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BIOTRANSFORMATION OF LOVASTATIN—III

EFFECT OF CIMETIDINE AND FAMOTIDINE ON *IN VITRO* METABOLISM OF LOVASTATIN BY RAT AND HUMAN LIVER MICROSOMES

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Abstract—The effects of the H_2-receptor antagonists, cimetidine and famotidine, on the microsomal metabolism of [^{14}C]lovastatin were investigated. Liver microsomes were prepared from control, phenobarbital- and 3-methylcholanthrene-pretreated rats and humans (male and female). Concentration-dependent inhibition of the metabolism of lovastatin (0.1 mM) was observed with cimetidine (0.1 to 1.0 mM). In contrast, famotidine at a similar concentration was a very weak inhibitor. The formation of 6′β-hydroxy-lovastatin, the major microsomal metabolite of lovastatin, was similarly inhibited. The results suggest that *in vivo* metabolic interaction with concomitantly administered lovastatin is less likely with famotidine than with cimetidine. Phenobarbital pretreatment produced 58% stimulation in overall metabolism, whereas 3-methylcholanthrene pretreatment had no effect relative to control rats (5.4 nmol/mg protein/min). Liver microsomes from phenobarbital-pretreated rats produced 67% more of the 6′β-hydroxy-lovastatin but 63–66% less of the 3″-hydroxy and 6′-exomethylene metabolites. Liver microsomes from 3-methylcholanthrene-treated rats also produced less 3″-hydroxy-lovastatin (49%) but similar quantities of the other two metabolites. 6′β-Hydroxy-lovastatin was a major metabolite with human liver microsomes. Interestingly with these microsomes, hydroxylation at the 3″-position of the molecule was a negligible pathway and hydrolysis to the hydroxy acid form was not observed. The formation of 6′-exomethylene-lovastatin was also catalyzed by human liver microsomes (0.5 to 0.8 nmol/mg protein/min).

H_2-receptor antagonists such as cimetidine, ranitidine and famotidine enjoy widespread use in the management of gastrointestinal ulcers and other acid-related diseases. These compounds are, to varying degrees, inhibitors of the cytochrome P-450 family of enzymes [1–4]. The inhibitory activity is attributed to their binding to cytochrome P-450 [5], and cimetidine is the most potent while the other two are only weak inhibitors [1–4]. Since this type of drug is consumed on a long-term basis, it may cause prolonged inhibition of drug-metabolizing enzymes and interact with biotransformation of other drugs. Lovastatin is a member of a new class of cholesterol-lowering drugs called 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors that is also prescribed widely. It has been shown to be a substrate for cytochrome P-450 which catalyzes its biotransformation to 6′β-hydroxy, 6′-exomethylene and 3″-hydroxy metabolites [6–8]. Lovastatin, like H_2-receptor antagonists, is also used chronically, and a potential for drug–drug interactions exists. In the present study, the interaction of cimetidine and famotidine with lovastatin was examined at the biochemical level *in vitro*. Liver microsomes prepared from control and induced (phenobarbital and 3-methylcholanthrene) rats and humans were used. The structures of two H_2-receptor antagonists and lovastatin are shown in Fig. 1.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Lovastatin (sp. act. 4.6 mCi/mmol), labeled at the $C_{1''}$ position, was synthesized as described previously [9] by the Labeled Compound Synthesis Group, Merck Sharp & Dohme Research Laboratories, Rahway, NJ. The radiochemical purity of [^{14}C]lovastatin was > 98% when analyzed by HPLC as described for incubated samples. Cimetidine and famotidine also were synthesized at the Merck Sharp & Dohme Research Laboratories, Rahway, NJ. NADPH, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. (St Louis, MO).

Preparation of liver microsomes. Liver microsomes were prepared from control, phenobarbital-treated (100 mg/kg/day for 4 days), and 3-methylcholanthrene-treated (25 mg/kg/day for 4 days) male Sprague–Dawley rats (200 g) and humans (male and female) as described [10]. Two human liver samples (male, 56 years; female, 44 years) with no known history of exposure to cytochrome P-450 modifying agents were used. Frozen liver samples were supplied by Dr C. VanBahr, Department of Clinical Pharmacology, Huddinge Hospital, Huddinge, Sweden.

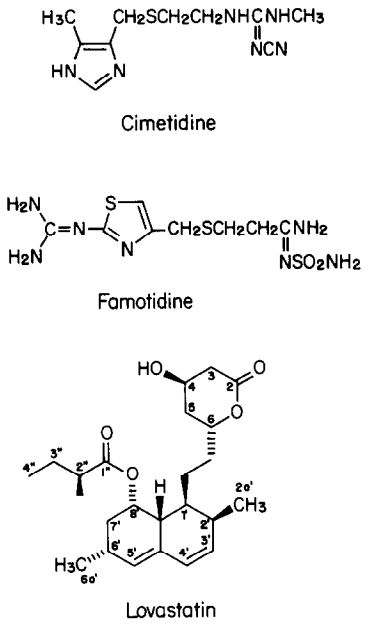


Fig. 1. Structures of cimetidine, famotidine and lovastatin.

Microsomal protein concentrations were measured according to the method of Lowry *et al.* [11] using bovine serum albumin as a standard.

Incubations. The standard incubation mixture contained 100 μmol potassium phosphate, pH 7.4, 6 μmol $MgCl_2$, 1 μmol NADP, 10 μmol glucose-6-phosphate, 0.7 units of glucose-6-phosphate dehydrogenase, 1.0 mg microsomal protein, 0.1 to 1.0 μmol cimetidine (dissolved in 10 μL of methanol) and 100 nmol [^{14}C]lovastatin (dissolved in 10 μL of acetone) in a final volume of 1.0 mL. The reaction was started by adding [^{14}C]lovastatin and incubated for 10 min at 37° in a 20 × 125 mm screw-capped flint glass tube under an air atmosphere using a Dubnoff metabolic shaker at 120 rpm. At the end of the incubation period the reaction was stopped by adding 1 mL acetone, and the mixture was extracted with 2 mL ethyl acetate. After centrifugation (3000 rpm, 10 min), the organic layer was carefully removed and concentrated to dryness under a stream of nitrogen. Concentrated extracts were stored at −70° until analysis by HPLC. Blank incubations consisted of either zero-time incubation or 10-min incubation with denatured microsomes. In a separate experiment, incubations were carried out as described, except that 0.1 to 1.0 μmol famotidine (dissolved in 10 μL dimethyl sulfoxide) was substituted for cimetidine.

HPLC analysis. A Gilson model 42 HPLC system equipped with a Kratos model 773 UV detector and a Ramona model 5-LS radioactivity detector were used for the analysis. Dried extracts from the incubations were reconstituted in 0.2 mL of *n*-propanol and microcentrifuged. Aliquots (20 μL) were injected on an Altex 5 μm Ultrasphere ODS column (4.6 × 250 mm) eluted with a linear gradient (1%/min) of 40 to 72% acetonitrile in 5 mM formic acid at a flow rate of 1.0 mL/min. The eluate was monitored at 238 nm, and various metabolites [6] of lovastatin were quantitated by the on-line radioactivity detector.

RESULTS

Metabolism of lovastatin by rat and human liver microsomes. The effects of inducers of the cytochrome P-450-dependent mixed-function oxidase system on the metabolism of [^{14}C]lovastatin by rat liver microsomes are shown in Table 1. Pretreatment of rats with phenobarbital stimulated metabolism by 58%, whereas 3-methylcholanthrene pretreatment had essentially no effect on the metabolism compared to control rats. Phenobarbital pretreatment of rats caused a 67% increase in the formation of the major 6′β-hydroxy metabolite and a 3-fold increase in the formation of unknown-1. Interestingly, it inhibited the formation of 3″-hydroxy and 6′-exomethylene metabolites by 66 and 63% respectively. Hydrolysis of lovastatin to the hydroxy acid form was also decreased (47%) by liver microsomes from phenobarbital-treated rats. 3-Methylcholanthrene pretreatment of rats produced 49% inhibition in the formation of 3″-hydroxy-lovastatin and a mild stimulation in the conversion to the hydroxy acid form. It had no effect on the formation of 6′β-hydroxy, 6′-exomethylene and unknown-1 metabolites.

Metabolism of [^{14}C]lovastatin by human liver microsomes is also shown in Table 1. Human liver microsomes catalyzed biotransformation of lovastatin primarily to 6′β-hydroxy-lovastatin. It accounted for 43 and 49% of total metabolites by male and female liver microsomes respectively. Conversion of lovastatin to unknown-1 was also a major pathway of metabolism, accounting for 50 and 38% of total metabolites by these microsomes. Interestingly, with human liver microsomes hydrolysis to the hydroxy acid form was not detected and the formation of the 3″-hydroxy metabolite was negligible. Dehydrogenation of lovastatin to the 6′-exomethylene metabolite was similar with human and control rat liver microsomes.

Effects of H_2-receptor antagonists on metabolism of lovastatin. When cimetidine (0.1 to 1.0 mM) was coincubated with [^{14}C]lovastatin (0.1 mM), liver microsomes from rat or human, and an NADPH-generating system, it inhibited the metabolism of lovastatin in a concentration-dependent manner (Fig. 2). Famotidine was a much weaker inhibitor. The inhibitory effects of cimetidine and famotidine on the metabolism of lovastatin were less pronounced with liver microsomes from phenobarbital-treated rats relative to liver microsomes from control and 3-methylcholanthrene-treated rats. At 1.0 mM, the percent inhibition displayed by cimetidine was 43, 26, 42, 27 and 41 for liver microsomes from control rats, phenobarbital-treated rats, 3-methylcholanthrene-treated rats, human male and human female respectively. The corresponding values for famotidine (1.0 mM) were 12, 4, 8, 6 and 16%. The effects of cimetidine and famotidine on the profiles of metabolites are shown in Tables 2 and 3 respectively. The effect of cimetidine on the formation of lovastatin metabolites was complex. The formation of the major metabolite, 6′β-hydroxy-

Table 1. Metabolism of [^{14}C]lovastatin by rat and human liver microsomes*

Microsomes	Metabolites (nmol)					Total Metabolism†	Recovery‡ (%)
	6′β-Hydroxy	3″-Hydroxy	6′-Exo methylene	Hydroxy acid	Unknown-1		
Rat							
Control	26.7 (52.9)§	4.7 (9.3)	6.2 (12.3)	3.8 (7.5)	9.1 (18.0)	53.99	93.6
Phenobarbital	44.7 (58.0)	1.6 (2.1)	2.3 (2.9)	2.0 (2.6)	26.4 (34.3)	85.43	90.1
3-Methylcholanthrene	26.9 (54.3)	2.4 (4.9)	6.5 (13.2)	4.8 (9.7)	8.9 (18.0)	52.61	94.3
Human							
Male	31.1 (43.1)	0.05 (0.1)	5.0 (6.9)	ND‖	36.1 (50.0)	83.77	86.3
Female	29.4 (48.9)	0.1 (0.2)	8.0 (13.3)	ND	22.7 (37.6)	64.95	92.7

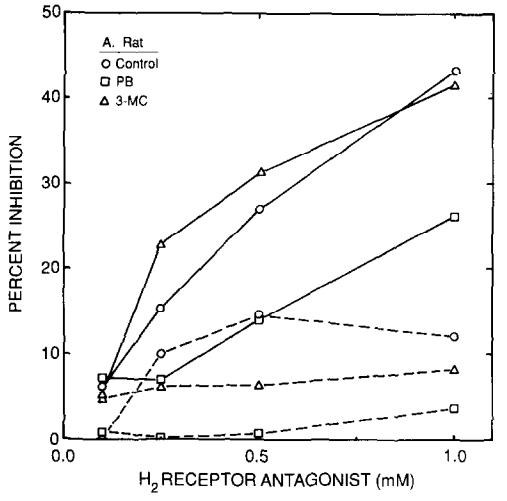
* Experimental conditions are as described under Materials and Methods. Results are averages of two separate experiments.

† Total metabolism denotes percentage conversion of substrate, i.e. the total radioactivity above blank which emerged from the column before the substrate.

‡ Recovery represents the percentage of the radioactivity due to metabolism emerging from the column in the defined metabolite fractions as compared with total radioactivity due to metabolism.

§ Numbers in parentheses represent individual metabolites as a percentage of total metabolites.

‖ Not detected.



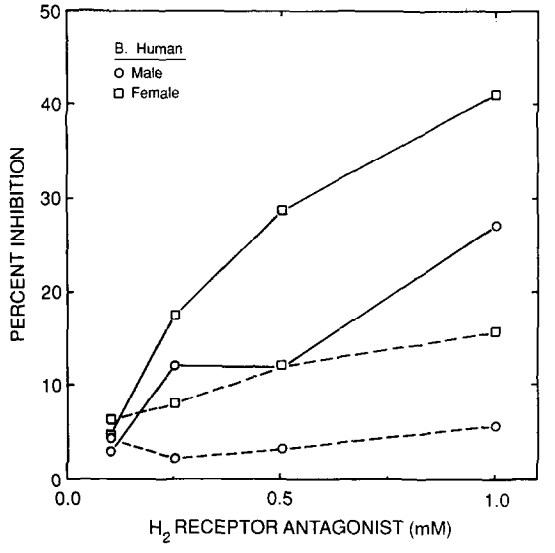
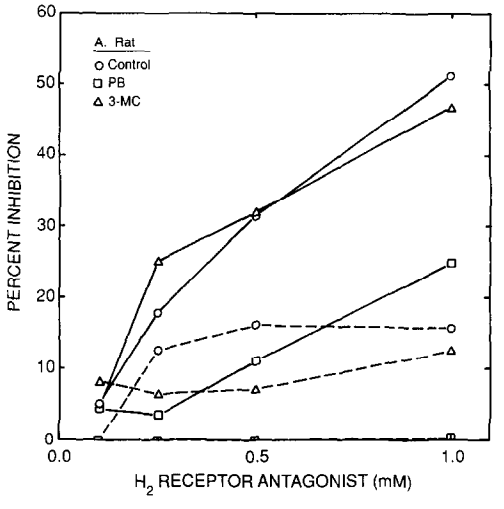


Fig. 2. Inhibition of lovastatin metabolism by cimetidine (solid lines) and famotidine (dashed lines) by rat (A) and human (B) liver microsomes. The absolute values for lovastatin metabolism are shown in Tables 2 and 3.

lovastatin, was inhibited in a concentration-dependent manner by all microsomal systems except male human liver microsomes (Fig. 3). With male human liver microsomes, cimetidine had a mild stimulatory effect on the conversion of lovastatin to 6′β-hydroxy-lovastatin and clearly stimulated conversion to 6′-exomethylene-lovastatin. Metabolism of lovastatin to 3″-hydroxy and 6′-exomethylene metabolites was decreased by cimetidine with liver microsomes from control and 3-methylcholanthrene-treated rats while increased with liver microsomes from phenobarbital-treated rats. Cimetidine inhibited formation of the unknown-1 metabolite by all five microsomal preparations by 40–61%. With rat liver microsomes, hydrolysis of lovastatin to the hydroxy acid form was not affected by famotidine, while cimetidine produced up to 43% stimulation.

DISCUSSION

The results of the present study clearly demonstrate that cimetidine inhibited biotransformation of lovastatin by rat and human liver microsomes in a concentration-dependent manner. Famotidine, on the other hand, was a very weak inhibitor of microsomal metabolism of lovastatin. These results are in agreement with the known inhibitory effect of cimetidine but not famotidine on the cytochrome P-450-dependent mixed-function oxidase system [1–4]. Cimetidine has been shown to inhibit activity of rat liver microsomal aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-*O*-deethylase, benzphetamine *N*-demethylase and 7-ethoxyresorufin-*O*-deethylase and binds strongly to human liver cytochrome P-450 [1, 2]. Famotidine has no effect on the activity of above enzymes and does not bind to human cytochrome P-450 [1, 2]. The inhibition of overall metabolism of lovastatin by H_2-receptor antagonists was



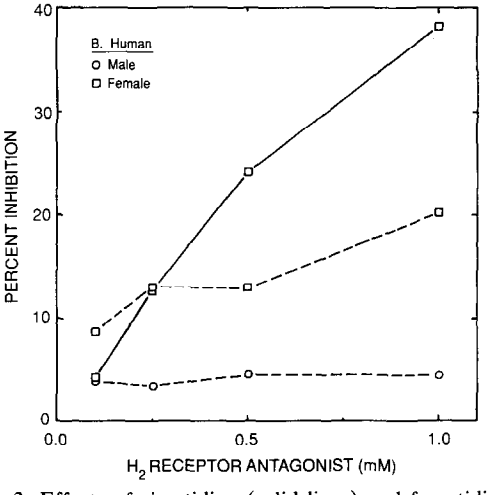


Fig. 3. Effects of cimetidine (solid lines) and famotidine (dashed lines) on the formation of 6′β-hydroxy-lovastatin by rat (A) and human (B) liver microsomes. The absolute values for the formation of 6′β-hydroxy-lovastatin are shown in Tables 2 and 3.

Table 2. Effect of cimetidine on metabolism of [^{14}C]lovastatin by liver microsomes*

Microsomes	Cimetidine (mM)	Metabolites (nmol/mg protein/10 min)						Total Metabolism†
		6′β-Hydroxy	3″-Hydroxy	Hydroxy acid	6′-Exo methylene	Unknown-1	Others	
Rat								
Control	0	24.94	4.60	3.29	5.82	8.34	2.86	49.85
	1.0	12.20	3.40	4.47	3.0	3.89	1.36	28.32
		(51.1)‡	(26.1)		(48.5)	(53.4)	(52.4)	(43.2)
Phenobarbital	0	44.80	1.91	1.82	2.01	25.89	9.14	85.57
	1.0	33.69	2.21	2.68	5.65	15.45	3.51	63.19
		(24.8)				(40.3)	(61.6)	(26.2)
3-Methyl	0	28.10	2.47	4.57	6.55	11.48	1.53	54.70
cholanthrene	1.0	14.96	1.91	5.58	4.62	4.44	0.45	31.96
		(46.8)	(22.7)		(29.5)	(61.3)	(70.6)	(41.6)
Human								
Male	0	27.13	ND§	ND	3.25	38.82	16.09	85.29
	1.0	28.41	0.09	ND	6.89	21.85	4.96	62.20
						(43.7)	(69.2)	(27.1)
Female	0	31.05	0.25	ND	7.65	26.30	6.23	71.48
	1.0	19.19	0.28	0.04	5.49	13.77	3.38	42.15
		(38.2)			(28.2)	(47.6)	(45.7)	(41.0)

* Experimental conditions are as described under Materials and Methods.
† Definition is as described in Table 1.
‡ Numbers in parentheses indicate percent inhibition.
§ Not detected.

Table 3. Effect of famotidine on metabolism of [^{14}C]lovastatin by liver microsomes*

Microsomes	Famotidine (mM)	Metabolites (nmol/mg protein/10 min)						Total Metabolism†
		6′β-Hydroxy	3″-Hydroxy	Hydroxy acid	6′-Exo methylene	Unknown-1	Others	
Rat								
Control	0	28.47	4.82	4.30	6.65	9.81	4.08	58.13
	1.0	24.03	4.12	4.23	6.47	6.92	5.37	51.14
		(15.6)‡	(14.5)	(1.6)	(2.7)	(29.5)		(12.0)
Phenobarbital	0	44.30	1.34	2.17	2.48	26.95	8.05	85.29
	1.0	44.13	0.35	2.04	3.24	24.13	8.28	82.17
		(0.4)	(73.9)	(6.0)		(10.5)		(3.7)
3-Methyl	0	25.77	2.36	5.06	6.51	6.35	4.46	50.51
cholanthrene	1.0	22.56	2.10	4.90	6.42	6.62	3.73	46.33
		(12.5)	(11.0)	(3.2)	(1.4)		(16.4)	(8.3)
Human								
Male	0	35.19	0.10	ND§	6.67	33.42	6.86	82.24
	1.0	33.64	ND	0.07	8.59	28.66	6.57	77.53
		(4.4)				(14.2)	(4.2)	(5.7)
Female	0	27.76	ND	ND	8.33	19.02	3.30	58.41
	1.0	22.16	0.12	0.16	7.57	16.82	2.40	49.23
		(20.2)			(9.1)	(11.6)	(27.3)	(15.7)

* Experimental conditions are as described under Materials and Methods.
† Definition is as described in Table 1.
‡ Numbers in parentheses indicate percent inhibition.
§ Not detected.

remarkably parallel with the inhibition of formation of the major 6′β-hydroxy metabolite except in the case of cimetidine with male human liver microsomes. Interestingly, with these microsomes cimetidine had a mild stimulatory effect. This lack of inhibition is difficult to explain at present. The stimulatory effect of cimetidine on the metabolism of lovastatin to the hydroxy acid form by rat liver microsomes, which is probably not a cytochrome P-450 catalyzed reaction, could be due to metabolic switching.

Human liver microsomes, like rat liver microsomes, catalyzed biotransformation of lovastatin primarily at the 6′ position to form 6′β-hydroxy and 6′-exomethylene metabolites. These two metabolites collectively represented 50 and 61% of total metabolites by male and female human liver microsomes respectively. They were also observed in plasma of humans receiving lovastatin.* Surprisingly, the 6′-hydroxymethyl derivative which was also formed *in vivo** was not detected as a metabolite by human liver microsomes. The other two pathways of metabolism of lovastatin by rat liver microsomes, hydrolysis of the lactone to the hydroxy acid form and hydroxylation at the 3″ position, were insignificant with human liver microsomes.

Inducers of the cytochrome P-450 family of enzymes had a mixed effect on the microsomal metabolism of lovastatin. Phenobarbital, which is an inducer of cytochrome P-450b and P-450e [12, 13], stimulated the metabolism while 3-methylcholanthrene, which predominantly induces cytochromes P-450c and P-450d [12, 13], had no effect on the total metabolism of lovastatin when compared to control. Furthermore, phenobarbital had differential effects on the formation of lovastatin metabolites. Although the formation of 6′β-hydroxy-lovastatin was induced by phenobarbital, the formation of 3″-hydroxy and 6′-exomethylene metabolites along with the hydroxy acid was inhibited. The formation of 3″-hydroxy-lovastatin was also inhibited by 3-methylcholanthrene, which had no effect on the formation of metabolites at the 6′ position. The data suggest that metabolism of lovastatin to 6′β-hydroxy, 3″-hydroxy and 6′-exomethylene derivatives is catalyzed by different forms of cytochrome P-450. Similar conclusions were derived from the effects of various inhibitors of cytochrome P-450 on the metabolism of lovastatin to these three metabolites in a recent study [7]. The effects of phenobarbital and 3-methylcholanthrene pretreatment on the metabolism of lovastatin correlated well with the inhibition of lovastatin metabolism by H_2-receptor antagonists from these microsomes. Phenobarbital stimulated metabolism of lovastatin and also lowered the inhibitory effect of cimetidine and famotidine, whereas 3-methylcholanthrene pretreatment, which had no effect on the rates of metabolism of lovastatin, did not diminish the inhibitory effect of H_2-receptor antagonists.

* Schwartz MS, Stubbs RJ, Thornton TJ, Vyas KP, Houck DR and Bayne WF, manuscript in preparation.

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