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THE INTERACTION OF OMEPRAZOLE WITH RAT LIVER CYTOCHROME P_{450}-MEDIATED MONOOXYGENASE REACTIONS *IN VITRO* AND *IN VIVO*

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Abstract—The effect of omeprazole on cytochrome P_{450}-mediated monooxygenase reactions was assessed in rat liver S9 utilising ethylmorphine-*N*-demethylase (EM) and ethoxycoumarin-*O*-deethylase (ECOD) activities. The inhibition of EM by omeprazole was judged to be predominantly reversible in mechanism. The average K_i for omeprazole was 40 ± 27 μM with EM and 76 ± 6 μM with ECOD in four separate rats. In preparations of rat hepatocytes the intrinsic clearance of diazepam was decreased substantially by 50 μM omeprazole (average inhibition 73%). In comparison 50 μM cimetidine inhibited the intrinsic clearance of diazepam by 50%. The relationship between these two *in vitro* models for drug interactions is discussed in the context of previously published drug inhibition data. Moreover, repeated administration of omeprazole to adult male rats ($500\ mg \cdot kg^{-1}$, 14 days, p.o.) resulted in statistical increases in liver weight, cytochrome P_{450} and ECOD activity. Thus omeprazole interacts with the mixed function oxidase system *in vitro* and *in vivo*.

Drug inhibition studies are often performed *in vitro* with subcellular preparations, providing valuable insight into the mechanism and potency of interactions with cytochrome P_{450} and other enzyme systems [1–3]. Such studies often have only limited predictive value for interactions *in vivo*, however, because they generally indicate the potential of a structure to interact with cytochrome P_{450} without indicating the extent of any such interaction. This arises from a number of reasons including the difficulty in comparing clinically used drugs studied *in vivo* with the probe compounds often employed *in vitro*. Studies with clinically used drugs in hepatocyte systems may therefore offer advantages in these respects in that the test compound is subject to elimination through metabolism. More importantly, it is difficult to account for the very many pharmacokinetic parameters which influence drug metabolism *in vivo*. We have previously used cultured rat hepatocytes successfully to investigate drug interactions with conjugation pathways [3]. In this study we have investigated the interaction between diazepam, a drug used extensively in the evaluation of clinical drug interactions, and omeprazole in cultured rat hepatocytes and have compared interactions with those in rat liver S9 systems using standard probe compounds.

The objective of the study is to assess the extent of prediction inherent in these *in vitro* techniques compared to reported clinical observations on the interaction of omeprazole with the cytochrome P_{450} system [4–8].

Omeprazole is a substituted benzimidazole which inhibits gastric acid secretion in animals and man [9]. Benzimidazole derivatives are known to inhibit hepatic microsomal mixed-function oxidase activity *in vitro* [10] and omeprazole has been reported to cause decreases in the plasma clearance of diazepam and phenytoin in man [7], despite the fact that omeprazole is rapidly eliminated by hepatic metabolism [7, 11]. We have, in addition, investigated the ability of omeprazole to induce the cytochrome P_{450} system in male rats to determine the possible importance of enzyme induction for *in vivo* interaction experiments involving multiple doses of omeprazole.

MATERIALS AND METHODS

Materials

Omeprazole (SK&F 95111; Batch 5) was synthesized and provided by the Department of Synthetic and Isotope Chemistry, SK&F, Welwyn. SK&F 92334-A, cimetidine hydrochloride (Batch 190/58), was obtained from the Pharmaceutical Development Department, SK&F Welwyn. Diazepam, *N*-desmethyldiazepam, 3-hydroxymethyldiazepam and oxazepam were kindly provided as a gift from Roche Products Limited, Welwyn Garden City, Herts.

Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, HEPES [4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid] and ethyleneglycol-bis-(β-amino ethyl ether) *N*,*N*′-tetra acetic acid (EGTA) were obtained from Sigma (Poole, Dorset, U.K.).

Ethylmorphine hydrochloride (EM) was supplied by May & Baker (Dagenham, Essex, U.K.), 7-ethoxycoumarin and 7-hydroxycoumarin by Aldrich Chemical Company (Dorset, U.K.). Deoxyribonuclease (type I, DNase), Insulin (bovine pancreas), bovine serum albumin (BSA, fraction V) and Trypan blue were obtained from Sigma (Poole, Dorset, U.K.). Collagenase from *Clostridium histolyticum* was obtained from Boehringer Corporation (London), Ltd.

Williams Medium E (WME) was obtained from Flow (Irvine, U.K.). Newborn calf serum, L-glutamine, penicillin, streptomycin and neomycin from Gibco (Uxbridge, U.K.). Soluble collagen was obtained from Worthington (Millipore, Corp., Paisley, U.K.). Multiwell plates (35 mm) were purchased from Sterilin (Teddington, Middlesex, U.K.).

Rats (Wistar, male) were obtained in the range 180–200 g from the SK&F colony and were housed on grade 6 greenwood granules in polypropylene cages. Free access to tap water and PRD pellets (Labsure, Poole, Dorset, U.K.) was provided.

Methods

Preparation of rat liver 10,000 g supernatant. Rats were killed by cervical dislocation, the livers excised rapidly and washed in ice-cold aqueous potassium chloride (KCl, 1.15% w.v; 0.154 M). All subsequent procedures were carried out at 4°. The tissue was blotted dry, weighed and homogenised in three volumes of KCl using a Potter-Elvehjem glass–Teflon homogeniser driven at 1200 rpm. The suspension was centrifuged (20 min, 10,000 *g*; Sorvall RC5-B centrifuge, SA-6000 rotor). The supernatant (10,000 *g*) was diluted (1:1 v/v) with KCl (0.154 M) and used directly.

Preparation of hepatocytes. Hepatocytes were prepared by the technique of Strom *et al.* [12]. Rats were killed by cervical dislocation, the livers removed and stored under ice-cold saline. Lobes were removed with a scalpel and a major vessel of the lobe cannulated with a polyethylene catheter (16 gauge, Medicut).

Perfusion was started immediately at a flow rate of 6 ml.min^{-1} and resulted in a blanching of the majority of the lobe. The perfusate consisted of aqueous sodium chloride (NaCl, 142 mM), KCl (7 mM), HEPES (10 mM, pH 7.4) and EGTA (40 μM). After 3–5 min, the perfusate was changed to NaCl (142 mM), KCl (7 mM), HEPES (10 mM, 7.4), aqueous calcium chloride ($CaCl_2$, 1 mM) and 1 mg.ml^{-1} collagenase and maintained on a recycle mode for 5–7 min. A Watson-Marlow 502 pump with a multichannel head was used to maintain flow and enabled several lobes to be perfused simultaneously. Temperature at the lobe was maintained at 37° by means of a standard water bath set at 40°, a 3° temperature loss occurring along the perfusion tubing.

The lobe was then removed from the perfusion apparatus and placed under cold buffer [NaCl (142 mM), KCl (7 mM), HEPES (10 mM, pH 7.4) containing 1% w/v BSA and 4 mg DNase I per 100 ml)]. The capsule was then gently folded back and the hepatocytes released by gentle agitation of the lobe. The cell suspension was then filtered through a nylon mesh (64 μm) and centrifuged (approximately 100 *g*, 5 min, 4°). Cells were resuspended in fresh buffer and the washing procedure repeated three times. Hepatocytes were then resuspended in buffer (final volume 10 ml). DNase I was omitted from the final two washes. Hepatocytes were then counted in a haemocytometer in the presence of 0.04% Trypan blue. Yields of 30–40 $\times$ 10^6 cells were commonly achieved from each lobe with a viability in excess of 90%.

For culture studies, hepatocytes were quickly diluted into culture medium consisting of Williams Medium E containing L-glutamine (4 mM), Streptomycin (100 μg.ml^{-1}), penicillin (100 IU.ml^{-1}), neomycin (100 μg.ml^{-1}), insulin (0.02 IU.ml^{-1}) and newborn calf serum (10% v/v). The cell suspension was then seeded on to 35 mm wells (Sterilin) which had been coated with soluble collagen. The culture dishes were then introduced into a 37° incubator (T.R. Heraeus) containing 5% CO_2 in a water saturated atmosphere. Cell attachment could be detected after about 1 hr in culture.

The protein content of culture dishes were determined by the method of Lowry *et al.* [13] after solubilization of the protein in 1.0 M NaOH.

Measurement of enzyme activities. Two cytochrome P-450-mediated activities were measured in rat liver 10,000 *g* supernatants. Each activity was measured at a range of substrate concentrations to enable K_m and V_{max} parameters to be determined. Ethylmorphine *N*-demethylation was measured by the liberation of formaldehyde from ethylmorphine [14, 15] in a final volume of 1 ml containing an NADPH generating system [NADP (0.5 mM), glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 unit)], enzyme source (250 μl 10,000 *g*), HEPES buffer (0.1 M, pH 7.4), semicarbazide (5 mM) and substrate. Incubation time was 15 min at 37°, the tubes being shaken at 100 cycles.min^{-1}.

7-Ethoxycoumarin *O*-deethylation [16] was measured by the formation of 7-hydroxycoumarin in a final assay volume of 1.5 ml which contained the NADPH generating system described above, enzyme source (500 μl, 10,000 *g* supernatant), HEPES buffer (0.1 M, pH 7.6) and substrate. Incubation time was 5 min at 37°, the tubes being shaken at 100 cycles.min^{-1}.

Metabolism of diazepam. The metabolism of diazepam by rat hepatocytes was measured at 15 min intervals over 120 min. Diazepam concentration in the culture medium was measured by h.p.l.c. utilizing a Hewlett-Packard 1090 liquid chromatograph fitted with autoinjector and a filter-photometric detector (230 nm). Quantification was performed with a Hewlett-Packard 3392A integrator. Separation was achieved on a Hewlett-Packard 5 μm ODS (100 $\times$ 2.1 mm) microbore column. An isocratic solvent system consisting of methanol (55%) and ammonium acetate (0.005 M, pH 6.0 adjusted with *o*-phosphoric acid, 45%) was employed with a flow rate of 0.5 ml.min^{-1}. Oven temperature was 70°. Retention time of standards in minutes in this system were as follows (N = number of replicate injections): oxazepam, 1.255 $\pm$ 0.002 (N = 40); 3-hydroxydiazepam, 1.506 $\pm$ 0.002 (N = 40); *N*-demethyldiazepam, 1.723 $\pm$ 0.004 (N = 40); Diazepam, 2.116 $\pm$ 0.002 (N = 40).

Standard curves were constructed from five concentration levels, injected in duplicate, for each compound.

Treatment of data. Kinetic data were analysed by various computer curve fitting routines. The Michaelis–Menten kinetic parameters, K_m and V_{max} were determined by a rectangular hyperbolae iterative fit programme from Wilkinson [17].

K_m^{app} and V_{max}^{app} are defined as the experimentally determined K_m and V_{max} respectively. K_i (inhibitor dissociation constant) was determined by a variety of techniques, dependent upon the observed mechanism of inhibition, e.g. the computer programme $RMICH_2$ was used in the case of competitive inhibition. The following equation for inhibition was assumed to apply:

$$V = \frac{V_{max}}{1 + \frac{K_m}{S}\left(1 + \frac{i}{K_i}\right) + \frac{i}{\alpha K_i}}$$

where $\alpha = K'_m/K_m = K'_i/K_i$ and i = inhibitor concentration. K'_m is the equilibrium dissociation constant for the binding of substrate to the enzyme–inhibitor complex. The parameter α relates to the mechanism of inhibition of the enzyme in the following way [18]:

α Value	Type of inhibition
0	Uncompetitive
1	Non-competitive
∞	Competitive

The disappearance of diazepam and omeprazole from the culture medium with time were analysed with an integrated form of the Michaelis–Menten equation (1).

$$\frac{d(C_o - C_t)}{dt} = \frac{V_m \cdot C_t}{K_m + C_t} \quad (1)$$

Integrating and rearranging:

$$\ln \frac{C_o}{C_t} = \frac{V_m}{K_m} \cdot t - \frac{(C_o - C_t)}{K_m} \quad (2)$$

If $C_o \ll K_m$ then

$$\ln \frac{C_o}{C_t} = \frac{V_m}{K_m} \cdot t \quad (3)$$

Further rearrangement of equation (2) (J. R. Gillette, personal communication) gives:

$$\frac{t}{C_o - C_t} = \frac{1}{V_m} + \frac{K_m}{V_m} \frac{\ln (C_o/C_t)}{C_o - C_t} \quad (4)$$

Hence equation (3) yields an estimate of intrinsic clearance (i.e. V_{max}/K_m) whereas equation (4) yields estimates of intrinsic clearance, K_m and V_m for the reaction.

RESULTS

Reversibility of interaction with cytochrome P-450

The reversibility of the interaction of omeprazole with EM in 10,000 *g* supernatant was investigated in two studies. Table 1 shows the effect of different preincubation times with omeprazole (50 μM) on EM activity. At 2.5 min omeprazole caused a 31% inhibition of EM activity compared with control (0.1% DMF). As the preincubation time with omeprazole increased, a small but steady increase in the effect of omeprazole on EM activity was noted. Thus at 20 min preincubation, the degree of inhibition of EM was increased to 40%. Since these data are not consistent with predominantly time-dependent activity loss a predominantly reversible mechanism of inhibition was assumed to be operating.

Table 1. The effect of preincubation with omeprazole (50 μM) on ethylmorphine *N*-demethylation by rat liver 10,000 g supernatant

Preincubation time (min)	Initial rate (nmol.min^{-1}.g liver^{-1}) Control	DMF (0.1%)	Omeprazole (50 μM)
2.5	207 ± 7	205 ± 4	142 ± 25
5.0	201 ± 8	198 ± 5	136 ± 5
10.0	196 ± 3	189 ± 7	123 ± 7
20.0	190 ± 6	193 ± 3	117 ± 2

Pooled supernatant from six rat livers was used to evaluate the reversibility of EM inhibition by omeprazole. Ethylmorphine was used at 363 μM (K_m concentration) and dimethylformamide (DMF, 0.1%) was used as cosolvent to dissolve omeprazole. Results are expressed as mean ± SD from six replicate tubes.

Table 2 shows the effect of preincubation of 10,000 *g* supernatants for 5 min at two concentrations of omeprazole (50 μM and 500 μM) on EM activity. Enzyme activity was then measured in the presence of 50 μM omeprazole. No significant difference was found in the final degree of inhibition by omeprazole suggesting a largely reversible mechanism of inhibition.

Interaction of omeprazole with EM and ECOD

The effect of omeprazole on EM activity in 10,000 *g* supernatant from rat liver is shown in Fig. 1 and these data were used to estimate K_m^{app} and V_{max}^{app} (Table 3) and K_i values (Table 4). In four separate rats omeprazole caused a concentration-dependent increase in K_m^{app} and a small

Table 2. The effect of preincubation (5 min) with different concentrations of omeprazole on ethylmorphine *N*-demethylation by rat liver 10,000 *g* supernatant

Omeprazole conc (μM) Preincubation	Assay	Initial rate (nmol.min^{-1}.g liver^{-1})
0	0	228 ± 9
50	50	73 ± 18
500	50	56 ± 17

Pooled supernatant from six rats was used to evaluate the reversibility of EM inhibition by omeprazole. The EM assay was performed at a single concentration of omeprazole and therefore if the inhibition is unchanged by the concentration during the preincubation period then a totally reversible process is involved. Results are expressed as mean ± SD from six replicates.

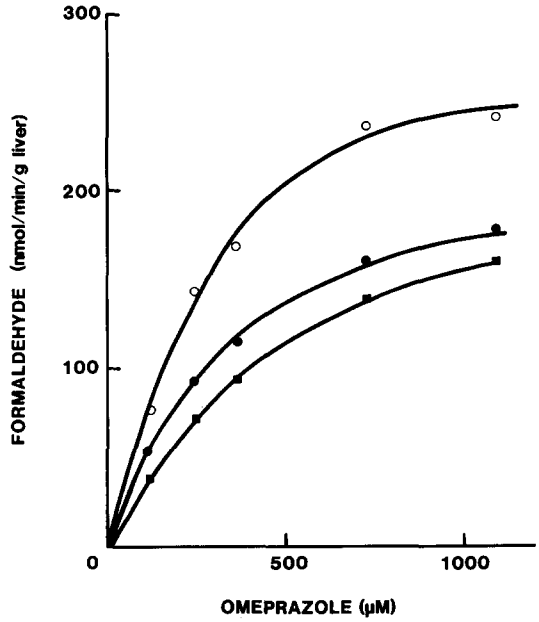

Fig. 1. The effect of omeprazole on EM activity in rat liver S9. Determinations were made in triplicate at 0 μM (open circles), 50 μM (close circles) and 100 μM (squares) omeprazole. One of four rats is presented.

Table 4. The apparent K_i for the inhibition of ethylmorphine *N*-demethylation by omeprazole in rat liver 10,000 *g* supernatant

Rat	K_i	α
	Calculated from Secondary Plots	
1	67	6
2	56	2
3	29	9
4	8	319
Mean ± SD	40 ± 27	84 ± 157
	Calculated from RMICH2	
1	43	
2	24	
3	23	
4	14	
Mean ± SD	26 ± 12	

decrease in V_{max}^{app}. The average K_i was calculated to be 40 ± 27 μM (range 8–67 μM) when calculated from secondary plots and 26 ± 12 μM (range 14–43 μM) when calculated by the RMICH2 programme. Taken together, this information suggests that a simple competitive mechanism of inhibition does not adequately describe the interaction.

The effect of omeprazole on ECOD activity in 10,000 *g* supernatant from rat liver is shown in Fig. 2 and these data were used to calculate K_m^{app} and V_{max}^{app} (Table 5) and K_i values (Table 6). In four separate rats a concentration-dependent increase in K_m^{app} was noted without any statistically significant change in V_{max}^{app}. This would indicate a completely competitive interaction with ECOD. Thus, calculated K_i values were 76 ± 6 μM (range 72–85 μM) by the secondary plot method and 70 ± 8 μM (range 64–82 μM) when calculated by the RMICH2 programme.

Interaction of omeprazole with diazepam metabolism by rat hepatocytes

Characteristics of diazepam metabolism in rat hepatocytes. Diazepam was found to be metabolised rapidly in the rat hepatocyte system. The rate of diazepam disappearance, expressed as the intrinsic clearance (Cl_{int}) was calculated with equation (3) for hepatocyte preparations from five rats. The value obtained was 1.2 ± 0.3 ml.hr^{-1}.mg protein^{-1} (range 0.6–1.4) suggesting that the hepatocyte preparations are highly reproducible in terms of metabolic capability for a given initial concentration of diazepam (data not shown).

The effect of initial diazepam concentration on the apparent clearance of diazepam was investigated (data not shown). The initial clearance of diazepam

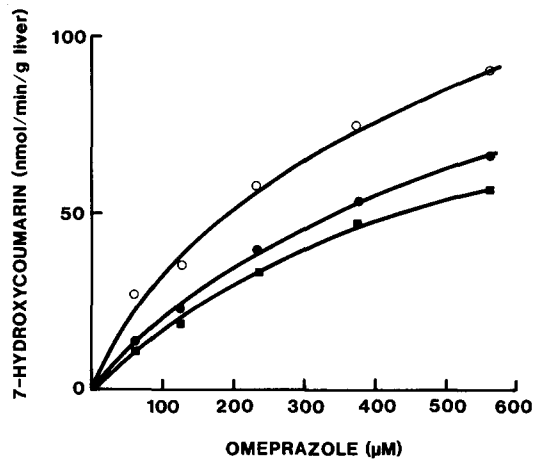

Fig. 2. The effect of omeprazole on ECOD by rat liver S9. Determinations were made in triplicate at 0 μM (open circles), 50 μM (close circles) and 100 μM (squares) omeprazole. One of four rats is presented.

Table 3. The effect of omeprazole on the K_m^{app} and V_{max}^{app} for ethylmorphine *N*-demethylation by rat liver 10,000 *g* supernatant

Rat	Omeprazole	K_m^{app} (μM)	V_{max}^{app} (nmol.min^{-1}.g liver^{-1})
Combined (1–4)	0	341 ± 45	293 ± 41
	50	580 ± 180	212 ± 31
	100	1163 ± 845	214 ± 44

Table 5. The effect of omeprazole on the K_m^{app} and V_{max}^{app} for ethoxycoumarin *O*-deethylation by rat liver 10,000 g supernatant

Rat	Omeprazole (μM)	K_m^{app} (μM)	V_{max}^{app} (nmol.min^{-1}.g liver^{-1})
Combined (1–4)	0	296 ± 78	128 ± 26
	50	495 ± 187	119 ± 34
	100	760 ± 357	138 ± 53

Table 6. The apparent K_i for inhibition of ethoxycoumarin *O*-deethylation by omeprazole in rat liver 10,000 *g* supernatant

Rat	K_i (μM)	α
	Calculated from Secondary Plots	
1	72	102
2	72	NC
3	74	159
4	85	9
Mean ± SD	76 ± 6	NC
	Calculated from RMICH2	
1	64	
2	82	
3	65	
4	67	
Mean ± SD	70 ± 8	

NC = Not calculated.

was markedly reduced at 50 μM diazepam compared to that at 15 μM diazepam suggesting that saturation of the metabolic pathways of diazepam was occurring. Data from this experiment were used to estimate an overall K_m and V_{max} for the disappearance of diazepam by employing an integrated form of the Michaelis–Menten equation (equation 4) and indicate that the rat hepatocyte system has a very low K_m of about 10 μM.

Authentic standards of diazepam and several metabolites were available and could be resolved by h.p.l.c. The identity and amount of metabolites accumulating in the culture medium after incubation with hepatocytes for 120 min was investigated both before and after hydrolysis with bacterial β-glucuronidase. After hydrolysis only about 40% of the metabolised diazepam could be accounted for in terms of authentic standards. The metabolites ident-

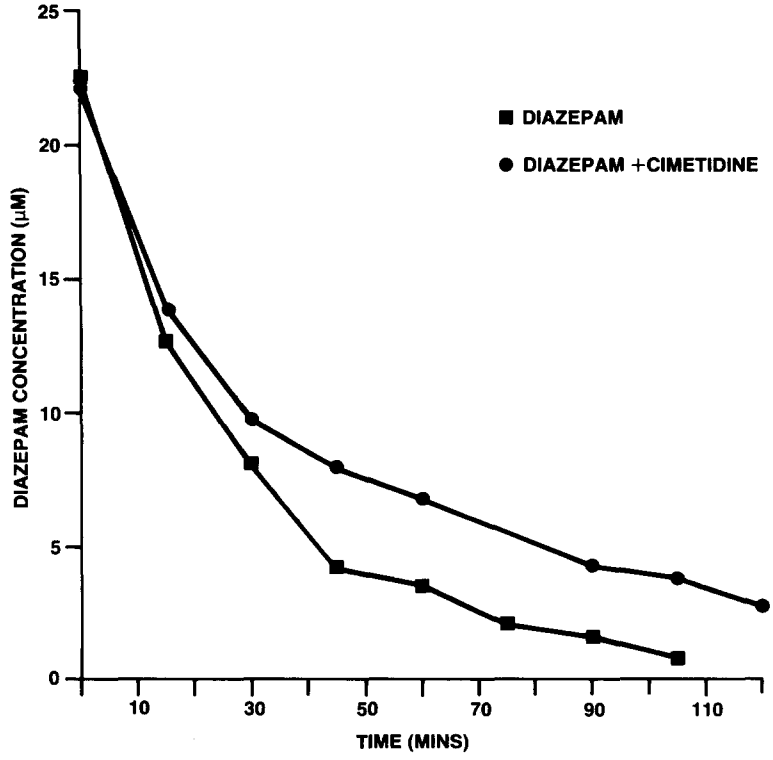

Fig. 3. The effect of cimetidine (50 μM) on diazepam clearance in cultured rat hepatocytes. Each data point represents a single determination. The experiment was repeated with essentially similar results. Clearance values are reported in the text.

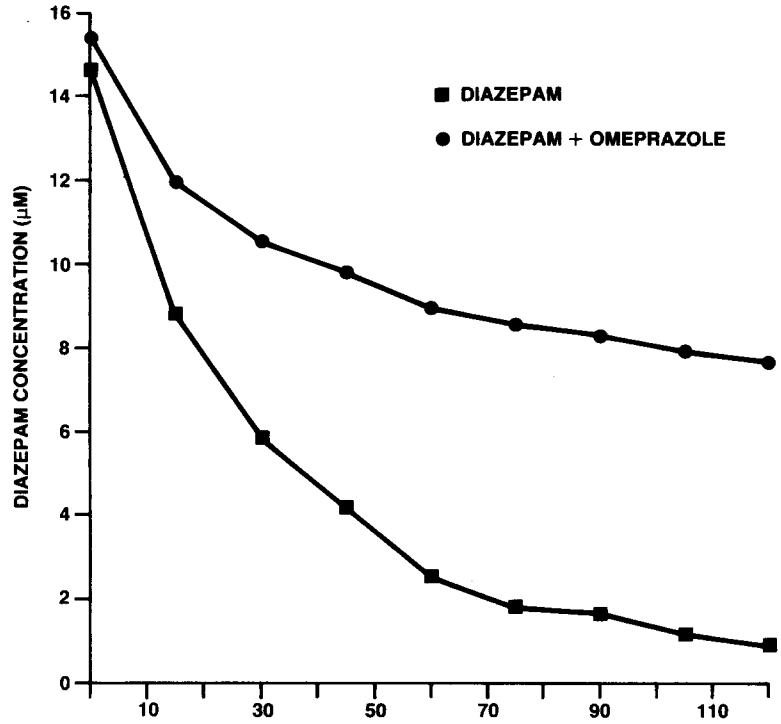

Fig. 4. The effect of omeprazole (50 μM) on diazepam clearance by rat hepatocytes. Each data point represents a single determination. The experiment was repeated with essentially similar results. Clearance values are reported in the text.

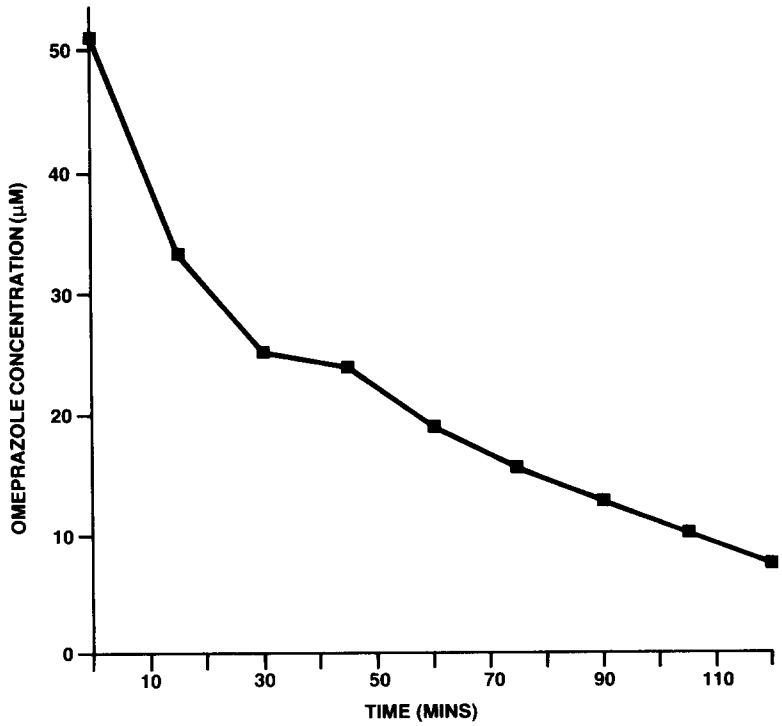

Fig. 5. Omeprazole disappearance in rat hepatocyte culture in the presence of diazepam. Each data point represents a single determination. The experiment was repeated with essentially similar results. Clearance values are reported in the text.

ified by co-injection were nordiazepam, temazepam and 4'-hydroxy-diazepam.

Interaction of cimetidine with diazepam metabolism

Figure 3 shows the inhibition of diazepam disappearance by cimetidine (50 μM). The inclusion of cimetidine in the culture medium caused an increase in the AUC and a decrease in calculated Cl_{int} from 1.2 ml.hr^{-1}mg protein^{-1} to 0.6 ml.hr^{-1}mg protein^{-1} indicating inhibition of diazepam metabolism by cimetidine.

Interaction of omeprazole with diazepam metabolism

The inhibition of diazepam metabolism by omeprazole was investigated in two separate preparations of rat hepatocytes (Fig. 4). In both preparations the inclusion of omeprazole (50 μM) in the incubation medium greatly increased the $AUC_{-\infty}$ for diazepam and decreased the calculated Cl_{int} value. In one preparation the Cl_{int} was decreased from 1.4 to 0.3 ml.hr^{-1}.mg protein^{-1} whilst in the other the Cl_{int} was decreased from 0.6 to 0.2 ml.hr^{-1}.mg protein^{-1}. The concentration of omeprazole was monitored in the culture medium throughout the incubation period (Fig. 5).

Induction study

The effect of repeated oral dosing of male rats with omeprazole (500 mg.kg^{-1}) is shown in Table 7. Cytochrome P_{450} activity was measured in liver S9 24 hr after the last dose. Omeprazole caused significant increases in liver weight, cytochrome P_{450} and ECOD activity but small and significant decreases in EM activity.

DISCUSSION

Only very limited data have been published concerning the interaction of omeprazole with cytochrome P_{450} in subcellular fractions. The IC_{50} of omeprazole with rat liver microsomes incubated with ethoxycoumarin, aminopyrine and *p*-nitroanisole has been reported [11] as has the K_i for interaction with ethoxycoumarin in microsomes from control, phenobarbital and 3-MC treated rats [19]. It is of interest that the K_i values obtained in our laboratories for EM and ECOD are an order of magnitude smaller (higher affinity) than those reported above.

Table 7. The effect of omeprazole (500 mg.kg^{-1}, p.o., 14 days) on liver weight, cytochrome P_{450}, EM and ECOD activity

Parameter	Vehicle	Omeprazole
Liver weight (*g*)	13.7 ± 1.6	15.9 ± 1.2 (P < 0.05)
Cytochrome P_{450} (nmol.g liver^{-1})	22.8 ± 3.2	30.9 ± 3.8 (P < 0.01)
EM 363 μM (nmol.min^{-1}.g liver^{-1})	167 ± 18	151 ± 11 (P < 0.01)
ECOD 186 μM (nmol.min^{-1}.g liver^{-1})	62.1 ± 4.0	166 ± 11 (P < 0.01)
ECOD 930 μM (nmol.min^{-1}.g liver^{-1})	103 ± 4	234 ± 23 (P < 0.01)

Results are expressed as mean ± SD from five male rats. All measurements were performed on Day 15 (24 hr after the last dose).

The average K_i for omeprazole was 40 ± 27 μM with EM in four separate rats and the average K_i for omeprazole was 76 ± 6 μM with ECOD in four separate rats. The mechanism of inhibition was shown to be predominantly reversible with EM. With ethoxycoumarin as substrate the inhibition was shown to be competitive in nature but with ethylmorphine the interaction could be best described as mixed. In comparison when inhibition by cimetidine was investigated we obtained K_i values of 147 μM with EM and 152 μM for ECOD. This greater *in vitro* potency of omeprazole compared to cimetidine is in agreement with work by Gugler and Jensen [7]. These authors suggested that in human microsomes the K_i of omeprazole is approximately three times lower than that of cimetidine, which is in close agreement with our results. The magnitude of the *in vitro* interaction of omeprazole with EM and ECOD would suggest a significant potential to interact with the cytochrome P_{450} system *in vivo*.

It is apparent, however, that many factors *in vivo* can influence the degree of observed inhibition other than the potency of interaction with cytochrome P_{450}. In order to assess whether some of these factors may be accounted for by utilizing an intact cellular system the inhibitory effect of omeprazole in rat hepatocytes on diazepam clearance was investigated and compared with cimetidine. The inclusion of cimetidine in the culture medium at similar concentrations caused a decrease in the calculated clearance of diazepam by 50%. In comparison omeprazole caused a 67% and 79% inhibition of diazepam clearance in two separate experiments. Omeprazole concentrations were monitored throughout the incubation period and were found to decrease from 50 μM to below 10 μM by 2 hr. It is therefore difficult to attribute the extended inhibition of diazepam metabolism to omeprazole alone and extended inhibition is possibly the consequence of the generation of inhibitory metabolites from omeprazole in the culture medium.

In these respects the hepatocyte system very closely models the clinical situation. Omeprazole is cleared from the bloodstream, with an apparent oral plasma clearance of 250 ml.kg^{-1}.hr in man [7]. Thus, no measurable plasma concentrations of omeprazole were seen 9 hr after drug administration and yet the inhibition of diazepam metabolism was still marked after 12 hr of dosing [7]. Similar observations were made by Webster *et al.* [19] in that the inhibition of antipyrine elimination in perfused rat liver was sustained for 4 hr even though omeprazole concentrations were undetectable by 90 min. This possible inhibitory effect of omeprazole metabolites on the cytochrome P_{450} system makes comparison of potency with the S9 interactions difficult but the target concentration of 50 μM omeprazole in the hepatocyte experiments approximated the K_i values obtained in the S9 system and achieved greater than 50% inhibition of diazepam clearance. Thus, omeprazole caused inhibition with all three substrates tested indicating that a variety of metabolic interactions will be seen with omeprazole in man.

It should be emphasised that species differences in cytochrome P_{450} isozyme content may be an impor-

tant factor in any prediction of interaction in man [20–22]. Thus, diazepam metabolism exhibits marked species differences in both rates and routes of metabolism [23–25]. Nordiazepam, temazepam and 4′-hydroxydiazepam were detected in the culture medium together with several unidentified metabolites but it is known that the 4′-hydroxylation pathway is of no importance in man and that only nordiazepam and temazepam accumulate on incubations with human hepatocytes (unpublished observations).

The induction of cytochrome P_{450} systems by oral dosing of omeprazole has not been directly demonstrated. Changes were seen in a number of parameters including liver weight, cytochrome P_{450} content and ECOD activity. The small decreases seen in EM activity may reflect changes in cytochrome P_{450} isozyme profile or inhibition of EM activity by residual drug or metabolite. However, the observation has clear implications for clinical interaction studies where repeated doses of omeprazole are administered [7].

In conclusion, *in vitro* experiments clearly predict the occurrence of interactions of omeprazole with the cytochrome P_{450} system *in vivo*. Studies in S9 or microsomal systems yield information on the potency and specificity of such interactions but not on the duration and overall extent of the interaction. In the case of omeprazole valuable further information was obtained from hepatocyte systems indicating that the duration of cytochrome P_{450} inhibition should outlast the presence of parent compound. Information may also be further optimised by the use of clinical substrates such as diazepam and in particular using hepatocytes from various species including man [26].

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REFERENCES

1. S. Rendic, F. Kajfez and H-H. Ruf, *Drug Metab. Disp.* **11**, 137 (1983).
2. H. G. Oldham and R. J. Chenery, *Biochem. Pharmac.* **34**, 2398 (1985).
3. S. Emery, H. G. Oldham, S. J. Norman ad R. J. Chenery, *Biochem. Pharmac.* **34**, 1415 (1985).
4. D. A. Henry, J. F. Gerkens, P. Brent and K. Somerville, *Lancet* **ii**, 46 (1984).
5. D. A. Henry, K. W. Somerville, G. Kitchingham and M. J. S. Langman, *Br. J. clin. Pharmac.* **18**, 195 (1984).
6. R. Gugler and J. C. Jensen, *Lancet* **i**, 969 (1984).
7. R. Gugler and J. C. Jensen, *Gastroenterology* **89**, 1235 (1985).
8. J. C. Jensen and R. Gugler, *Archs Pharmac.* **239** (Suppl) R16 Abst. 62 (1985).
9. H. Larsson, H. Mattson, G. Sundell and E. Carlsson, *Scand. J. Gastroenterol* **20**, 23 (1985).
10. M. Murray, A. J. Ryan and P. J. Little, *J. Med. Chem.* **25**, 887 (1982).
11. C. G. Regardh, M. Gabrielsson, K. J. Hoffman, I. Lofberg and I. Skanberg, *Scand. J. Gastroenterol.* **20**, 79 (1985).
12. S. C. Strom, R. C. Jirtle, R. S. Jones, D. L. Novicki, M. R. Rosenberg, A. Novotny, G. Irons, J. R. McLain and G. Michalopoulos, *J. natn. Cancer Inst.* **68**, 771 (1982).
13. O. H. Lowry, J. J. Roseborough, A. L. Carr and R. J. Randall, *J. biol. Chem.* **193**, 253 (1951).
14. J. L. Holtzman, T. E. Gram, P. C. Gigon and J. R. Gillette, *Biochem. J.* **110**, 407 (1968).
15. T. Nash, *Biochem. J.* **55**, 416 (1953).
16. V. Ullrich and P. Weber, *Hoppe-Seyler's Z. Physiol. Chem.* **353**, 1171 (1972).
17. G. N. Wilkinson, *Biochem. J.* **60**, 324 (1961).
18. J. L. Webb, in *Enzyme and Metabolism Inhibitors,* Vol. 1, pp. 55–60. Academic Press, New York (1963).
19. L. K. Webster, D. B. Jones, G. W. Mihaly and R. A. Smallwood, *J. Pharm. Pharmac.* **36**, 470 (1984).
20. F. P. Guengerich, G. A. Dannan, S. T. Wright, M. V. Martin and L. S. Kaminsky, *Biochem.* **21**, 6010 (1982).
21. D. W. Nebert and M. Negishi, *Biochem. Pharmac.* **31**, 2311 (1982).
22. I. R. Phillips, E. A. Shepard, A. Ashworth and B. R. Rabin, *Proc. natn. Acad. Sci. U.S.A.* **82**, 983 (1985).
23. S. M. Andrews and C. A. Griffiths, *Xenobiotica* **14**, 751 (1984).
24. T. W. Guentert, *Progress Drug Metab.* Vol. 8, Chap. 5, p. 241 (1984).
25. M. A. Schwartz, B. A. Koechlin, E. Postma, S. Palmer and G. Krol, *J. Ther. Pharmac. exp. Ther.* **149**, 423 (1965).
26. A. Guillouzo, P. Beaune, M. N. Gascoin, J. M. Begue, J. P. Campion, P. F. Guengerich and C. Guguen-Guillouzo, *Biochem. Pharmac.* **34**, 2991 (1985).