Differential Stereoselective Pharmacokinetics of Pantoprazole, a Proton Pump Inhibitor in Extensive and Poor Metabolizers of Pantoprazole—A Preliminary Study

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ABSTRACT Pantoprazole (PAN) is a proton pump inhibitor that is administered as a racemic mixture. The pharmacokinetics of PAN enantiomers were investigated in extensive metabolizers (EMs) and apparent poor metabolizers (PMs) of PAN who received a single 40, 60, or 80 mg oral dose of racemic PAN as enteric-coated formulation. In the EMs, the serum concentrations of (–)-PAN were slightly higher than those of (+)-PAN at each dose level. The (+)/(–) ratios for the area under the concentration-time curve (AUC) and the half-life were 0.58–0.89 and 0.62–0.88, respectively. In the PMs, the serum concentrations of both enantiomers were much higher than those in the EMs at each dose level and significant differences in pharmacokinetics of (+)- and (–)-PAN were observed. The half-lives for (+)-PAN were 2.67–3.77 times longer than those for (–)-PAN. The AUCs for (+)-PAN were 2.65–3.45 times greater than those for (–)-PAN. Therefore, the metabolism of (+)-PAN is impaired to a greater extent than (–)-PAN in the PMs, which resulted in the stereoselective disposition of PAN in the PMs. It has been suggested that the EMs and the PMs of PAN could be differentiated by determining the (+)/(–) enantiomer ratio in serum at one time point, possibly 2–6 h after oral dosing, because the (+)/(–) enantiomer ratios in the PMs were opposite those in the EM subjects. *Chirality 9:17–21, 1997*

KEY WORDS: proton pump inhibitor; pantoprazole; stereoselective pharmacokinetics; genetic polymorphism; human

Pantoprazole (PAN), 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole, is a substituted benzimidazole sulfoxide and a selective and long-acting proton pump inhibitor (PPI).[1] In healthy Caucasian subjects, PAN was well tolerated after single and multiple intravenous and oral administration and produced a dose-dependent reduction in gastric acid output.[2–5] PAN is currently under phase 2 clinical trial as an antiulcer drug in Japan.

Compounds which contain tricoordinated sulfur atoms in a pyramidal structure can exist in different optically active forms, and this is the case with the sulfoxide center of PAN, which is used clinically as a racemate (Fig. 1). Omeprazole,[6] the first registered substance of this class, and other PPIs, including lansoprazole[7] and E3810,[8] are also chiral benzimidazole sulfoxides and administered as racemic mixtures.

Cairns et al.[9] recently reported the plasma concentration-time profiles of omeprazole enantiomers after intravenous administration to a volunteer who seemed to be an extensive metabolizer (EM). They reported no significant differences in disposition between the (+)- and (–)-enantiomers. However, there is no published report on stereoselective disposition of other PPIs, including PAN in humans. Furthermore, no information on the effect of the genetically determined polymorphisms on stereoselective pharmacokinetics of PPIs is currently available.

In this study, the pharmacokinetics of PAN enantiomers after single oral administration of racemic PAN as enteric-coated tablets at doses of 40, 60, and 80 mg have been investigated in EMs and poor metabolizers (PMs) of PAN found during the phase 1 clinical study conducted in Japan.

MATERIALS AND METHODS

Test Compound

Enteric-coated 20 mg tablets of PAN sodium sesquihydrate, sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]-sulfinyl]-1*H*-benzimidazolide sesquihy-

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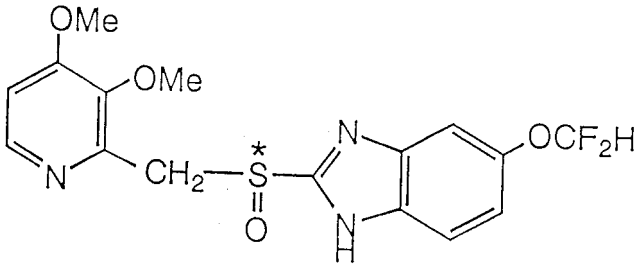

Fig. 1. Chemical structure of pantoprazole.

drate, purity 100.6%, and placebo tablets were supplied by Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). The dose was expressed as equivalents of PAN.

Subjects

Forty healthy Japanese male volunteers participated in the study after being fully informed of the purpose and risks involved. The study was conducted at the Sekino Hospital (Tokyo, Japan). Volunteers gave written informed consent to participate in the study, which was approved by the Institutional Review Board.

Study Design and Sample Collection

Forty healthy volunteers were divided into five groups of eight each (groups 1–5). Ascending doses of PAN (20, 40, 60, 80, and 100 mg) were administered to groups 1–5, respectively. In each study, six volunteers received PAN and two volunteers received placebo in a fasted state. The drug was administered orally with 120 ml of water. Serial blood samples were collected at 0 (predose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24 h after dosing. All samples were stored frozen at −20°C until analysis.

The pharmacokinetics of PAN were determined with a non-stereoselective analytical method, and three of 30 volunteers (one PM in the 40, 60, and 80 mg dose studies, respectively) proved to be the PMs of PAN, showing much lower clearance of PAN compared to the remaining EMs (unpublished data).

In the present study, serum samples obtained from one PM and one EM in the 40, 60, and 80 mg oral dose studies, respectively, were selected for the determination of PAN enantiomers to investigate the stereoselective disposition of PAN.

Analytical Method

Concentrations of PAN enantiomers in serum were determined by a stereoselective high-performance liquid chromatographic (HPLC) method developed by Tanaka et al.[10] Enantiomers were separated on a cellulose-based chiral stationary phase (Chiralcel OJ-R) following on-line solid phase sample clean-up with a column switching device. A mixture of acetonitrile and 50 m*M* sodium perchlorate (25:75, v/v) was used as the mobile phase at a flow rate of 0.5 ml/min. PAN enantiomers were detected by monitoring the column effluent at 290 nm. The limit of quantitation for serum was 0.10 μg/ml. The intraday and interday accuracy and precision, as indicated by relative error (RE, %) and coefficient of variation (CV, %) were within 14% above 0.25 μg/ml and within 16% at 0.1 μg/ml. Serum concentrations were expressed as PAN equivalents. Representative chromatograms of the serum samples obtained from an EM and a PM 3 h after a single 40 mg oral dose are shown in Figure 2.

Pharmacokinetic and Statistical Analyses

The software used for the pharmacokinetic analysis was TopFit[11] on an IBM compatible personal computer. The

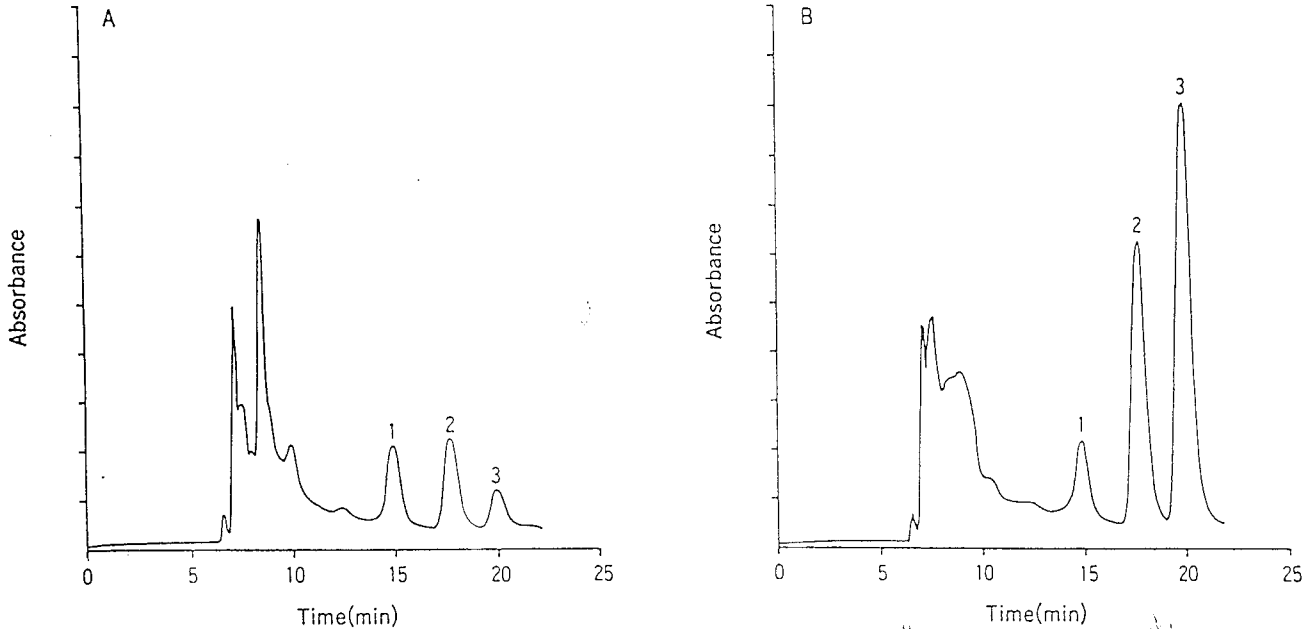

Fig. 2. Representative chromatograms of serum samples obtained from an EM **(A)** and a PM **(B)** 3 h after a single 40 mg oral dose. Peak identification: 1, IS; 2, (−)-PAN; 3, (+)-PAN.

pharmacokinetic parameters for PAN enantiomers were determined using model-independent methods. The terminal phase rate constant (λ_z) was determined by least-square regression of the logarithm of serum concentration on time over the terminal phase. The half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. Maximum serum concentration (C_{max}) was obtained from the measured values. The area under the concentration-time curve (AUC) was determined to the last quantifiable serum concentration using the linear trapezoidal rule and extrapolated to infinity using the terminal phase rate constant.

RESULTS

All volunteers completed the study, and no serious adverse events were reported or observed. There were no clinically significant changes in blood pressure, pulse rate, body temperature, or electrocardiogram. There were no drug-related changes in hematological and biochemical parameters.

The serum concentration-time profiles of (+)-PAN and (–)-PAN after single 40, 60, and 80 mg oral doses of racemic PAN to the PMs and the EMs of PAN are shown in Figure 3A–C. The pharmacokinetic parameters for each enantiomer are presented in Table 1. Figure 4 represents the change in the (+)/(–) ratios of serum concentrations in the EMs and the PMs.

In the EMs, the serum concentrations of (–)-PAN were slightly higher than those of (+)-PAN at each dose level. The (+)/(–) ratios of C_{max} ranged 0.77–0.99. The $t_{1/2}$ values for (+)-PAN (0.57–1.30 h) were shorter than those of (–)-PAN (0.92–1.48 h), resulting in the (+)/(–) ratios of 0.62–0.88. The serum concentrations of (+)-PAN were below the limit of quantitation 4, 6, and 8 h after 40, 60, and 80 mg doses, respectively. The (+)/(–) ratios of the initial serum drug concentrations were in the range 0.81–0.89, and the ratios gradually decreased to 0.45–0.74 with time. The (+)/(–) ratios of the AUC (0–∞) were in the range 0.58–0.89.

In the PMs, the serum concentrations of both enantiomers were much higher than those in the EMs at each dose level, and a significant difference in the pharmacokinetics of (+)- and (–)-PAN was observed. PMs showed significantly higher serum concentrations of (+)-PAN than (–)-PAN. The (+)/(–) ratios of C_{max} ranged 1.26–1.41. The $t_{1/2}$ values for (+)-PAN (5.34–8.63 h) were much longer than those of (–)-PAN (1.76–2.36 h), resulting in the (+)/(–) ratios of 2.67–3.77. The (+)/(–) ratios of the initial serum drug concentrations in the PMs were in the range 0.98–1.19. The ratios increased with time to reach more than 5 in the PMs 8–10 h after dosing. The (+)/(–) ratios of the AUC (0–∞) were in the range 2.65–3.45. The (+)/(–) enantiomer ratios of the serum concentrations in the PMs were opposite those in the EMs.

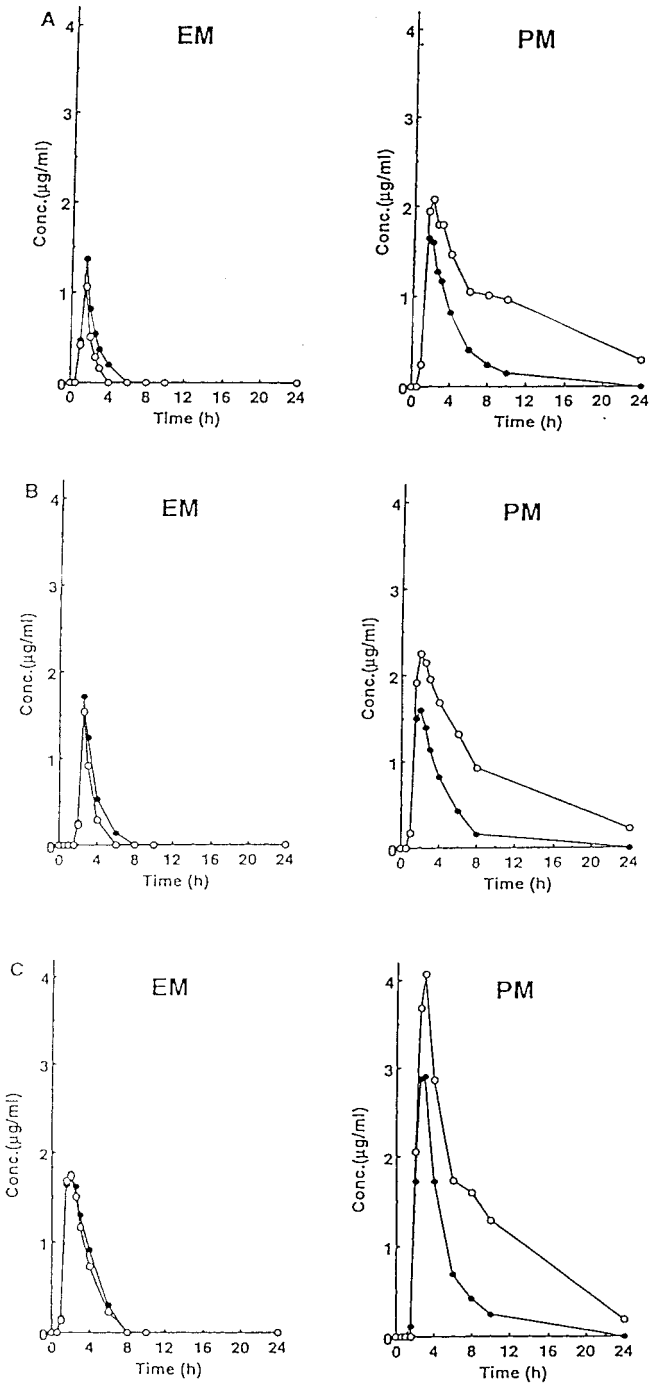

Fig. 3. Serum concentrations of (+)- (○) and (–)-pantoprazole (●) after single oral administration of racemic pantoprazole to EMs and PMs at doses of 40 **(A),** 60 **(B),** and 80 **(C)** mg.

DISCUSSION

It has been reported that in Caucasian populations there were apparent PMs of PAN who showed much lower clearance of PAN compared to the remaining EMs.[3] Serum concentrations for PMs were several times greater than those for EMs. The lower clearance of PAN in the PMs, without any noticeable difference in the distribution volume, compared with the EMs was reflected in a prolonged apparent terminal half-life estimate for the PMs. The PMs of PAN were also observed during the phase 1 clinical study conducted using healthy male Japanese volunteers (unpublished results). It has been reported that a few individuals exhibit a lower metabolic clearance (higher systemic exposure) of omeprazole than the majority of healthy volunteers,[12] and this polymorphism cosegregates with the

TABLE 1. Pharmacokinetic parameters of the enantiomers of pantoprazole (PAN) after single oral administration to extensive metabolizers (EM) and poor metabolizers (PM)

Dose (mg)	AUC (μg · h/ml)			C_{max} (μg/ml)			$t_{1/2}$ (h)		
	(+)-PAN	(–)-PAN	(+)/(–)	(+)-PAN	(–)-PAN	(+)/(–)	(+)-PAN	(–)-PAN	(+)/(–)
EM									
40	1.26	2.19	0.58	1.06	1.37	0.77	0.57	0.92	0.62
60	2.01	2.98	0.68	1.55	1.72	0.90	0.61	0.96	0.64
80	4.96	5.56	0.89	1.74	1.76	0.99	1.30	1.48	0.88
PM									
40	24.35	7.06	3.45	2.08	1.65	1.26	8.63	2.36	3.66
60	22.00	6.59	3.34	2.25	1.60	1.41	6.64	1.76	3.77
80	30.05	11.35	2.65	4.07	2.90	1.40	5.34	2.00	2.67

AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; $t_{1/2}$, half-life.

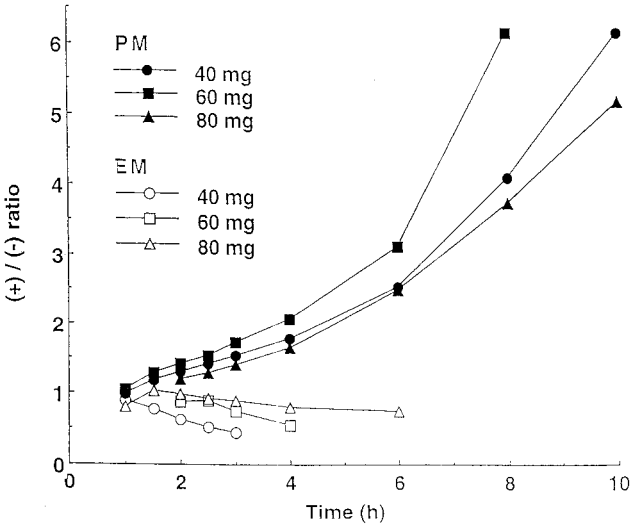

Fig. 4. Ratios of (+)- and (–)-pantoprazole serum concentrations after single oral administration of racemic pantoprazole to EMs and PMs at doses of 40, 60, and 80 mg.

S-mephenytoin hydroxylase (CYP2C19) phenotype.[13,14] Recently, Simon et al.[15] reported, using human hepatic microsomes, that the metabolism of PAN was also mediated by the polymorphically distributed isozyme CYP2C19.[15] Although the PMs of PAN in this study have not been phenotype with mephenytoin retrospectively, it is possible that the PMs of PAN are also the PMs of mephenytoin, the incidence of which is higher in Asian populations than in Caucasians.[16]

The PPIs including PAN, omeprazole, and lansoprazole are chiral benzimidazole sulfoxides used clinically as racemates. However, only very limited information on the stereoselective disposition of PPIs has been reported.[9] Furthermore, no information on the effect of the genetically determined polymorphism on the stereoselective metabolism of the PPIs is currently available. Using the stereoselective assay methodology,[10] it was possible in the present study to describe the pharmacokinetics of the individual PAN enantiomers in EMs and PMs of PAN.

The present pharmacokinetic data show a relatively small difference in disposition between (+)- and (–)-PAN in EMs but a significant difference in PMs. It has been suggested that PAN undergoes a stereoselective first pass effect to very limited extent since the (+)/(–) ratios of the initial serum drug concentrations in both EMs and PMs were in the range 0.81–1.19. The ratios gradually decreased to 0.45–0.74 with time in the EMs, whereas the ratios increased to greater than 5 in the PMs up to 8–10 h. These data indicate that the stereoselective disposition observed in the PMs is due to greater impairment in the metabolism of (+)-PAN than (–)-PAN in PMs. It may be postulated that the different enzymes of PAN biotransformation may each preferentially metabolize either (+)- or (–)-PAN. In the EMs, the stereoselectivity of the multiple enzymes for metabolism is balanced so that there are no significant differences in disposition between (+)- and (–)-PAN. PMs lack at least one enzyme which must preferentially eliminate the (+)-PAN so that stereoselective pharmacokinetics are observed. It is possible that the disposition of (+)-PAN is related to CYP2C19 to a greater extent compared to its (–)-enantiomer. Further in vitro and in vivo studies, however, are required to identify the cytochrome P450s involved in the metabolism of PAN enantiomers.

There are no significant differences in pharmacological activities between (+)- and (–)-PAN in experimental animals (unpublished data), both anantiomers being converted by a sequence of reactions to the same active form, which has no chiral center. PAN is a drug that is well tolerated, and no dose-related side effects have been reported in clinical trials. Thus, no stereoselective differences in pharmacological effects of PAN are expected even in PM patients, and it would be of little clinical significance for the antisecretory efficacy of PAN that the drug is administered as a racemate.

It has been suggested that EMs and PMs of PAN could be differentiated by determining the (+)/(–) enantiomer ratio in serum at one time point, possibly 2–6 h after oral dosing because the (+)/(–) enantiomer ratios in the PMs were opposite those in the EM subjects. Further studies in larger numbers of individuals of each phenotype are necessary to test this hypothesis.

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LITERATURE CITED

1. Simon, W.A., Keeling, D.J., Laing, S.M., Fallowfield, C., Taylor, A.G. BY 1023/SK&F 96022: biochemistry of a novel (H^++K^+)-ATPase inhibitor. Biochem. Pharmacol. 39:1799–1806, 1990.
2. Simon, B., Müller, P., Marinis, E., Lühmann, R., Huber, R., Hartmann, R., Wurst, W. Effect of repeated oral administration of BY 1023/SK&F 96022–a new substituted benzimidazole derivative—on pentagastrin-stimulated gastric acid secretion and pharmacokinetics in man. Aliment. Pharmacol. Ther. 4:373–379, 1990.
3. Pue, M.A., Laroche, J., Meineke, I., de May, C. Pharmacokinetics of pantoprazole following single intravenous and oral administration to healthy male subjects. Eur. J. Clin. Pharmacol. 44:575–578, 1993.
4. Bliesath, H., Huber, R., Hartmann, M., Lühmann, R., Wurst, W. Dose linearity of the pharmacokinetics of the new H^+/K^+-ATPase inhibitor pantoprazole after single intravenous administration. Int. J. Clin. Pharmacol. Ther. 32:44–50, 1994.
5. Simon, B., Müller, P., Hartmann, M., Bliesath, H., Lühmann, R., Huber, R., Bohnenkamp, W., Wurst, W. Pentagastrin-stimulated gastric acid secretion and pharmacokinetics following single and repeated intravenous administration of the gastric H^+,K^+-ATPase-inhibitor pantoprazole (BY 1023/SK&F96022) in healthy volunteers. Z. Gastroenterol. 28:443–447, 1990.
6. Massoomi, F., Savage, J., Destache, C.J. Omeprazole: a comprehensive review. Pharmacotherapy 13:46–59, 1993.
7. Barradell, L.B., Faulds, D., McTavish, D. Lansoprazole, a review of its pharmacodynamic and pharmacokinetic properties and its therapeutic efficacy in acid-related disorders. Drugs 44:225–250, 1992.
8. Yasuda, S., Ohnishi, A., Ogawa, T., Tomono, Y., Hasegawa, J., Nakai, H., Shimamura, Y., Morishita, N. Pharmacokinetic properties of E3810, a new proton pump inhibitor, in healthy male volunteers. Int. J. Clin. Pharmacol. Ther. 32:466–473, 1994.
9. Cairns, A.M., Chiou, R.H.-Y., Rogers, J.D., Demetriades, J.L. Enantioselective high-performance liquid chromatographic determination of omeprazole in human plasma. J. Chromatogr. B 666:323–328, 1995.
10. Tanaka, M., Yamazaki, H. Direct determination of pantoprazole enantiomers in human serum by reversed-phase high-performance liquid chromatography using a cellulose-based chiral stationary phase and column-switching system as a sample cleanup procedure. Anal. Chem. 68:1513–1516, 1996.
11. Tanswell, P., Koup, J. TopFit: a PC-based pharmacokinetic/pharmacodynamic data analysis program. Int. J. Clin. Pharmacol. Ther. Toxicol. 31:514–520, 1993.
12. Andersson, T., Cederberg, C., Edvardsson, G., Heggelund, A., Lundborg, P. Effect of omeprazole treatment on diazepam plasma levels in slow versus normal rapid metabolizers of omeprazole. Clin. Pharmacol. Ther. 47:79–85, 1990.
13. Andersson, T., Regårdh, C., Dahl-Puustinen, M., Bertilsson, L. Slow omeprazole metabolizers are also poor S-mephenytoin hydroxylators. Ther. Drug Monit. 12:415–416, 1990.
14. Chiba, K., Kobayashi, K., Manabe, K., Tani, M., Kamataki, T., Ishizaki, T. Oxidative metabolism of omeprazole in human liver microsomes: cosegregation with S-mephenytoin 4′-hydroxylation. J. Pharmacol. Exp. Ther. 266:52–59, 1993.
15. Simon, W.A. Pantoprazole—which cytochrome P450 isozymes are involved in its biotransformation [Abstract]? Gut 37(Suppl. 2):A135, 1995.
16. Sohn, D.R., Shin, S.G., Ishizaki, T. S-Mephenytoin pharmacogenetics and its clinical implications in Asian ethnic populations. Asia Pac. J. Pharmacol. 9:287–301, 1994.