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In vitro studies on the interaction of famotidine with liver microsomal cytochrome P-450

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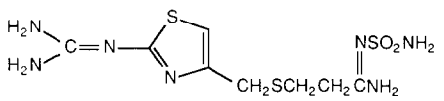
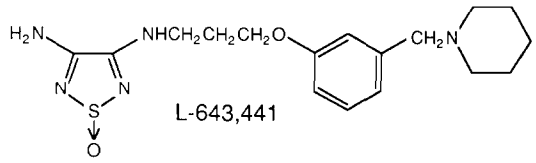
Cimetidine, an H_2-receptor antagonist widely used to treat peptic ulcers, has been found to impair the elimination of other drugs that are metabolized by the hepatic mixed-function oxidase system [1–6]. The interference of cimetidine with oxidative drug metabolism is believed to be due to its binding to cytochrome P-450 [7], which thereby decreases the interaction between cytochrome P-450 and other substrates. Because of the widespread use of H_2-receptor blocking agents, it is important that newly developed therapeutic agents in this class be examined for their ability to interact with cytochrome P-450 in order to assess the potential risk of drug interactions.

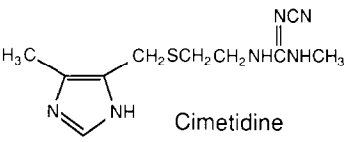
Famotidine, 3-[[[2[(aminoiminomethyl) amino]-4-thiazoyl]methyl]thiol]-*N*-(aminosulfonyl) propanimidamide, is a new H_2-receptor antagonist and has been shown to be more potent and longer-lasting than cimetidine in inhibiting gastric acid secretion in animals and humans [8–12]. Famotidine is structually different from cimetidine in having a thiazole nucleus instead of an imidazole ring (Fig. 1). To examine the potential of drug interactions *in vivo*, the *in vitro* interaction of famotidine with rat and human liver microsomal cytochrome P-450 and its subsequent inhibition of drug metabolism were compared with those of cimetidine. L-643,441, *N*-[3-[3-[1-piperidinylmethyl]-phenoxy]-propyl]-1,2,5-thiadiazole-3,4-diamine-1-oxide, another newly synthesized H_2-receptor antagonist, is also included in this study for comparison.



Famotidine



L-643,441



Cimetidine

Fig. 1. Structures of H_2-receptor antagonists.

Materials and methods

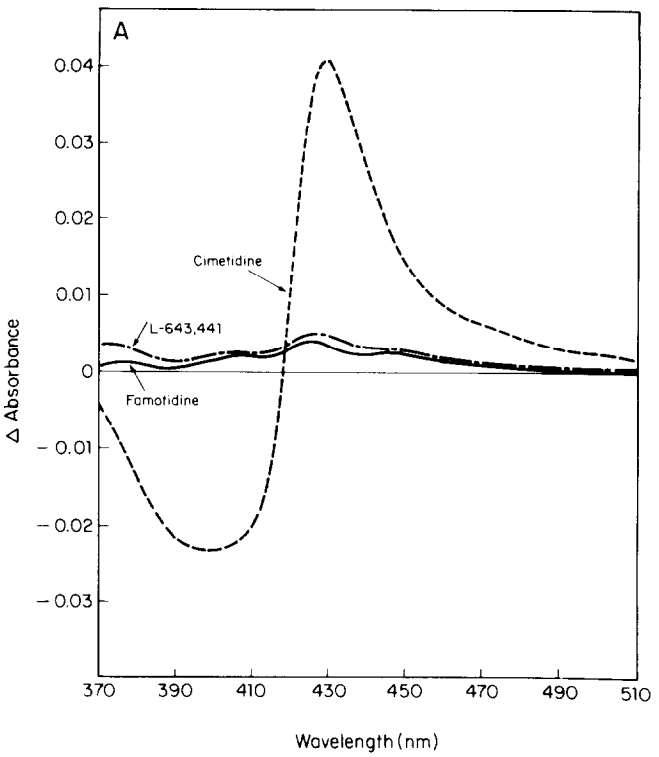
Liver microsomes from untreated, phenobarbital (PB) treated (100 mg/kg/day, i.p. for 4 days), and 3-methylcholanthrene (3-MC) treated (40 mg/kg/day, i.p. for 4 days) male Sprague–Dawley rats were prepared by differential centrifugation. Frozen liver samples from human kidney donors were supplied by Dr. C. Van Bahr, Department of Clinical Pharmacology, Huddinge Hospital, Huddinge, Sweden. Substrate-induced difference spectra were recorded on an Aminco DW-2a spectrophotometer according to Schenkman *et al.* [13]. The O-deethylation of 7-ethoxycoumarin was measured fluorometrically by following the formation of 7-hydroxycoumarin according to the method of Ullrich and Webster [14] with some modifications [15]. The N-demethylation of *d*-[*N*-methyl-^{14}C]benzphetamine was measured by liquid scintillation counting of the [^{14}C]formaldehyde that was trapped *in situ* as the semicarbazone derivative [16]. Famotidine, L-643,441 and cimetidine were synthesized at the Merck Sharp & Dohme Research Laboratories, Rahway, NJ.

Results

Spectral studies on the interaction of famotidine and L-643,441 with microsomal cytochrome P-450. The interaction of famotidine and L-643,441 with cytochrome P-450 was examined by the difference spectra obtained with liver microsomes from untreated or treated rats. Cimetidine was also examined as a positive control. When cimetidine was added at a concentration of 300 μM to liver microsomal suspensions from untreated as well as treated rats, a pronounced difference spectrum with a peak at 431 nm and a trough at approximately 400 nm was obtained. Cimetidine was the only compound to show this type II binding spectrum with all of the rat liver microsomal preparations. L-643,441 gave small spectral changes with liver microsomes from PB- and 3-MC-treated rats. Famotidine, when added at the same concentration, demonstrated little or no difference spectra with all of the rat liver microsomal preparations (Fig. 2).

The interaction of these three H_2-receptor antagonists with cytochrome P-450 was also examined in liver microsomes prepared from human kidney donors. Representative difference spectra for human liver microsomes are illustrated in Fig. 3. At a 300 μM concentration, famotidine and L-643,441 showed little or no difference spectra with human liver microsomes, whereas cimetidine gave a noticeable spectral change.

Effects of famotidine and L-643,441 on cytochrome P-450-catalyzed drug oxidations. The effects of famotidine, L-643,441, as well as cimetidine on the cytochrome P-450-catalyzed O-deethylation of 7-ethoxycoumarin and the N-demethylation of benzphetamine were examined. In these studies, the concentrations of substrate used in the incubations were near the K_m values (i.e. 0.1 mM for 7-ethoxycoumarin in rat and human liver microsomal systems, 0.1 and 0.02 mM for benzphetamine in rat and human liver microsomal systems respectively). Less than saturating substrate concentrations provide a more sensitive measurement if the H_2-receptor antagonists (ranging from 0.25 to 2 mM) cause any inhibition. Table 1 shows that cimetidine caused a substantial concentration-dependent inhibition of both *O*-deethylase and *N*-demethylase activities. L-643,441 caused similar inhibition, except for *N*-demethylase activity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Wavelength (nm)

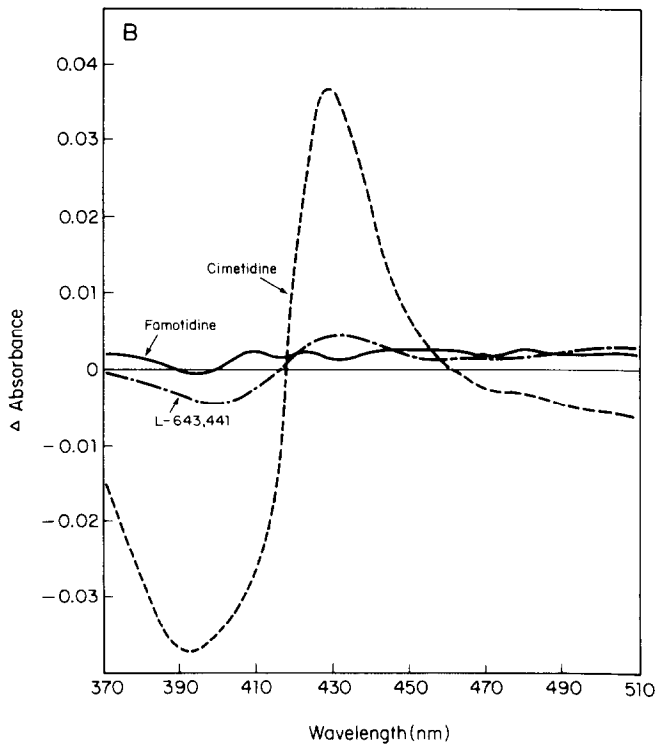
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Fig. 2((A) and (B)

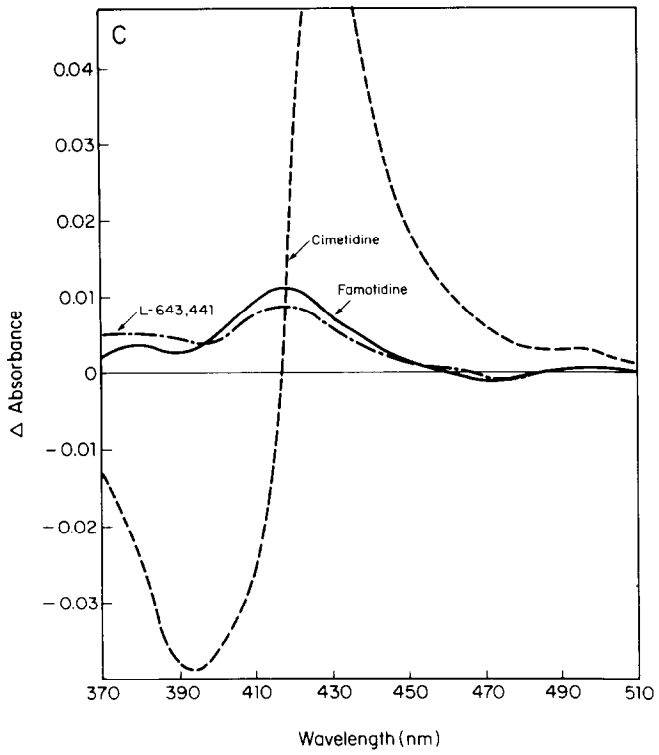

Fig. 2. Difference spectra of liver microsomes from (A) untreated, (B) PB-treated and (C) 3-MC-treated rats in the presence of 300 μM famotidine (———), or L-643,441, (· · · · ·) or cimetidine (- - - - -).

Table 1. Inhibition of microsomal 7-ethoxycoumarin and benzphetamine metabolism by H_2-antagonists*

Antagonist	Concentration (mM)	Inhibition of 7-ethoxycoumarin O-deethylation (%)			Inhibition of benzphetamine N-demethylation (%)	
		Untreated	PB	3MC	Untreated	PB
Cimetidine	0.25	18	10	0	24	5
	0.50	26	19	4	38	18
	1.00	35	35	11	57	24
	2.00	48	45	20	70	34
Famotidine	0.25	3	0	0	0	5
	0.50	5	4	0	0	4
	1.00	0	10	0	1	6
	2.00	21	25	0	6	10
L-643,441	0.25	13	16	0	3	5
	0.50	21	17	0	7	7
	1.00	29	39	2	7	13
	2.00	50	55	18	14	24

* The antagonists were added as DMSO solutions to the incubation mixture. Values represent percent inhibition relative to control samples containing only the DMSO solvent. The 7-EC (0.1 mM) was incubated at 37° for 10 min. No inhibition corresponds to: untreated, 0.105 nmol/mg/min; PB, 0.545 nmol/mg/min; 3-MC, 1.44 nmol/mg/min. Benzphetamine (0.1 mM) was incubated at 37° for 10 min with untreated microsomes or 5 min with PB-treated microsomes. No inhibition corresponds to: untreated, 24.7 nmol/mg/min; PB, 97.5 nmol/mg/min.

Table 2. Effects of H_2-antagonists on human liver microsomal benzphetamine *N*-demethylase activity

Antagonist	Concentration (mM)	Inhibition of metabolism* (%) Individual livers Male	Female	Average
Cimetidine	0.25	0 6 8 2 1	16 13 29 9 1	9
	0.50	4 3 15 5 11	9 0 28 10 0	9
	1.00	11 16 24 10 16	11 31 25 23 3	17
	2.00	24 28 35 16 29	20 27 32 36 17	26
Famotidine	0.25	0 2 0 0 0	12 0 9 20 4	5
	0.50	0 6 0 2 0	18 0 26 11 12	8
	1.00	0 6 5 0 2	1 1 25 20 4	6
	2.00	0 9 5 0 0	6 0 27 14 0	6
L-643,441	0.25	0 6 0 0 0	0 12 27 10 0	6
	0.50	0 3 0 1 0	22 13 42 0 2	8
	1.00	0 6 9 2 0	25 18 30 14 3	11
	2.00	4 10 3 8 5	26 10 43 24 18	15

* Values represent percent inhibition relative to control samples containing only the DMSO solvent. No inhibition corresponds to: 0.14, 0.14, 0.10, 1.56, 0.07, 0.20, 0.20, 0.85, 0.25 and 0.42 nmol/mg/min. Benzphetamine (0.02 mM) was incubated with microsomes at 37° for 10 min.

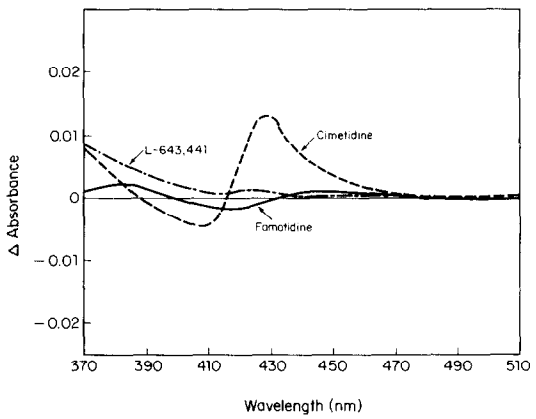

Fig. 3. Difference spectra of human liver microsomes in the presence of 300 μM famotidine (———), or L-643,441 (· · · · ·), or cimetidine (- - - - -).

with microsomes from untreated rats. In contrast, famotidine caused little or no inhibition in both P-450-dependent reactions, thus indicating its lack of significant interaction with microsomal cytochrome P-450.

To test the *in vitro* effect of famotidine and L-643,441 on drug metabolism in humans, liver microsomes prepared from five male and five female donors were incubated with these compounds and benzphetamine (0.02 mM). The mean inhibition observed for ten human liver microsomal samples of the benzphetamine N-demethylation is summarized in Table 2. The order of the inhibitor effect of these compounds was found to be similar to that found in studies with rat liver microsomes.

Discussion

Examination of the effects of newly developed therapeutic agents on the *in vitro* metabolism of model compounds represents a simple, first step evaluation of the potential risk of drug interaction. In view of the known species differences in drug metabolism, liver microsomes prepared from both humans and rodents should be used. In our studies, cimetidine was shown to interact with both rat and human cytochromes P-450 and to inhibit the oxidative metabolism of 7-ethoxycoumarin and benzphetamine *in vitro*, confirming the reported cimetidine drug interactions *in vivo* [3, 5, 6]. In contrast, famotidine showed little interaction with cytochrome P-450 and insignificant effects on the *in vitro* metabolism of 7-ethoxycoumarin and benzphetamine, suggesting that the drug interaction potential of this compound, *in vivo*, is low. Because of the presence of multiple cytochrome P-450 isozymes [17], we may have not probed the interaction of famotidine with all the potential P-450 species. Nevertheless, the fact that benzphetamine and 7-ethoxycoumarin metabolism by uninduced and induced rat liver microsomes as well as human liver microsomes was minimally affected suggests that famotidine does not interact with a variety of cytochrome P-450 isozymes. This suggestion is consistent with the recent report by Lin *et al.* [18] that famotidine has no effect on the *in vivo* metabolism and elimination of antipyrine, hexobarbital and warfarin in rats.

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REFERENCES

1. J. Puurunen and O. Pelkoner, *Eur. J. Pharmac.* **55**, 335 (1979).
2. D. A. Henry, J. A. MacDonald, G. Kitchingman, G. D. Bell and M. J. S. Langman, *Br. med. J.* **281**, 775 (1980).
3. A. Somogyi and R. Gugler, *Clin. Pharmacokinet.* **7**, 23 (1982).

4. K. J. Breen, R. Bury, P. V. Desmond, M. L. Mashford, B. Morphett, B. Westwood and R. G. Shaw, *Clin. Pharmac. Ther.* **31**, 297 (1982).
5. M. C. Gerber, G. A. Tejwani, N. Gerber and J. R. Bianchine, *Pharmac. Ther.* **27**, 353 (1985).
6. E. M. Sorkin and D. L. Darvey, *Clin. Pharm.* **17**, 110 (1983).
7. S. Rendic, F. Kajfez and H. H. Ruf, *Drug Metab. Dispos.* **11**, 137 (1983).
8. J. L. Smith, M. A. Gamal, A. N. Chremos and D. Y. Graham, *Dig. Dis. Sci.* **30**, 308 (1985).
9. R. G. Pendleton, M. L. Torchiana, C. Chung, P. Cook, S. Wiese and B. V. Clineschmit, *Archs int. Pharmacodyn. Thér.* **266**, 4 (1983).
10. T. Takagi, M. Takeda and H. Maeno, *Archs int. Pharmacodyn. Thér.* **256**, 49 (1982).
11. D. M. Campoli-Richards and S. P. Clissold. *Drugs* **32**, 197 (1986).
12. Y. Imai, M. Inada, S. Tamura, S. Noda, S. Kawata, Y. Minami and S. Tarui, *Pharmac. Res. Commun.* **18**, 629 (1986).
13. J. B. Schenkman, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **3**, 113 (1967).
14. V. Ullrich and P. Webster, *Hoppe-Seyler's Z. physiol. Chem.* **353**, 1171 (1972).
15. W. F. Greenlee and A. P. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
16. P. E. Thomas, A. Y. H. Lu, D. Ryan, S. B. West, J. Kawalek and W. Levin, *J. biol. Chem.* **251**, 1385 (1976).
17. A. Y. H. Lu and S. B. West, *Pharmac. Rev.* **31**, 277 (1980).
18. J. H. Lin, D. M. Coochetto, K. C. Yeh and D. E. Duggan, *Drug Metab. Dispos.* **14**, 649 (1986).

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Identification of a cytochrome P-450 in human fetal liver related to glucocorticoid-inducible cytochrome P-450HLp in the adult*

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The cytochromes P-450 are a multigene family of microsomal hemoproteins prominently found in the liver. These isozymes may be distinguished by differences in not only their primary structure, but also their substrate specificities and regulation of expression [1, 2]. Recent studies of human liver have confirmed that, in adult tissue, there are numerous forms of cytochrome P-450 structurally and functionly related to those found in experimental animals. These include: HLp, the glucocorticoid inducible, erythromycin *N*-demethylase homologous to P-450p in the rat and to LM3c in the rabbit [3]; HLj, the human ethanol-inducible *N*-nitrosodimethylamine demethylase orthologous to rat P-450j and rabbit LM3a [4]; HLc and HLd, the human orthologs of polycyclic aromatic hydrocarbon-inducible P-450c and P-450d, respectively, in the rat [5]; and HLx, a human isozyme related to the family of constitutive isozymes in untreated animals [6]. The human fetus has also been shown to actively metabolize many substrates of the cytochromes P-450 [7–10]. In addition, there are reports that microsomes isolated from human fetal livers contain proteins immunochemically-related to adult human liver cytochromes P-450 [9, 11]. In this report, we examined microsomes prepared from human fetal livers for the presence of proteins immunochemically-related to the five well characterized human cytochromes P-450 currently under study in our laboratory.

Methods

Liver specimens. Fetuses were obtained from therapeutic abortions performed prior to 12 weeks of gestation. The livers were removed, frozen in liquid nitrogen, and stored at −80°. Adult liver specimens were obtained from patients who had not received drugs known to induce HLp [3, 4] and were undergoing hepatic lobectomy or were brain dead renal transplant donors. Patient code numbers refer to individual specimens. Microsomes were prepared and stored as previously described [3]. Protein concentration was determined colorimetrically [12].

Antibody preparation and immunoblot analysis. Antibodies which specifically recognize human liver cytochrome P-450 HLj [4], HLc and HLd [5], or HLx [6] were prepared as described in the indicated references. Polyclonal antibodies against HLp were raised in goats as previously described [13]. Immunoblot analyses were performed and quantitated as previously described [3].

Results and discussion

Microsomes isolated from human fetal livers were subjected to electrophoresis in polyacrylamide gels, transferred to nitrocellulose filters, and then exposed to one of the anti-cytochrome P-450 antibodies. Analysis of these immunoblots (Fig. 1) demonstrated that the fetal liver microsomes did not contain detectable levels of proteins immunochemically-related to HLj [4], HLc and HLd [5], or HLx [6]. These results are in keeping with developmental studies in rats which indicate that P-450c and P-450d [14, 15] and P-450j (F. J. Gonzalez, personal communication, cited with permission) are absent in fetal liver, but then rise to adult levels during the neonatal period. To our knowledge, P-450g, a rat liver counterpart of HLx in humans, has not been studied in the rat fetus.

Cytochrome P-450p has been reported to be undetectable in rat fetal liver [15]. However, each of the human fetal microsomal samples we examined contained a protein that reacted with anti-HLp antibodies and displayed an electrophoretic mobility identical to that of HLp (Fig. 1). Scanning densitometry of these immunoblots demonstrated that the average amount of the HLp-related protein in five fetal preparations was 0.07 nmol HLp/mg protein (range, 0.03 to 0.09) (Table 1). By comparison, the average amount of immunoreactive HLp in hepatic microsomes prepared from adult specimens was 0.12 nmol/mg protein (range, 0.07 to 0.19) (Table 1). Thus, the concentration of immunoreactive HLp in adults is almost twice that of the HLp-related protein in fetal liver. However, in both adult and fetal microsomes, the amounts of the immunoreactive HLp

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