$
T_{max} (h)	2.50 ±0.71	3.79 ±1.10	$p<0.05$
MRT (h)	4.72 ±0.99	5.30 ±0.93	n.s.
E_{22} (mg)	3.167±1.109	2.372±0.441	n.s.

Parameter	Tablet D		
	Low	High	*t*-test
$V_{1.5}$ (mg/h)	0.080±0.071	0.028±0.060	n.s.
$V_{2.5}$	0.948±0.346	0.532±0.463	n.s.
V_{max} (mg/h)	0.948±0.346	0.794±0.393	n.s.
T_{lag} (h)	1.86 ±0.51	2.35 ±0.49	n.s.
T_{max} (h)	2.50 ±0.00	3.40 ±0.69	$p<0.05$
MRT (h)	4.87 ±0.46	7.36 ±3.06	n.s.
E_{22} (mg)	2.613±0.287	2.674±0.913	n.s.

Parameter	Tablet E		
	Low	High	*t*-test
$V_{1.5}$ (mg/h)	1.193±0.403	0.273±0.428	$p<0.05$
$V_{2.5}$	0.879±0.627	0.671±0.624	n.s.
V_{max} (mg/h)	1.377±0.223	1.188±0.367	n.s.
T_{lag} (h)	1.03 ±0.49	2.60 ±1.04	$p<0.05$
T_{max} (h)	1.90 ±0.55	3.20 ±1.38	n.s.
MRT (h)	3.52 ±1.18	5.23 ±0.86	$p<0.05$
E_{22} (mg)	3.135±0.465	2.764±0.642	n.s.

Values are means±S.D. n.s., not significant at $p=0.05$. *MRT*, mean residence time.

absorbed as fast as I from the sugar-coated tablet (Fig. 2).

As shown in Fig. 3, however, it could not be considered that I in tablet C dissolved in the stomach even in the subjects with anacidity, since II was excreted later after the administration of tablet C than after that of tablet F in the anacidity group.

As shown in Fig. 4, the excretion rate–time curves of II after administration of tablet D (apparent critical dissolution pH 5.5) were distinctly different from those of the sugar-coated tablet in the three groups; absorption of I from tablet D was slower than from tablet F. Further, the bioavailability of tablet D did not depend on the gastric acidity of the subjects (Table III). These results suggest that the tablet with an apparent critical dissolution pH of 5.5

TABLE III. Correlation Coefficients between Gastric Acidity[a] and Bioavailability of Pyridoxal Phosphate

Parameter	Tablet					
	A	B	C	D	E	F
V_{max}	0.355	−0.016	−0.578[b]	−0.093	−0.172	0.136
T_{lag}	—[c]	—[c]	0.680[b]	0.318	0.459	−0.254
T_{max}	—[c]	—[c]	0.547	0.527	0.323	−0.119
MRT	—[c]	—[c]	0.481	0.245	0.470	0.471
$V_{1.5}$	0.377	−0.418	−0.307	−0.218	−0.525	0.044
$V_{2.5}$	0.458	−0.257	−0.781[b]	−0.121	−0.204	−0.239
E_{22}	0.160	−0.207	−0.627[b]	−0.030	−0.450	0.100

a) Gastric acidity was represented by the logarithm of absorbance at 520 nm. *b*) Significant at $p=0.05$. *c*) Not calculated because no absorption due to the drug was detected in several subjects.

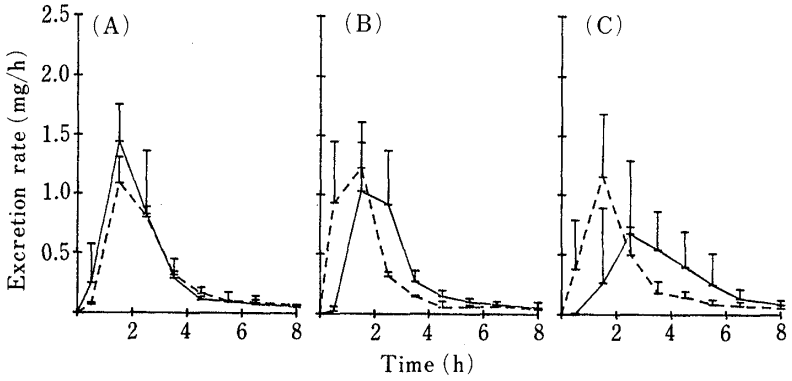

Fig. 2. Urinary Excretion Rate Profiles of Pyridoxic Acid after Oral Administration of Tablets E and F in Various Gastric Acidity Groups

Solid line, tablet E (an enteric-coated tablet with apparent critical dissolution pH of 4.5); dotted line, tablet F (a sugar-coated tablet); vertical line, standard deviation.
(A), anacidity ($n=2$); (B), low acidity ($n=3$); (C), high acidity ($n=7$).

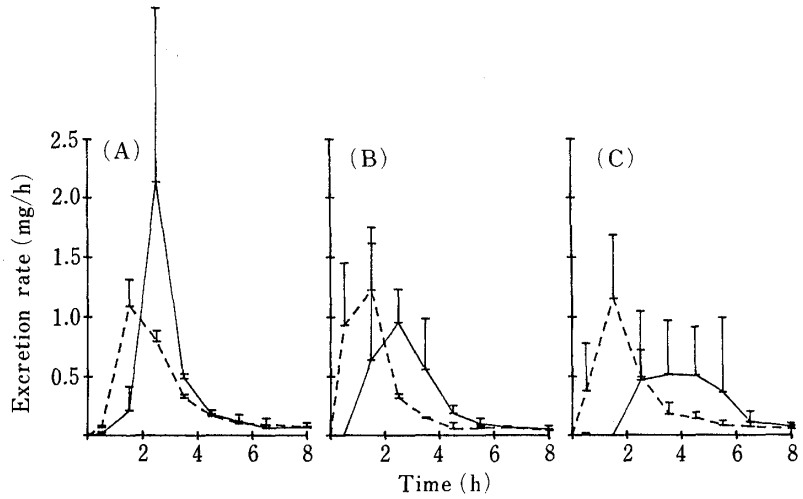

Fig. 3. Urinary Excretion Rate Profiles of Pyridoxic Acid after Oral Administration of Tablets C and F in Various Gastric Acidity Groups

Solid line, tablet C (an enteric-coated tablet with apparent critical dissolution pH of 6.6); dotted line, tablet F (a sugar-coated tablet); vertical line, standard deviation.
(A), anacidity ($n=2$); (B), low acidity ($n=3$); (C), high acidity ($n=7$).

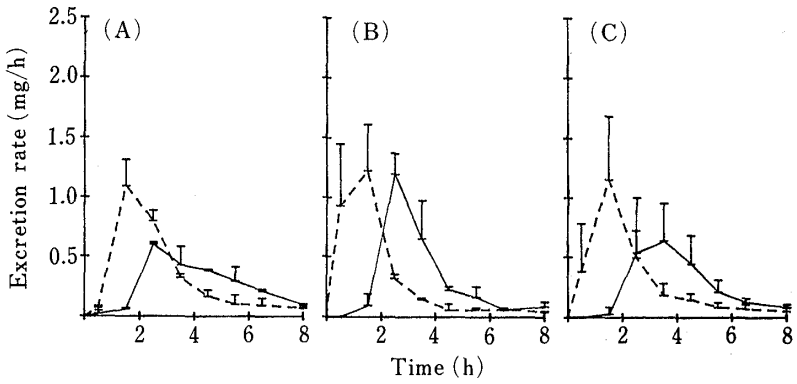

Fig. 4. Urinary Excretion Rate Profiles of Pyridoxic Acid after Oral Administration of Tablets D and F in Various Gastric Acidity Groups

Solid line, tablet D (an enteric-coated tablet with apparent critical dissolution pH of 5.5); dotted line, tablet F (a sugar-coated tablet); vertical line, standard deviation.
(A), anacidity ($n=2$); (B), low acidity ($n=3$); (C), high acidity ($n=7$).

does not disintegrate in the stomach at any level of gastric acidity, but disintegrates rapidly after reaching the upper part of the intestine, because the pH of the upper part of the intestine should be low enough for tablet D to disintegrate even in the normal and high gastric acidity groups, (probably near 5.5 to 6.6).

Using a radiocapsule for gastro-intestinal pH measurement, Shibuya reported that the pH values of the empty stomach in thirty subjects varied from less than 2 to 5.5.[11] The results of bioavailability tests on tablets E and D suggest that the pH of the empty stomach in the anacidity group was higher than 4.5 but not higher than 5.5, while that that in the high gastric acidity group was lower than 4.5. These results raise the possibility of dissolution of drugs in enteric-coated tablets having a critical dissolution pH of 4.5 in the stomach of patients with anacidity.

Shibuya found in fifty-one subjects that the pH values at the entrance of the duodenum varied from 3.5 to 7.5, and those at the entrance of the jejunum from 4 to 7.[11] The pH values at the duodenum were also reported to vary from 2.7 to 6.6.[12] Since the gastric fluid flows into the intestine intermittently, the pH at the upper part of the intestine must be affected by the pH of gastric fluid. These data indicate that the pH of the upper part of the intestine of subjects with high gastric acidity might be relatively low. This is presumably the reason why absorption of I from tablet C (apparent critical dissolution pH 6.6) was delayed in the subjects showing high gastric acidity. On the other hand, pH lower than 5.5 cannot be maintained for long even in the high gastric acidity group, because absorption of I from tablet D (apparent critical dissolution pH 5.5) was not affected by the gastric acidity.

It is not surprising that the bioavailability of the sugar-coated tablet F did not depend on gastric acidity, since it showed pH-independent dissolution.[4] The low bioavailability of tablets A and B which had a high apparent critical dissolution pH (6.6) and long lag times for dissolution at pH 6.6 did not depend on the gastric acidity of subjects. Both tablets probably pass through the absorption segment without disintegration in some subjects, because a long time is required for disintegration at higher pH than 6.6. Whether these tablets are available *in vivo* or not may be determined by the transit rate in the digestive tract rather than by the gastro-intestinal pH.

Though the amount of I absorbed from the enteric-coated tablets did not differ between the high and low acidity groups (Table II), the rate of bioavailability was affected by the gastric acidity. Enteric-coated tablets are designed to protect the mucosa of the stomach from irritation by drugs, or to protect drugs from degradation by acid or enzymes secreted in the

stomach. On the other hand, drugs in enteric-coated tablets should be dissolved and absorbed rapidly once the tablets are transferred into the intestine. The drug may dissolve in the stomach of a patient with anacidity when an enteric-coated tablet with a critical dissolution pH of 4.5, such as table E, is administered. In this case, the drug may be degraded enzymatically or may irritate the stomach mucosa, even if it is not degraded by acid catalysis. On the other hand, drug absorption from an enteric-coated tablet with a critical dissolution pH 6.6, despite the short lag time, may be delayed in subjects with high gastric acidity, even though complete absorption occurs. Complete drug absorption cannot be guaranteed when an enteric-coated tablet with a critical dissolution pH of 6.6 and having a long lag time is administered.

Conclusion

The enteric-coated tablets which showed short lag times in dissolution at pH 6.6 (tablets C, D and E) were estimated to be bioequivalent with a sugar-coated tablet in the previous study.[4] However, I is likely to dissolve in the stomach of patients with anacidity when administered in an enteric-coated tablet with a critical dissolution pH of 4.5. On the other hand, the absorption of I is likely to be delayed in subjects with high gastric acidity from an enteric-coated tablet with a critical dissolution pH of 6.6, even though there is a short lag in dissolution at that pH. It can be concluded that in order to get full and rapid absorption of a drug from an enteric-coated tablet irrespective of the gastric acidity of the subjects, a critical dissolution pH of 5.5 is most appropriate.

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