Biochemical Pharmacology, Vol. 42, No. 2, pp. 347–355, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

THE H^+,K^+-ATPase INHIBITOR PANTOPRAZOLE (BY1023/SK&F96022) INTERACTS LESS WITH CYTOCHROME P450 THAN OMEPRAZOLE AND LANSOPRAZOLE

W. ALEXANDER SIMON,* CHRISTIAN BÜDINGEN, SUSANNE FAHR, BURKHARD KINDER and MARGA KOSKE

Byk Gulden Pharmazeutika, D-7750 Konstanz, Germany

(*Received* 7 *August* 1990; *accepted* 9 *March* 1991)

Abstract—The gastric acid antisecretory compound omeprazole (5-methoxy-2-((4-methoxy-3,5-dimethyl-2-pyridinylmethyl)-sulphinyl)-1*H*-benzimidazole), a member of the new class of H^+,K^+-ATPase inhibitors, is known to interact with the metabolism of other drugs *in vitro* and *in vivo*. In this study, two other substituted benzimidazoles, pantoprazole (5-difluoromethoxy-2-((3,4-di-methoxy-2-pyridinylmethyl)-sulphinyl)-1*H*-benzimidazole) and lansoprazole (2-((3-methyl-4-(2,2,2-trifluoro-ethoxy)-2-pyridinylmethyl)-sulphinyl)-1*H*-benzimidazole) are compared for their ability to inhibit cytochrome P450 dependent biotransformation *in vitro* with regard to three representative reactions: O-dealkylation of 7-ethoxycoumarin (EC), N-demethylation of ethylmorphine (EM) and hydroxylation of lonazolac (Lona). These reactions can be seen in microsomes from phenobarbital pretreated rats representing the cytochrome P450IIB1 subfamily. As shown in presence of known inhibitors of cytochrome P450, e.g. SK&F 525A, metyrapone, chlorpromazine and nitrendipine, different enzymes seem to be responsible for these three indicator reactions of the cytochrome P450IIB1 complex. These reactions are inhibited to a different extent by the three H^+,K^+-ATPase inhibitors. Pantoprazole shows the lowest inhibitory activity versus the three reactions (K_i, μmol/L): EC, 138; EM, 104; Lona, 128. A greater effect is observed with omeprazole: EC, 38; EM, 68; Lona, 20. Lansoprazole exceeds omeprazole in inhibiting the three cytochrome P450 dependent enzymes: EC, 17; EM, 34; Lona, 8. In microsomes from untreated rats with the predominant cytochrome P450IIA1 subfamily as well as in microsomes from isopropanol treated rats (induction of cytochrome P450IIE1) which catalyse only lonazolac hydroxylation to a detectable amount, the latter reaction was inhibited by pantoprazole with a somewhat lower K_i of 77 whereas the values for omeprazole and lansoprazole remained unchanged in comparison to those found in microsomes from phenobarbital pretreated rats. The biotransformation rate of the substituted benzimidazoles themselves in microsomes from control and induced rats is lowest for pantoprazole followed by lansoprazole and omeprazole.

The knowledge of drug interactions with the liver microsomal mixed-function oxidase system is becoming more and more important in the safety assessment of new compounds developed for clinical use. This is particularly important in multi drug therapy, because the pharmacodynamics of two simultaneously given drugs may be dramatically altered by the influence of one drug on the metabolism of the other drug. One drug may interact with a second by a range of different mechanisms such as chemical incompatibility, displacement of protein binding, enzyme induction and interference with drug metabolism, i.e. biotransformation. The latter involves the transformation of specific moieties in a substrate molecule creating new functional groups which are generally more hydrophilic compared to the parent molecule. The route of biotransformation as well as the affinity of drugs for cytochrome P450 can be assessed by measuring their influence on the liver microsomal mixed-function oxidase system *in vitro*.

The phase I reactions of biotransformation include O- and N-dealkylation of ethers and amines, respectively, and the *p*-hydroxylation of aromatic ring systems. The basic chemistry involved in various reactions is probably quite uniform, and the selectivity of different cytochrome P450s for various substrates or reactions is thought to be the result of distinctions in the three-dimensional binding sites of the enzymes [1]. Therefore the metabolism of test compounds may be influenced to a different extent in the presence of known inhibitors of cytochrome P450. 7-Ethoxycoumarin is used as the substrate for O-dealkylation [2] and ethylmorphine for N-dealkylation [3]. For aromatic ring hydroxylation, lonazolac was previously introduced as the substrate for biotransformation studies [4]. These reactions are catalysed by enzymes which are believed to belong to the enzymes of the cytochrome P450IIB subfamily due to their inducibility by phenobarbital. In non-induced microsomes from rat (cytochrome P450IIA), only lonazolac hydroxylation can be detected to a measurable amount.

Substituted pyridylmethylsulphinyl-benzimidazoles are a new class of inhibitors of the gastric H^+,K^+-ATPase [5–11]. This enzyme acts as a proton pump enabling H^+ to be secreted into the stomach [12, 13]. Pantoprazole, omeprazole and lansoprazole inhibit the proton pump in isolated gastric glands after stimulation by dibutyryl cyclic AMP in an equipotent range of 0.5 to 1.0 μmol/L

* Author for correspondence: Dr W. A. Simon, Byk Gulden Pharmazeutika, Postfach 100310, D-7750 Konstanz, Germany.

[8]. Recently, omeprazole has been shown to interact with 7-ethoxycoumarin dealkylase [14, 15] and ethylmorphine demethylase [15] *in vitro* and to be a stronger inhibitor of cytochrome P450 dependent enzymes than pantoprazole both *in vitro* and *in vivo* [16].

The purpose of this study was to compare and characterize the biotransformation of pantoprazole, omeprazole and lansoprazole in microsomes and their influence on the three cytochrome P450 dependent reactions with the substrates 7-ethoxycoumarin, ethylmorphine and lonazolac.

MATERIALS AND METHODS

Chemicals

The source of the drugs used were as follows: pantoprazole, omeprazole, lansoprazole, lonazolac (3-(*p*-chlorophenyl)-1-phenylpyrazole-4-acetic acid), and nitrendipine were synthesized at the Chemical Department, Byk Gulden Pharmazeutika, Konstanz, Germany; metyrapone (2-methyl-1-2-di-(3-pyridyl)-1-propanone, Aldrich, Milwaukee, U.S.A.); chlorpromazine (Bayer AG, Leverkusen, Germany); SK&F 525A (proadifen, Smith Kline & French, Welwyn, U.K.); 7-ethoxycoumarin, ethylmorphine (Sigma, München, Germany); other chemicals were from E. Merck (Darmstadt, Germany) and were of analytical grade. The benzimidazole sulphoxides were dissolved in dimethylsulphoxide.

Preparation of liver microsomes

Female rats (Sprague–Dawley, 180–200 g, Ivanovas, Kißlegg, Germany) were fed Altromin® standard diet No. 1324 with free access to water with and without 0.1% phenobarbital for 7 days, respectively, before being killed by decapitation. Induction of cytochrome P450IIE1 was achieved by oral administration of 10 mL aqueous isopropanol solution (25% v/v) per kilogram body weight 24 hr before being killed by decapitation [17]. The livers were excised and homogenized (Potter-Elvejhem, 860 rpm, six strokes) in three volumes of sodium phosphate buffer (100 mmol/L, pH 7.4) and KCl (150 mmol/L). The homogenate was centrifuged for 20 min at 10,000 *g* with 13,000 rpm (Sorvall RC5B, SS 34 rotor) and the pellet discarded. The supernatant was further centrifuged for 60 min at 29,000 rpm (Kontron TGA 50, Type 30 rotor). The pellets were resuspended in the same volume sodium phosphate buffer and centrifuged again for 20 min with 29,000 rpm in the same rotor. The washed pellets were resuspended in one third of the volume of the same buffer (final protein concentration 25–30 mg/mL) and stored at −80°.

Assay of enzymes

7-Ethoxycoumarin (EC) deethylase. The O-deethylation of 7-ethoxycoumarin to the highly fluorescent product, 7-hydroxycoumarin (HC), is a particularly sensitive indicator of the monoxygenase activity as a function of the cytochrome P450 complex [2].

Incubation: 0.44 mg protein were incubated in 100 mmol/L Tris*–HCl buffer, pH 7.4, in the presence of 10, 15, 30 and 100 μmol/L, respectively, of EC plus test compound in a concentration range of 0 to 10 μmol/L lansoprazole, 0 to 50 μmol/L omeprazole and 0 to 100 μmol/L pantoprazole in a final volume of 3 mL. The reaction was started with 0.3 mmol/L $NADPH_2$. After 15 min of incubation at 37°, the reaction was terminated by adding consecutively 125 μL trichloroacetic acid (15% w/v) and 2 mL chloroform. After vigorous shaking and centrifugation for 5 min, 1 mL of the organic phase was extracted with 2 mL 0.01 mol/L NaOH plus 1 mol/L NaCl, and a concentration of the 7-hydroxycoumarin in the alkaline phase was determined fluorimetrically, the excitation and emission wavelengths used were 336 and 460 nm, respectively. 7-Hydroxycoumarin in predetermined concentrations was used as standard. The K_m for 7-ethoxycoumarin was found to be 44 μmol/L with the specific activity of 1.2 nmol/L HC/mg/min. Linearity of HC formation with time was observed up to 30 min in the presence of 0.3 mg protein and the EC concentration indicated above.

Lonazolac hydroxylase activity. Lonazolac is exclusively metabolized *in vitro* on its aromatic ring moiety to hydroxy-lonazolac (3-(4-chlorophenyl)-1-(4-hydroxyphenyl)pyrazole-4-acetic acid) [4]. This biotransformation can directly be followed by detecting the metabolite by HPLC. The tested compounds at the highest concentrations used in this study did not interfere with this analysis. Linearity with time up to 30 min for the formation of the metabolite was observed for all concentrations of lonazolac used in the assay up to 0.3 mg protein/mL. The K_m for lonazolac on liver microsomes from untreated female rat was found to be 19.8 ± 4 μmol/L with a V_{max} of 4.2 nmol hydroxy-lonazolac/mg/min. For microsomes from phenobarbital stimulated female rats these values were 42 ± 7.6 μmol/L with a V_{max} of 11.4 ± 2.3 nmol hydroxy-lonazolac/mg/min.

Incubation: 0.25 mg protein were incubated in 100 mmol/L Tris–HCl buffer, pH 7.4, in the presence of lonazolac (25, 50 and 100 μmol/L) and test compound (0–300 μmol/L). The reaction was started by addition of $NADPH_2$, final concentration 0.3 mmol/L, then incubated at 37° for 15 min. The reaction was stopped by freezing in liquid nitrogen, samples were then thawed and centrifuged at 20,000 *g* to remove protein prior to analysis by HPLC. Separation of lonazolac (substrate) and its metabolite, hydroxy-lonazolac, was performed on a reversed phase column (Nucleosil C 8.5 μm, column 125 × 4.6 mm) and LiCHROPREP RP-2 (precolumn 12 × 4 mm). A gradient of acetonitrile (10–45%) and 10 mmol/L KH_2PO_4 buffer, pH 7.4, was used as eluent on the analytical column over 15 min (flow: 1 mL/min).

Ethylmorphine N-demethylase. Linear time dependence of formaldehyde production was obtained during a 25 min period in the concentration range between 0.1 and 10.0 mmol/L of ethylmorphine up

* Abbreviations used: Tris, α,α,α-tris(hydroxymethyl)-methylamine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

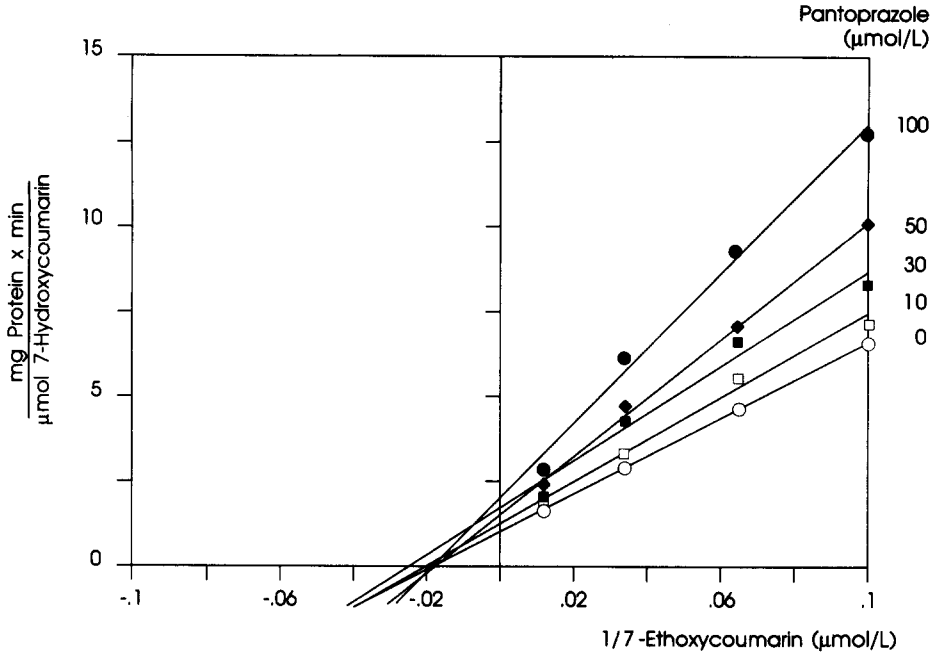

Fig. 1. Effect of pantoprazole on ethoxycoumarin-*O*-dealkylase. Lineweaver–Burk plot for inhibition of ethoxycoumarin-*O*-dealkylase by pantoprazole. Microsomal protein (0.44 mg) was incubated in 100 mmol/L Tris–HCl buffer, pH 7.4, in the presence of 10, 15, 30 and 100 μmol/L ethoxycoumarin plus pantoprazole in the concentration range 0 to 100 μmol/L for 15 min at 37°. The data shown are from a representative experiment out of the four performed.

to 2 mg protein/mL. The formaldehyde formation was measured as described [18].

Incubation: 1.8 mg protein were incubated in 50 mmol/L Hepes–Tris buffer, pH 7.4, 150 mmol/L KCl and 3 mmol/L $NADPH_2$ in the presence of the test compound (concentration range 10 to 100 μmol/L) for 5 min at 37°. After addition of ethylmorphine (0.3 and 1.0 mmol/L) the incubation was continued for 15 min in a final volume of 500 μL. The reaction was stopped by addition of 200 μL trichloroacetic acid (15% w/v) and by centrifugation for 10 min with 2500 rpm at 2000 *g*. Supernatant (500 μL) was mixed with the Nash reagent and incubated for 12 min at 60°. Absorbance at 410 nm was followed on a KONTRON UVICON 860 spectrophotometer and converted to concentration on the basis of a standard curve obtained with similarly treated formaldehyde solutions of appropriate concentrations. The K_m value for ethylmorphine was found to be 326 ± 10 μmol/L with the V_{max} of 0.88 ± 0.24 nmol formaldehyde/mg/min.

Incubation of the test compounds and analysis by HPLC

Incubation was performed as described for lonazolac hydroxylase and in the presence of 10 μmol/L test compound. The reaction was stopped at the indicated times. The HPLC analysis was performed as described for lonazolac.

Protein assay

Protein was determined according to Lowry *et al.* [19] using bovine serum albumin as standard.

Evaluation

The results were qualitatively and quantitatively evaluated using graphical plots according to Lineweaver and Burk (primary scheme: reciprocal velocity in the presence of various inhibitor concentrations versus reciprocal substrate concentrations, secondary scheme for K_i determination: slope from primary scheme versus inhibitor concentrations) [20], Dixon (reciprocal velocity versus inhibitor concentrations) [21] and Cornish-Bowden (substrate concentrations over velocity versus inhibitor concentrations) [22].

RESULTS

Cytochrome P450II dependent biotransformation of pantoprazole, omeprazole and lansoprazole in rat microsomes

The metabolism of the test compounds in microsomes was analysed in order to demonstrate that they are substrates of cytochrome P450 dependent enzymes. Each of the three compounds was metabolized in microsomes from untreated rats (cytochrome P450IIA1 [23]) with the slowest rate for pantoprazole followed by lansoprazole and omeprazole. In microsomes from phenobarbital treated rats, however, the metabolism of the three compounds is considerably increased which is consistent with the contribution of the cytochrome P450IIB subfamily to their biotransformation (Table 1) [23]. The three compounds have different affinities to the two cytochrome P450 subfamilies IIA and IIB.

Table 1. Biotransformation of pantoprazole, omeprazole and lansoprazole in microsomes from control and phenobarbital induced rat

	% Remaining parent compound					
	Control			Phenobarbital treated		
Time of incubation (min)	15	30	60	15	30	60
Pantoprazole	100	100	92	68	57	53
Omeprazole	69	59	37	33	27	22
Lansoprazole	86	78	72	45	31	28

Table 2. Inhibitory potency of compounds which interact with cytochrome P450

	K_i (μmol/L)		
	7-Ethoxycoumarin dealkylase	Lonazolac hydroxylase	Ethylmorphine demethylase
SK&F 525A	<1	<1	6
Metyrapone	3	118	74
Nitrendipine	4	<1	32
Chlorpromazine	14	20	>500

K_i determination on rat microsomes for 7-ethoxycoumarin dealkylase and lonazolac hydroxylase according to Lineweaver and Burk and for ethylmorphine demethylase according to Dixon.

Interaction of standard compounds with the three cytochrome P450 dependent reactions

The four compounds, proadifen, metyrapone, nitrendipine and chlorpromazine, known as inhibitors of cytochrome P450 dependent enzymes, were studied in the three enzymatic reactions. As shown in Table 2, the inhibitory potencies of the four compounds versus the three enzymes were completely different demonstrating that the three indicator reactions for cytochrome P450 seem to represent three different enzymes or binding sites.

Interaction of pantoprazole, omeprazole and lansoprazole with 7-ethoxycoumarin-O-dealkylase

In the presence of pantoprazole, a concentration dependent inhibition of the dealkylase activity was observed (Lineweaver–Burk plot, Fig. 1). The slope of each line was plotted versus the inhibitor concentration and the intersection point of the regression line with the ordinate taken as the K_i value. The K_i for pantoprazole was 135 μmol/L. Omeprazole showed a stronger interaction with cytochrome P450 which is indicated by the K_i value of 38.5 μmol/L, which is then followed by the K_i value for lansoprazole of 17 μmol/L (Table 3). The three compounds showed mixed-type inhibition for 7-ethoxycoumarin dealkylase (data only shown for pantoprazole, Fig. 1).

Interaction of pantoprazole, omeprazole and lansoprazole with lonazolac hydroxylation

The three compounds were tested in microsomes from treated and untreated rats, respectively. The kinetic analysis of the interaction of pantoprazole with lonazolac hydroxylation in microsomes from control rats shows mixed-type inhibition in the double reciprocal graph of specific activity versus lonazolac concentration (Fig. 2a). In the analysis according to Dixon (not shown) and Cornish-Bowden (Fig. 3a), however, this inhibition seems to be competitive. This is not the case for inhibition of lonazolac hydroxylation by pantoprazole in phenobarbital induced microsomes where we found a non-competitive type of inhibition (Figs 2b and 3b). Likewise, the K_i values for pantoprazole differ in both types of microsome whereas the K_i values for omeprazole and lansoprazole do not differ (Table 4). The ranking order for inhibition of lonazolac hydroxylation by the three compounds is mainly in parallel to that shown above for the O-dealkylation of 7-ethoxycoumarin (Table 3). In both types of microsome, omeprazole showed competitive inhibition for lonazolac hydroxylase (Fig. 3c) whereas for lansoprazole non-competitive inhibition was observed (not shown). The interaction of the three compounds with lonazolac hydroxylase was also examined in microsomes which were obtained from rats induced by isopropanol. This treatment is believed to induce the cytochrome P450 subfamily IIE1. However, the K_i values of the three H^+,K^+-ATPase inhibitors were unaltered in comparison to the control (Table 4).

Interaction of pantoprazole, omeprazole and lansoprazole with ethylmorphine demethylase

Among the three model systems for cytochrome P450 the demethylation of ethylmorphine is characterized by its low substrate affinity, i.e. the high K_m value for ethylmorphine of 326 μmol/L which has been found also by others [3, 14]. Pantoprazole, up to 100 μmol/L, inhibited this activity in a manner that resulted in a linear Dixon

Table 3. Inhibitory potencies of pantoprazole, omeprazole and lansoprazole on cytochrome P450

Inhibitor	K_i (μmol/L) 7-Ethoxycoumarin dealkylase	Lonazolac hydroxylase	Ethylmorphine demethylase
Pantoprazole	138 ± 12.3 (4)	128 ± 4.6 (4)	104 ± 9.1 (8)
Omeprazole	38 ± 2.7 (4)	20 ± 3.6 (4)	68 ± 8.7 (4)
Lansoprazole	17 ± 4.3 (3)	8 ± 1.4 (3)	34 ± 3.6 (5)

Values are means ± SE (the number of separate experiments is given in parentheses).
K_i determination in rat liver microsomes for 7-ethoxycoumarin dealkylase and lonazolac hydroxylase according to Lineweaver and Burk and for ethylmorphine demethylase according to Dixon.

plot (not shown). However, for omeprazole and lansoprazole linearity was only seen up to 30 μmol/L. The non-linearity at higher concentrations of these two compounds indicated that the inhibition was partial. For these compounds K_i values were calculated from the intersection points of the linear portion of the lines in the Dixon plot and given in Table 3. Again, for pantoprazole the highest K_i value was obtained.

DISCUSSION

In this study, three benzimidazole sulphoxides representing the new class of proton pump inhibiting anti-secretory compounds, pantoprazole, omeprazole and lansoprazole were compared with respect to their inhibitory potency on cytochrome P450 dependent enzymes. One of these compounds, omeprazole, has already been shown in animal experiments [16] and clinical studies [15] to interact with cytochrome P450. Three *in vitro* test systems have been used to assess the interactions with cytochrome P450 in the present study. Two of them are commonly used for inhibition studies, the 7-ethoxycoumarin dealkylase and ethylmorphine demethylase. Both reactions can only be seen in microsomes from phenobarbital treated rats with the analysis used. This fact indicates that these enzymes are members of the cytochrome P450IIB subfamily. Very low 7-ethoxycoumarin dealkylase activity could also be found in microsomes from isopropanol treated rats which indicates that this enzyme may also be a member of the cytochrome P450IIE1 subfamily. However, the activity was not sufficiently high for inhibition studies. The third one, the lonazolac hydroxylase, was recently established in our laboratory [4]. The metabolite of lonazolac, 3-(4-chlorophenyl)-1-(4-hydroxyphenyl)pyrazole-4-acetic acid, formed in this system is easily detectable by HPLC and the method represents an excellent diagnostic procedure in interaction studies. Lonazolac hydroxylation could be observed in microsomes from untreated and phenobarbital treated rats which represents the cytochrome P450IIA1 and P450IIB subfamily, respectively. In isopropanol induced microsomes, the specific activity of lonazolac hydroxylase is not enhanced in comparison to the control (data not shown). Similar results were obtained in isopropanol treated rats with respect to the K_i value of the three test compounds for lonazolac hydroxylation, which are in the range of the control rats. Therefore, the conclusion can be drawn that these three compounds interfere with the three enzymes of at least two subfamilies of the major cytochrome P450II family whereas the third one, cytochrome P450IIE1, inducible by ethanol and isopropanol, does not metabolize the substrates to a measurable extent. Relevant interactions of the three benzimidazole sulphoxides with the cytochrome P450IIE1 dependent enzymes might, therefore, be excluded. However, the question arose as to whether there was any need to check a new compound in more than one *in vitro* system. Unfortunately, no comparisons are possible so far between different cytochrome P450 dependent enzymatic systems and different inhibitors, and data are not available in the literature to answer this question. Inhibition data differ qualitatively and quantitatively from laboratory to laboratory [24]. Even worse, there is no agreement among scientists to use K_i values only if obtained from experiments using at least two substrate concentrations. The comparison by the IC_{50}, the concentration of inhibitor required for 50% inhibition, is only possible in cases where the mechanism of inhibition is known. For this reason we compared the three systems with the aid of some known inhibitors. The results in Table 2 justify the use of these three *in vitro* systems in order to mimic the broad substrate specifity and great diversity of the drug metabolizing enzyme system *in vivo*. In the presence of inhibitors for cytochrome P450, the three substrates 7-ethoxycoumarin, ethylmorphine and lonazolac, respectively, seem to be metabolized by three different enzymes or at least by different binding sites. As long as no data on a new class of compounds exist for drug interactions *in vivo*, *in vitro* interaction studies of new drugs using a range of substrates may be considered as a useful way to gain insight in this area. The benzimidazole sulphoxides are metabolized *in vitro* to a negligible extent to the corresponding sulphones during incubation, which are themselves poor inhibitors of the drug metabolizing enzymes (personal observations). The low interaction of pantoprazole in comparison to the other two compounds with the three enzymes is obvious. This low affinity to

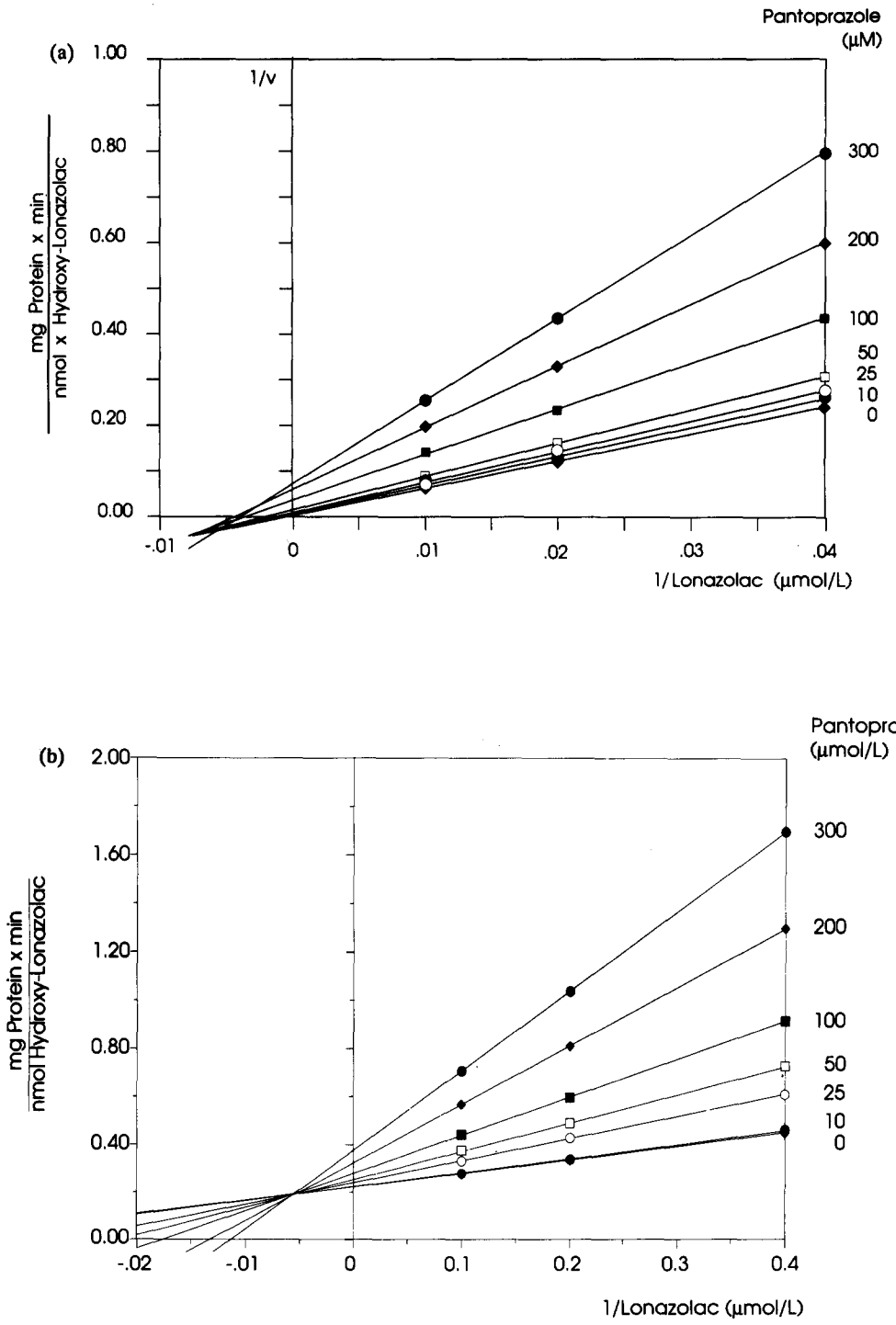

Fig. 2. Effect of pantoprazole on lonazolac hydroxylase. Lineweaver–Burk plot for inhibition of lonazolac hydroxylation by pantoprazole in microsomes from untreated rats (a) and from phenobarbital treated rats (b). Microsomal protein (0.25 mg) and 0.3 mmol/L $NADPH_2$ were incubated in the presence of 25, 50 and 100 µmol/L lonazolac and 0–300 µmol/L pantoprazole in 100 mmol/L Tris–HCl buffer, pH 7.4, at 37° for 15 min. The data shown are from a representative experiment out of the four performed.

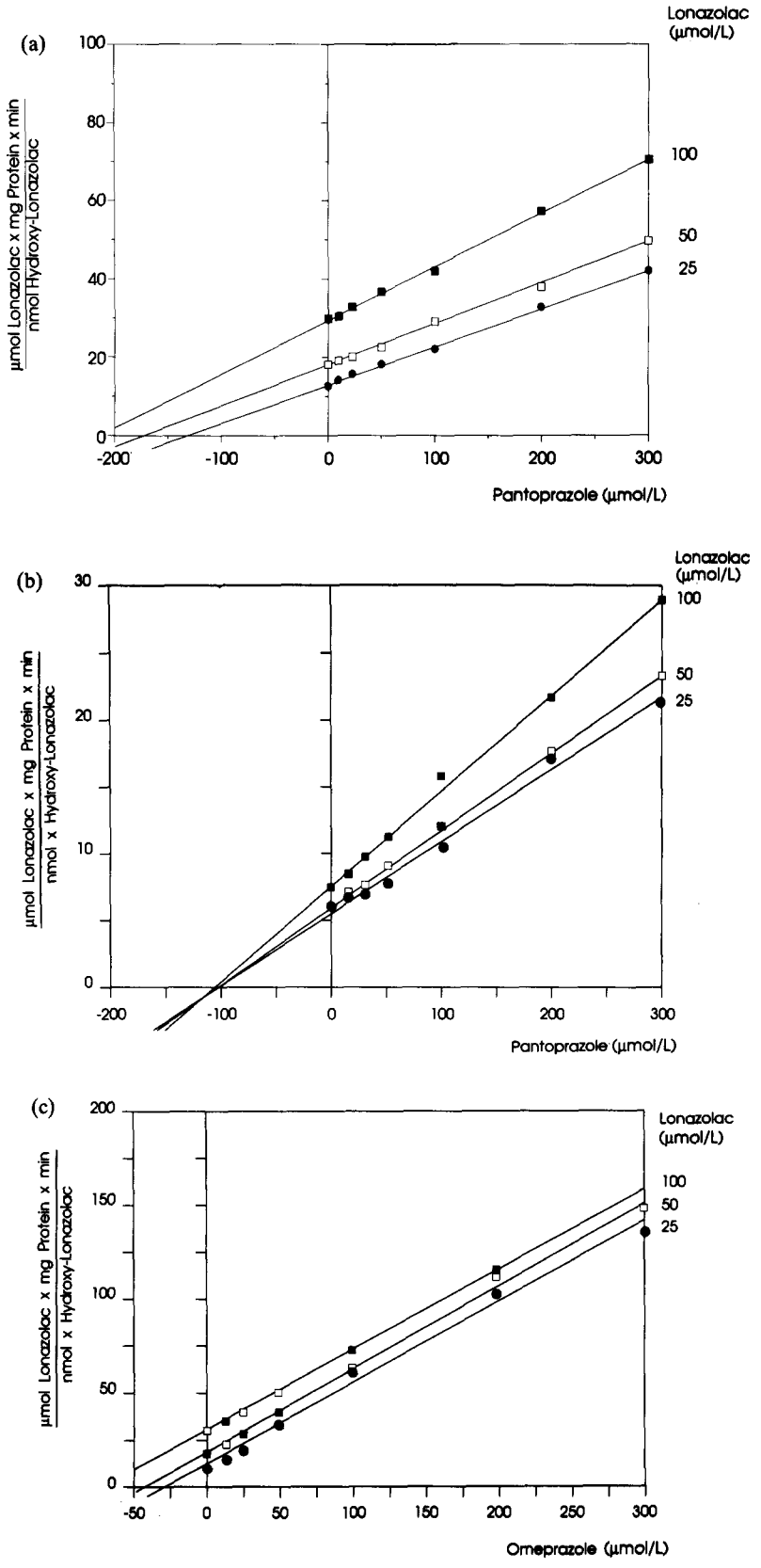

Fig. 3. Cornish-Bowden plot, effect of pantoprazole on lonazolac hydroxylase in microsomes from untreated rats (a) and of pantoprazole (b) and omeprazole (c) on lonazolac hydroxylase in microsomes from phenobarbital treated rats.

Table 4. Inhibition of lonazolac hydroxylase by pantoprazole, omaprazole and lansoprazole in microsomes from untreated rats (control) and from rats treated with phenobarbital and isopropanol

	K_i (μmol/L)		
	Control	Phenobarbital	Isopropanol
Pantoprazole	77 ± 5.0 (2)	128 ± 4.6 (4)	70 (1)
Omeprazole	20 ± 4.3 (2)	20 ± 3.6 (4)	24 (1)
Lansoprazole	8 ± 1.5 (2)	8 ± 1.4 (4)	6 (1)

Values are means ± SE (the number of experiments is given in parentheses).

cytochrome P450 was also demonstrated by the time course of biotransformation in control and induced microsomes where we obtain, in both cases, the lowest rate for pantoprazole. Interestingly, omeprazole showed the highest rate for biotransformation in control and phenobarbital induced microsomes in comparison to pantoprazole and lansoprazole which is not in the same ranking order as shown in the interaction studies. It is obvious that the benzimidazole sulphoxides might have different affinities to the enzymes responsible for their own biotransformation in comparison to those responsible for biotransformation of 7-ethoxycoumarin, ethylmorphine and lonazolac. In control microsomes, pantoprazole and omeprazole showed competitive inhibition versus lonazolac whereas in phenobarbital induced microsomes the K_i for pantoprazole is increased and the inhibition seems to be more of the mixed type. This is consistent with the observation that the K_i has also changed and that in phenobarbital induced microsomes an additional enzyme or binding site might metabolize lonazolac which is not accessible for pantoprazole in a competitive way. Omeprazole and lansoprazole do not interact at all with this additional binding site for lonazolac although their metabolism was increased in phenobarbital induced microsomes. The most pH sensitive benzimidazole sulphoxide among the three tested [8], lansoprazole, interacts very strongly and non-competitively with lonazolac hydroxylase in both types of microsomes. The K_i for pantoprazole obtained in the three cytochrome P450IIB dependent systems is in the range of 104 to 135 μmol/L showing similar low affinity in all three *in vitro* systems investigated. The K_i values for inhibition by omeprazole is in the concentration range of 19 to 68 μmol/L. The pronounced inhibition of cytochrome P450 in presence of lansoprazole with the range of K_i values between 7.4 and 34 μmol/L is of the mixed type similar to pantoprazole and omeprazole for 7-ethoxycoumarin dealkylase.

In vitro studies need to be confirmed by *in vivo* findings. Administration of omeprazole (40 mg over 7 days) in man prolonged the half-life of diazepam from 37 to 85 hr [15]. In rats, 50% prolongation of the diazepam effect was observed after dosing of 281 μmol/kg omeprazole. The same effect was obtained after 170 μmol/kg lansoprazole but was not found below 1000 μmol/kg pantoprazole [16, 25]. In pharmacological studies in rats, the range of ID_{50} (μmol/kg; p.o.) for inhibition of acid secretion is between 150 and 300 μmol/kg for omeprazole and between 25 and 40 μmol/kg for pantoprazole [16]. Whereas the ID_{50} values for inhibition of acid secretion and those for 50% prolongation of diazepam effect for omeprazole coincide with this concentration range, this is not the case for pantoprazole where we observe concentrations at least one order of magnitude higher necessary for a 50% prolongation of the diazepam effect in comparison to the ID_{50} for acid secretion. For lansoprazole only interaction studies with diazepam are available and these show a 50% prolongation already at 170 μmol/kg.

Although it was possible to demonstrate the interaction of the benzimidazole sulphoxides with cytochrome P450 in animals, these studies were performed only with one substrate. In contrast to the interaction with the diazepam metabolism, the biotransformation of propranolol seems not to be affected by omeprazole [26]. Therefore, the conclusion for biotransformation studies *in vivo* as well as *in vitro* should be to use a range of various substrates for cytochrome P450 which are metabolized by different typical pathways of biotransformation. This procedure should provide us with sufficient information about the interaction of a new drug in the body.

In summary, the use of the three different enzymatic systems, 7-ethoxycoumarin dealkylase, ethylmorphin demethylase and lonazolac hydroxylase, for testing interactions with cytochrome P450 seems to be helpful for evaluating possible drug interactions *in vivo*. The three biotransformation reactions are catalysed by three different enzymes. In all experiments, pantoprazole showed much lower affinity to cytochrome P450 than omeprazole or lansoprazole.

REFERENCES

1. Guengerich FP, Characterization of human microsomal cytochrome P-450 enzymes. *Annu Rev Pharmacol Toxicol* **29**: 241–264, 1989.
2. Greenlee WF and Poland A, An improved assay of 7-ethoxycoumarin O-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J Pharmacol Exp Ther* **205**: 596–605, 1978.
3. Thompson JA and Holtzman JL, Kinetics of ethylmorphine *N*-demethylase at low substrate concentrations and its implications concerning the role of

the stimulation of NADPH-cytochrome P-450 reductase activity in mixed-function oxidase activity. *Biochem Pharmacol* **26**: 1803–1807, 1977.

4. Simon WA and Hopley JP, HPLC and its application for rapid analysis of cytochrome P-450 mediated xenobiotic metabolism *in vitro*. *Third European Congress of Biopharmaceutics and Pharmacokinetics Proceedings*, *Freiburg* Vol. II, pp. 606–612, 1987.
5. Fellenius E, Berglindh T, Brändström A, Elander B, Helander HF, Olbe L, Sachs G, Sjöstrand SE and Wallmark B, The inhibitory action of substituted benzimidazoles on isolated oxyntic glands and H^+-K^+-ATPase. In: *Hydrogen Ion Transport in Epithelia* (Eds. Schulz I, Sachs G, Forte J and Ullrich KJ), pp. 193–202. Elsevier, Amsterdam, 1980.
6. Fellenius E, Berglindh T, Sachs G, Olbe L, Elander B, Sjöstrand SE and Wallmark B, Substituted benzimidazoles inhibit gastric acid secretion by blocking (H^+,K^+)ATPase. *Nature* **290**: 159–161, 1981.
7. Wallmark B, Sachs G, Mardh S and Fellenius E, Inhibition of gastric (H^+,K^+)-ATPase by the substituted benzimidazole, picoprazole. *Biochim Biophys Acta* **728**: 31–38, 1983.
8. Simon WA, Sturm E and Kohl B, Inhibition of (H^+,K^+)-ATPase by 2-((2-pyridylmethyl)-sulphinyl)-benzimidazoles: improved pH selectivity of di-alkoxypyridyl-derivatives. *Gastroenterol Int* **1**: 1157 (abstract), 1988.
9. Kohl B, Sturm E, Simon WA, Schaefer H and Keeling DJ, Selection of BY1023/SK&F96022 from a new series of H^+,K^+-ATPase inhibitors. *Gastroenterology* **96**: A 264 (Abstract), 1989.
10. Simon WA, Keeling DJ, Laing SM, Fallowfield C and Taylor AG, BY1023/SK&F96022: biochemistry of a novel ($H^+ + K^+$)-ATPase inhibitor. *Biochem Pharmacol* **39**: 1799–1806, 1990.
11. Satoh H, Inatomi N, Nagaya H, Inada I, Nohara A and Maki Y, Antisecretory activity of AG-1749, a proton pump inhibitor. *Jpn J Pharmacol* **40**: 226 P (abstract), 1986.
12. Ganser AL and Forte JG, K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochim Biophys Acta* **307**: 169–180, 1973.
13. Chang H, Saccomani G, Rabon E, Schackmann R and Sachs G, Proton transport by gastric membrane vesicles. *Biochim Biophys Acta* **464**: 313–327, 1977.
14. Chenery RJ, Ayrton A, Oldham HG, Norman SJ and Standring P, The interaction of omeprazole with rat liver cytochrome P450-mediated monoxygenase reactions *in vitro* and *in vivo*. *Biochem Pharmacol* **37**: 1407–1414, 1988.
15. Gugler R and Jensen JC, Omeprazole inhibits oxidative drug metabolism. *Gastroenterology* **89**: 1235–1241, 1985.
16. Kromer W, Postius S, Riedel R, Simon WA, Hanauer G, Brand U, Gönne S and Parsons ME, BY 1023/SK&F96022, a novel gastric proton pump inhibitor, potently inhibits acid secretion but lacks relevant cytochrome P-450 interactions. *J Pharmacol Exp Ther* **254**: 129–135, 1990.
17. Tu YY, Peng R, Chang Z and Yang S, Induction of a high affinity nitrosamine demethylase in rat liver microsomes by acetone and isopropanol. *Chem Biol Interact* **44**: 247–260, 1983.
18. Nash T, The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* **55**: 416–421, 1953.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Lineweaver H and Burk D, The determination of enzyme dissociation constants. *J Am Chem Soc* **56**: 658–666, 1934.
21. Dixon M, The determination of enzyme inhibitor constants. *Biochem J* **55**: 170–171, 1953.
22. Cornish-Bowden A, A simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. *Biochem J* **137**: 143–144, 1974.
23. Nebert DW and Gonzalez FJ, P450 genes: structure, evolution, and regulation. *Annu Rev Biochem* **56**: 945–993, 1987.
24. Netter KJ, Inhibition of oxidative drug metabolism in microsomes. *Pharmac Ther* **10**: 515–535, 1980.
25. Hanauer G, Graf U and Meißner T, *In vivo* cytochrome P450 interactions of the newly developed H^+,K^+-ATPase inhibitor pantoprazole (BY1023/SK&F96022) compared to other anti-ulcer drugs. *Methods Findings* **13**: 63–68, 1991.
26. Henry D, Brent P, Whyte I, Mihaly G and Devenish-Meares S, Propanolol steady-state pharmacokinetics are unaltered by omeprazole. *Eur J Clin Pharmacol* **33**: 369–373, 1987.