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# Bioavailability of Pyridoxal Phosphate from Enteric-Coated Tablets. I. Apparent Critical Dissolution pH and Bioavailability of Commercial Products in Humans

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The critical dissolution pH values for forty-eight enteric-coated tablets of pyridoxal phosphate (PAL-P) commercially available in Japan were determined by using an oscillating basket apparatus with buffers of various pH. The tablets were divided into three groups according to their critical dissolution pH (pH <4.5, 4.5—5.5 and 5.5—6.6). The bioavailabilities of five enteric-coated and a sugar-coated tablets of PAL-P were studied in humans by measuring 4-pyridoxic acid (PiA), one of the main metabolites of PAL-P, in the urine. Two enteric-coated tablets whose critical dissolution pH values were 4.5—5.5 and <4.5, and one enteric-coated tablet having a critical dissolution pH of 5.5—6.6 and showing a relatively short lag in dissolution at this pH were bioequivalent with the sugar-coated tablet, though the absorption of the drug from the enteric-coated tablets was significantly delayed. However, the other two enteric-coated tablets having long lags in dissolution at their critical dissolution pH of 5.5—6.6 were found to show inferior bioavailabilities in both rate and extent.

**Keywords**——bioavailability; pyridoxal phosphate; enteric-coated tablets; sugar-coated tablets; human; apparent critical dissolution pH

In 1964, Levy and Hollister found that the physiological availability of aspirin from an enteric-coated tablet was low, though the tablet satisfied the official USP disintegration test for enteric-coated tablets.[1] An enteric-coated tablet of aminosalicyclic acid, which satisfied all USP specifications, was found intact in the feces.[2] Enteric-coated tablets of erythromycin which did not dissolve at pH below 6.5 *in vitro* were bioinequivalent with other enteric-coated tablets,[3] and enteric-coated tablets which did not disintegrate *in vitro* at pH 6 did not disintegrate in the intestine rapidly,[4] though all of them met the JP disintegration test. The pH values of the media used in the USP (<XIX) and JP (<IX) disintegration tests were higher than 7.0. Thus, the reports mentioned above showed that some concern was justified regarding the pH values of the disintegration or dissolution test media, and the critical dissolution pH values of enteric coated tablets.

In this study, we determined the apparent critical dissolution pH of forty-eight enteric-coated tablets of pyridoxal phosphate (PAL-P) commercially available in Japan, and studied the relationships of the bioavailabilities of PAL-P in humans and the critical dissolution pH, using some of these enteric-coated tablets and a sugar-coated tablet.

## Experimental

**Materials**——Forty-eight enteric- and five sugar-coated tablets of PAL-P (10 mg) were obtained as commercial

products in Japan. 4-Pyridoxic acid (PiA) was synthesized from pyridoxal (PAL) according to the method of Heyl.[5] All other chemicals were of reagent grade.

**Determination of Apparent Critical Dissolution pH**——The dissolution rate of PAL-P from the enteric-coated tablets was measured by following the absorption at 390 nm, using the oscillating basket method.[6] The dissolution media used were as follows: pH 1.2, 1st fluid (J.P. IX); pH 4.5 and 5.5, 0.1 M acetate buffer; pH 6.6, 0.1 M phosphate buffer and pH 7.5, 2nd fluid (J.P. IX). The lowest pH at which an enteric-coated tablet dissolved within 3 h was designated as its apparent critical dissolution pH.

**Infrared (IR) Spectra of Coating Films**——Water-insoluble coating films were dissolved in a mixture of chloroform and methanol (1 : 1, v/v), the mixture was spotted on a warmed KBr tablet, and the IR spectrum was determined (IRA-102, Japan Spectroscopic Co., Ltd., Tokyo). IR spectra of authentic cellulose acetate phthalate (CAP), 2-methyl-5-vinylpyridine-methylacrylate-methacrylic acid co-polymer (MPM-47®) and purified shellac were also determined.

**Bioavailability Test**——Twelve male subjects (aged 21 to 52, average 31 years old, and weighing 54 to 69, average 61 kg) were divided into six groups and a $6 \times 6$ latin square design was employed for five enteric-coated tablets and one sugar-coated tablet of PAL-P. A washout period of a week was allowed between treatments. After overnight fasting, subjects received a tablet of PAL-P with 200 ml of water, and urine samples were collected at 1, 2, 3, 4, 5, 6, 7, 9, 11 and 22 h after the administration and stored frozen until assay.

**Relationship between Dose and Excreted Amount of PiA**——Three different doses (5, 10, 15 mg) of PAL-P in aqueous solutions were given orally to five subjects (other than the participators in the bioavailability test) in the fasting state by a three-way cross-over design, and urine samples were collected.

**Correlation between Blood Levels of PAL and the Excretion Rate of PiA**——Two subjects each received an enteric-coated tablet of PAL-P and blood was sampled at the mid points between the urine collecting times. Blood samples were treated with trichloroacetic acid[7] and stored frozen until assay.

**Assay**——PAL in human whole blood was determined by the method of Masukawa *et al.*[7] PiA in the urine was determined fluorometrically by the method of Reddy *et al.*[8] using an Amberlite CG-120 column instead of a Dowex 50 column. Tablets were analyzed for PAL-P by grinding five tablets to powder and dissolving the powder in 50 ml of 0.1 M $KH_2PO_4$ containing 0.1 mg/ml nicotinamide as an internal standard. Then 5 $\mu$l of supernatant was injected into the HPLC and PAL-P was detected at 254 nm. Column, 3.9 i.d. $\times$ 300 mm $\mu$Bondapak $C_{18}$; mobile phase, methanol–0.1 M $KH_2PO_4$ (1 : 9, v/v); flow rate, 2 ml/min.

**Statistical Analyses**——PiA is constantly excreted as one of the metabolites of the vitamin $B_6$ group. The basal excretion rates (44 to 62 $\mu$g/ml), which were observed during the lag time after administration of an enteric-coated tablet, were subtracted from the observed excretion rates of PiA for estimating the bioavailability. Excretion rates of PiA at the mid points between urine sampling times ($V_t$), cumulative amounts of urinary excretion of PiA to infinity ($E_\infty$), peak excretion rate ($V_{max}$), time to reach peak excretion rate ($T_{max}$), mean residence time ($MRT$),[9] and lag time in excretion ($T_{lag}$) were estimated. The $E_\infty$ values were calculated by the method of Wagner[10] and $T_{lag}$ was calculated by fitting the data to an apparent two-compartment model by the nonlinear least-squares method (MULTI) using a microcomputer.[11] These parameters were statistically subjected to analysis of variances (ANOVA), and the differences among the formulations were examined by the least-significant-difference method.[12] ANOVA and multiple range tests for a randomized block design instead of for a latin square were performed for $T_{lag}$, $T_{max}$ and $MRT$ among tablets C, D, E and F, because some subjects showed no absorption of PAL-P from tablets A and B.

## Results

### Apparent Critical Dissolution pH of Commercial Enteric-Coated Tablets of PAL-P

None of the enteric-coated tablets dissolved in the J.P. IX disintegration test medium No. 1 (pH 1.2) in 2 h. Forty-eight commercially available enteric-coated tablets of PAL-P were divided into 3 groups according to their apparent critical dissolution pHs, 6.6, 5.5 and 4.5. Most of the enteric coated tablets (39 products) belonged to the second group, three products to the first group and six products to the third group. Five enteric-coated tablets (A, B, C, D and E, Table I) and a sugar-coated tablet F were chosen according to their apparent critical dissolution pHs and lag times in dissolution for bioavailability studies.

### IR Sepctra of Coating Films

CAP and MPM-47® in tablet A, shellac and CAP in tablet B, and CAP in tablets C and D were identified. No characteristic absorption spectra were obtained in the case of tablet E.

### Correlation between Blood Level of PAL and Excretion Rate of PiA

It is not known whether after oral administration of PAL-P it is absorbed intact or as

TABLE I. Classification of Commercially Available Pyridoxal Phosphate Tablets According to Their Apparent Critical Dissolution pH and Time Lag in Dissolution

| Product | Content of PAL-P (mg)[b] | Apparent critical dissolution pH | Lag in dissolution (min)[a] pH | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1.2 | 4.5 | 5.5 | 6.6 | 7.5 |
| A | 11.03 | 6.6 | × | × | × | 87 | 28 |
| B | 12.00 | 6.6 | × | × | × | 35 | 21 |
| C | 12.30 | 6.6 | × | × | × | 15 | 9 |
| D | 10.10 | 5.5 | × | × | 5 | 5 | 14 |
| E | 10.38 | 4.5 | × | 21 | 7 | 8 | 7 |

*a*) The oscillating basket method was used. *b*) Determined by the high performance liquid chromatography (HPLC) method. ×, The drug did not dissolve within 3 h.

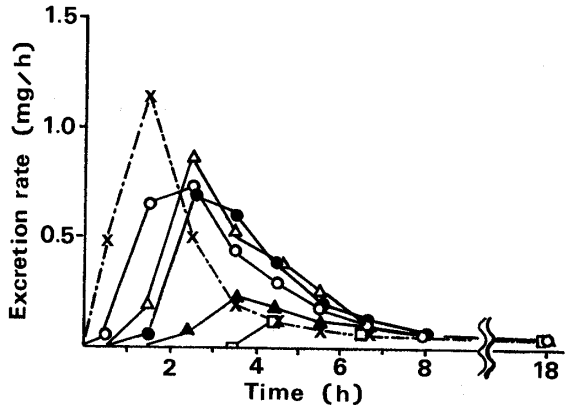


Fig. 1. Average Excretion Rate Curves of Pyridoxic Acid after Oral Administration of Pyridoxal Phosphate Tablets

▲, tablet A; □, tablet B; △, tablet C; ●, tablet D; ○, tablet E; ×, tablet F.

Tablets A, B, C, D and E were enteric-coated tablets and tablet F was a sugar-coated tablet. The results of multiple range tests for $V_{0.5}$ and $V_{1.5}$ were as follows: $V_{0.5}$, F>E>C>D>B>A; $V_{1.5}$, F>E>C>D>A>B.

PAL, the hydrolyzed product of PAL-P, in the human digestive tract. In rats and rabbits, PAL-P was found to be absorbed as PAL.[13]

After oral administration of PAL-P, blood levels of PAL and urinary excretion rates of PiA were significantly correlated in two subjects ($r=0.846$ and $r=0.906$, respectively). Therefore bioavailability of PAL-P could be determined by measuring urinary excretion rates of PiA nearly as precisely as by measuring PAL in blood.

**Relationship between Dose and $E_\infty$**

The values of $E_\infty$ for PiA after oral administration of 5, 10 and 15 mg of PAL-P in solution were 1.373, 2.741 and 5.503 mg, respectively. The bioavailability was linearly related to dose up to 10 mg.

**Bioavailability Test**

The results of bioavailability tests of six formulations are summarized in Table II and Fig. 1. The values in Table II were corrected for the content of PAL-P. The results of ANOVA for $E_\infty$ indicate that equivalent amounts of PAL-P were absorbed from tablets C, D and E and the sugar-coated tablet F. On the other hand, in four subjects among twelve, PAL-P was not absorbed at all from tablets A and B. The average $E_\infty$ values after the administration of tablets A and B were less than 50% of those after other tablets, and $V_{max}$ values after administration of tablets A and B were significantly lower than those after other enteric-coated tablets.

The results of ANOVA and multiple range tests for $T_{lag}$, $V_{0.5}$ and $V_{1.5}$ showed significantly delayed absorption of PAL-P from enteric-coated tablets C, D and E compared

TABLE II. Bioavailability Parameters of Pyridoxal Phosphate Enteric-Coated Tablets and Results of ANOVA and Multiple Range Tests

| Parameter | Tablet | | | | | | Result of ANOVA | Multiple range test[a] |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | | |
| $V_{max}$[b] (mg/h) | 0.343 | 0.291 | 1.428 | 0.859 | 1.250 | 1.236 | $p<0.01$ | C>E>F>D>A>B |
| $E_{\infty}$[b] (mg) | 1.393 | 1.319 | 2.734 | 2.756 | 2.968 | 2.995 | $p<0.01$ | F>E>C>D>A>B |
| $T_{lag}$ (h) | 3.80[c] | 7.55[c] | 2.56 | 2.12 | 1.95 | 0.43 | $p<0.01$[d] | F<E<D<C |
| $T_{max}$ (h) | 6.69[c] | 9.25[c] | 3.25 | 3.00 | 2.67 | 1.33 | $p<0.01$[d] | F<E<D<C |
| $MRT$ (h) | 8.43[c] | 11.93[c] | 5.06 | 6.33 | 4.52 | 3.45 | $p<0.01$[d] | F<E<C<D |

*a*) The multiple range test was done by the least significant difference method. The mean values for treatments not underscored by the same line differ significantly. *b*) Values were corrected for the content of pyridoxal phosphate in each tablet; corrected value = observed value × 100/content (%). *c*) Mean of subjects who absorbed the drug. *d*) ANOVA was performed according to a randomized block design.

with the sugar-coated tablet F. Among the enteric-coated tablets, tablet E, whose apparent critical dissolution pH was 4.5, showed the highest $V_{1.5}$, which suggested faster absorption than from the other enteric-coated tablets.

## Discussion

PAL is known to be absorbed rapidly[14] and transformed to PAL-P by PAL kinase *in vivo*,[15] even if PAL-P is hydrolyzed to PAL in the stomach. Because a sugar-coated tablet of PAL-P was bioequivalent with enteric-coated tablets, as shown in this study, there may be little practical need for enteric-coated tablets of PAL-P.

Thus, though the absorption was delayed significantly from all the enteric-coated tablets tested in this study as compared with the sugar-coated tablet, PAL-P was equivalently well absorbed from tablets C, D and E (which showed rapid dissolution at pH 6.6 *in vitro*) and the sugar-coated tablet. On the other hand, the enteric-coated tablets A and B, which showed long lags in dissolution at pH 6.6 and no dissolution at pH 5.5, were bioinequivalent with the other enteric-coated tablets. Rapid drug dissolution at pH 6.6 seems to be necessary for an enteric-coated tablet to be fully absorbed. MPM 47® and shellac may contribute to the low bioavailability of tablets A and B.

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