Selective Inhibition of Acetaminophen Oxidation and Toxicity by Cimetidine and Other Histamine H_2-Receptor Antagonists In Vivo and In Vitro in the Rat and in Man

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Abstract. Acetaminophen-induced hepatotoxicity results from hepatic enzymatic oxidation of acetaminophen to a toxic, electrophilic intermediate. Acetaminophen is ordinarily eliminated after conjugation with glucuronic acid and sulfate to nontoxic derivatives. Cimetidine has been shown to inhibit the hepatic oxidation of a number of drugs and to protect rats from acetaminophen-induced hepatic necrosis. The aim of this study was to define the mechanism by which cimetidine reduced acetaminophen-induced hepatic necrosis and to determine whether inhibition of formation of the reactive metabolite(s) of acetaminophen occurred also in man. In vivo cimetidine pretreatment decreased covalent binding of [^{3}H]acetaminophen to the liver from 552±23.8 to 170±31.6 nmol/g protein 2 h after a toxic dose of acetaminophen in 3-methylcholanthrene pretreated rats ($P < 0.05$). Cimetidine pretreatment also significantly reduced the rate of hepatic glutathione depletion. Both cimetidine and metiamide produced dose-dependent inhibition of acetaminophen oxidation in vitro, whereas inhibition by ranitidine and cimetidine sulfoxide was quantitatively less. Inhibition of acetaminophen oxidation by cimetidine and metiamide was primarily competitive with an inhibition constant (K_i) of 130±16 and 200±50 μM, respectively. By contrast, cimetidine inhibited acetaminophen glucuronidation minimally with a K_i of 1.39±0.23 mM. Similar results were obtained using human liver microsomes as a source of enzymes. In a dose-related fashion, cimetidine also reduced acetaminophen-induced toxicity to human lymphocytes when incubated with microsomes and NADPH. Pharmacokinetics of acetaminophen elimination were studied in normal volunteers with and without co-administration of cimetidine 300 mg every 6 h. In normal volunteers, cimetidine decreased the fractional clearance of the oxidized (potentially toxic) metabolites of acetaminophen more than the conjugated metabolites. This finding confirmed the hypothesis that cimetidine is a relatively selective inhibitor of the oxidation of acetaminophen to reactive metabolites in man as well as in animals. When considered together with the results of previous studies showing improved survival and decreased hepatotoxicity in acetaminophen-poisoned animals, the present results provide a rational basis for assessing possible benefits of cimetidine treatment of acetaminophen overdoses in man.

Introduction

Acetaminophen is a commonly used analgesic that has been shown to cause dose-related hepatic necrosis in both laboratory animals and in humans after massive doses (1, 2). This dose-related hepatotoxicity is believed to result from metabolic conversion of acetaminophen to a highly reactive, electrophilic intermediate by the cytochrome P-450 mixed function oxidase complex (1). Once formed this toxic intermediate may alkylate tissue macromolecules unless detoxified by further conjugation with glutathione. Cytochrome P-450-mediated oxidation is a minor pathway in the overall biotransformation and elimination of acetaminophen. After usual therapeutic doses only a small amount of this toxic intermediate is formed. The major pathways in acetaminophen biotransformation are the phase II conjugation reactions: glucuronidation and sulfation (3). These latter two

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pathways account for ~80–90% of the metabolism of acetaminophen, except after large doses, when sulfation may become saturated (4). The oxidized metabolites of acetaminophen are excreted in urine as mercapturic acid and cysteine conjugates, which represent degradation products of the acetaminophen-glutathione adduct. At low doses of acetaminophen these compounds serve as an index of the potentially toxic oxidation of acetaminophen while the glucuronide and sulfate conjugates represent nontoxic elimination. Current therapy of acetaminophen overdoses includes administration of *N*-acetylcysteine, which protects against necrosis by increasing synthesis of glutathione, providing active sulfate, and/or by reduction of the electrophilic intermediate (*N*-acetyl-*p*-benzoquinoneimine) back to the parent compound (5).

Cimetidine, a 4–5 substituted imidazole used widely as a histamine H_2-receptor antagonist, has been demonstrated to cause dose-related inhibition of cytochrome P-450-mediated oxidation both in vivo and in vitro (6–8). This inhibition of cytochrome P-450-mediated drug metabolism by cimetidine seems to be related to its imidazole structure rather than to its H_2-receptor antagonist potency. Ranitidine, a substituted furan, exhibits higher H_2-receptor antagonist potency, but is far less active than cimetidine in inhibiting drug oxidation (6–8). Furthermore, this inhibition of oxidative drug metabolism appears to be relatively selective, as glucuronidation of drugs has been reported to be unaffected by cimetidine both in vivo and in vitro (7, 9). Thus, cimetidine (and related imidazole derivatives) should theoretically prevent the toxic oxidation of acetaminophen while allowing nontoxic conjugation to continue unaffected. Several studies have recently demonstrated cimetidine to be as effective as *N*-acetylcysteine in preventing acetaminophen-induced hepatic necrosis and death in rats (10–12). The aims of the present studies were to extend these observations, to define the mechanism by which cimetidine prevents acetaminophen hepatotoxicity and to determine whether cimetidine might protect against hepatic necrosis in man.

Methods

Animals. Male Fischer 344 rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) weighing 100–175 g were pretreated with a single intraperitoneal dose of 3-methylcholanthrene (3-MC)[1] (Eastman Laboratory and Specialty Chemicals, Eastman Kodak Co., Rochester, NY) 20 mg/kg in corn oil to increase oxidative drug metabolism and were used 72 h later.

Chemicals. Cimetidine, cimetidine sulfoxide, an oxidized metabolite of cimetidine, and metiamide were kindly supplied by Smith, Kline & French Laboratories (Philadelphia, PA). Ranitidine was kindly provided by Glaxo Inc., Research Division (Fort Lauderdale, FL). Acetaminophen, cysteine, GSH, glutathione reductase, NADPH, and uridine diphosphoglucuronic acid (UDPGA), ammonium salt, were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]Acetaminophen (9.3 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Synthetic standards of acetaminophen-cysteine, acetaminophen, and acetaminophen glucuronide were donated by Dr. Josiah Tam of McNeil Laboratories (Fort Washington, PA). All other reagents were of the highest grade commercially available.

In vivo covalent binding of [^{3}H]acetaminophen and glutathione depletion. Animals were pretreated with 3-MC 72 h before receiving 500 mg/kg of acetaminophen and 200 μCi/kg of [^{3}H]acetaminophen in saline as a single intraperitoneal injection. There were three animals in each group at each time point. Half of the rats received 150 mg/kg of cimetidine and the other half received an equal volume of saline (0.1 ml i.p.) 30 min before the acetaminophen dose. Rats were killed at 30, 60, 90, and 120 min after receiving acetaminophen. The livers were removed, blotted dry with filter paper, weighed, and homogenized in 20 ml of 10% trichloroacetic acid (TCA) in 0.01 N HCl. The precipitate was washed extensively with methanol until radioactivity in the supernatant was decreased to background levels. Precipitated protein was solubilized with 2.0 N NaOH overnight and aliquots were removed for counting by liquid scintillation spectrometry or for determination of protein using the method of Lowry et al. (13). Results were expressed as nanomoles acetaminophen covalently bound per gram of liver protein after solubilization.

Total hepatic glutathione levels in the initial supernatant after homogenization of the liver were measured using the method of Tietze (14). Results were expressed as micrograms of glutathione per gram liver (wet weight).

Enzyme assays. Hepatic microsomes were prepared from 3-MC-pretreated rats by the method of Franklin and Estabrook (15). In preliminary experiments all reactions were demonstrated to be linear with time and protein content for the conditions used.

Acetaminophen oxidation. Oxidation of acetaminophen was measured by in vitro covalent binding of [^{3}H]acetaminophen to microsomes as previously described (10). Formation of a covalent adduct of oxidized acetaminophen with cysteine by microsomes in the presence of NADPH was measured by a modification of the method of Corcoran et al. (16). After termination of the reaction by addition of an equal volume of ice-cold methanol, 4-fluorophenol was added as an internal standard. Precipitated protein was removed by centrifugation and an aliquot of supernatant was injected into a high-performance liquid chromatograph (HPLC) (Waters Associates, Millipore Corp., Milford, MA) using a μBondapak reverse-phase C_{18} column (Waters Associates) and a mobile phase containing 1% acetic acid/methanol/ethyl acetate (90:5:0.1) at a flow rate of 2.0 ml/min, monitoring the effluent at 254 nm. Retention time of acetaminophen-cysteine adduct was 4.8 min. The acetaminophen-cysteine adduct was quantified by determination of peak-height ratios compared with a synthetic standard.

Acetaminophen glucuronidation. Formation of acetaminophen glucuronide was assayed by incubation of microsomes in the presence of acetaminophen and 5.0 mM UDPGA in 50 mM Tris–Cl pH 7.4 containing 10 mM Mg^{++}. The reaction was carried out for 30 min at 37°C in a shaking incubator and terminated by addition of an equal volume of ice-cold methanol. 4-Fluorophenol was added as internal standard. Acetaminophen glucuronide was measured by HPLC on a μBondapak reverse-phase C_{18} column using 1% acetic acid/methanol/ethyl acetate (90:5:0.1) as the mobile phase at a flow rate of 2.0 ml/min. Acetaminophen glucuronide was quantitated by peak-height ratio using a synthetic standard of acetaminophen glucuronide. Retention time was 3.3 min.

Metabolism of acetaminophen by human microsomes. Liver was obtained from a cadaveric renal transplant donor who died as a result of intracranial bleeding. The liver was removed at surgery and perfused

1. *Abbreviations used in this paper:* Cl_o, oral clearance; HPLC, high-performance liquid chromatograph(y); 3-MC, 3-methylcholanthrene; UDPGA, uridine diphosphoglucuronic acid.

with 4.0 liter of ice-cold saline and frozen at −70°C for 2 wk before preparation of microsomes as described above (15). Oxidation of acetaminophen and formation of acetaminophen glucuronide were determined as described above.

Acetaminophen-induced human lymphocyte toxicity. Lymphocytes were separated from heparinized venous blood collected from normal volunteers by centrifugation through Ficoll-Paque (Pharmacia Fine Chemicals Piscataway, NJ) (17). Hepatic microsomes were prepared from the livers of Fischer 344 rats, which had been pretreated with an intraperitoneal dose of 20 mg/kg of 3-MC 72 h before killing. Lymphocytes (1×10^6) were incubated for 3 h with 20 mM acetaminophen, an NADPH-generating system (17), and varying concentrations of cimetidine (0.1–1.7 mM) in the presence of 2 mg/ml of microsomes and O_2 according to the method of Spielberg (17). Lymphocytes were washed, transferred to culture medium, and incubated an additional 21 h at 37°C. Lymphocyte toxicity was measured by inability to exclude trypan blue dye at 24 h (200 cells scored in each of triplicate tubes) and by release of lactate dehydrogenase into the medium. Controls for each experiment included lymphocytes incubated without either acetaminophen, or microsomes, or NADPH-generating system. Lymphocyte survival was typically 95% in all controls.

Effects of cimetidine on the metabolism of acetaminophen in normal volunteers. The distribution and elimination of acetaminophen were studied in normal human volunteers. All subjects gave written informed consent and the protocol was approved by the institutional review committee for clinical investigation. After an overnight fast, each subject was given 1.5 g of acetaminophen in capsules (Tylenol, extrastrength) with 100 ml of water. Venous blood was collected from an indwelling heparin lock and plasma separated and frozen at −20°C until determination of plasma acetaminophen concentration. Samples were collected at 0, 0.5, 0.75, 1.0, 2, 3, 4, 6, and 8 h after oral acetaminophen administration. Urine was collected from each subject for 24 h, beginning at the time of acetaminophen dosing. Plasma acetaminophen concentrations and urinary acetaminophen metabolite concentrations were determined as previously described (18). The oral clearance (Cl_o) of acetaminophen from plasma was determined by calculation of the area under the curve after linear regression of the logarithm of plasma concentration vs. time as follows:

$$Cl_o = \text{Dose}/AUC_0,$$

assuming absorption to be rapid and complete. Plasma half-life for the elimination phase ($t_{1/2}$) and volume of distribution for the elimination phase were determined as previously described (18). The fractional clearance of acetaminophen by each metabolic pathway (glucuronidation, sulfation, and potentially toxic oxidation) was calculated by multiplying the total plasma clearance of acetaminophen by the fractional recovery of each metabolite in 24-h urine samples. After base-line kinetics for elimination of acetaminophen were obtained, each subject was given cimetidine 300 mg per os four times daily for 48 h. To determine the effects of cimetidine upon acetaminophen elimination, the above study was repeated as described above beginning 1 h after the first a.m. dose of cimetidine. Cimetidine was continued every 6 h for a total of 4 doses in the next 24 h. Pharmacokinetic parameters for acetaminophen elimination were again calculated as described above.

Statistics. Results are expressed as a mean±SEM for all groups unless otherwise indicated. The *t* test for paired samples was used to compare differences in acetaminophen pharmacokinetic parameters before and after administration of cimetidine. The unpaired, two-tailed *t* test was used to compare results between groups for in vitro studies. A value of $P < 0.05$ was used as the minimum level of statistical significance.

Results

In vivo covalent binding of [^{3}H]acetaminophen. In control animals receiving acetaminophen only, covalent binding of acetaminophen increased linearly with time reaching a maximum of 552±23.8 nmol acetaminophen bound/g liver protein at 90 min after injection of acetaminophen. By contrast, in animals pretreated with cimetidine the covalent binding of acetaminophen was significantly lower at all time points ($P < 0.05$) and reached a maximum of 170±31.6 nmol acetaminophen bound/g liver protein at 120 min (Fig. 1 *A*).

Hepatic glutathione depletion by acetaminophen. Hepatic glutathione was rapidly depleted in animals receiving acetaminophen only, reaching a minimum of 13.9±1.28% of initial levels at 60 min after injection of acetaminophen. Total glutathione stores also decreased with time after administration of acetaminophen in rats pretreated with cimetidine, but at a slower rate reaching a minimum of 30.3±1.91% of initial values at 120 min after injection of acetaminophen (Fig. 1 *B*). Total hepatic glutathione concentration in cimetidine-pretreated rats was significantly greater than those in control animals up to

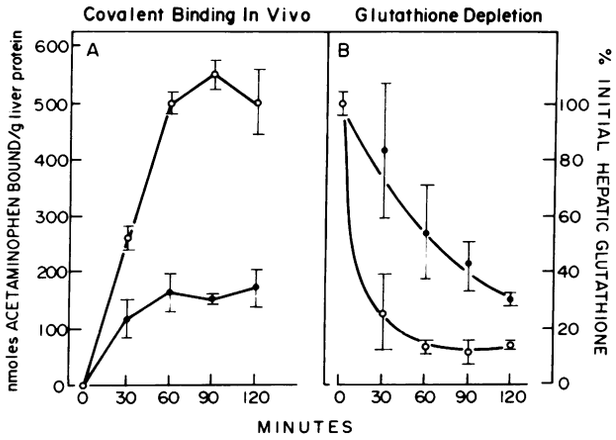

Figure 1. Covalent binding of [^{3}H]acetaminophen to liver proteins in vivo. In control animals (open circles) receiving 500 mg/kg of [^{3}H]acetaminophen (200 μCi/kg), covalent binding increased with time reaching a maximum of 552±23.8 nmol acetaminophen bound/g liver protein at 90 min. In animals pretreated with 150 mg/kg of cimetidine (filled circles) 30 min before receiving the same dose of acetaminophen, significantly less [^{3}H]acetaminophen was bound, reaching a maximum of 170±31.6 nmol/g liver protein at 120 min ($P < 0.05$) (*A*). Hepatic glutathione depletion after a toxic dose of acetaminophen. The initial mean total hepatic glutathione levels at $t = 0$ were similar in control animals (open circles) and those pretreated with 150 mg/kg of cimetidine (filled circles) 30 min before the zero time averaging 1,543±122 μg/g liver (wet weight). Glutathione levels decreased more slowly in cimetidine-pretreated animals than in controls after 500 μg/kg of acetaminophen given intraperitoneally at time zero. At all time points the total glutathione content was significantly higher in cimetidine-pretreated rats than in controls ($P < 0.05$) (*B*).

120 min ($P < 0.05$). Administration of cimetidine alone to 3-MC-pretreated rats did not significantly alter hepatic glutathione content (10).

Acetaminophen oxidation. Incubation of [^{3}H]acetaminophen with rat hepatic microsomes in the presence of NADPH and oxygen resulted in covalent binding of 5.21±0.64 pmol/mg microsomal protein per min at an acetaminophen concentration of 0.1 mM. Incubation of microsomes in the presence of either cimetidine or metiamide resulted in a dose-dependent decrease in the covalent binding of [^{3}H]acetaminophen. In the presence of 0.8 mM cimetidine, covalent binding was 75.8±3.9% of control activity, whereas in the presence of 0.8 mM metiamide, covalent binding was slightly lower, 62.9±5.7% of control (n = 5). This degree of inhibition was significantly greater than that observed for either ranitidine or cimetidine sulfoxide, neither of which inhibited covalent binding by >10% at 0.8 mM inhibitor concentration ($P < .05$), (Fig. 2).

A dose-dependent inhibition of the formation of the acetaminophen-cysteine adduct was observed for both cimetidine and metiamide at a substrate concentration of 1.0 mM acetaminophen. For these two H_2-receptor antagonists only 44% of control activity was present at inhibitor concentrations of 1.0 mM. Ranitidine and cimetidine sulfoxide were far less potent in inhibiting the formation of acetaminophen-cysteine adduct. At similar inhibitor concentrations (1.0 mM) activity was 78% of control for ranitidine and 80% for cimetidine sulfoxide. At higher substrate concentrations (10 mM acetaminophen), similar inhibition was observed for 1.0 mM cimetidine, 50% of control activity, and for 1.0 mM metiamide, 54% of control activity. However, essentially no inhibition occurred with 1.0 mM ranitidine or 1.0 mM cimetidine sulfoxide (Fig. 3). Inhibition of the cysteine adduct formation by cimetidine was mixed, but primarily competitive, when plotted using Lineweaver-Burk plots of 1/v vs. 1/s (Fig. 4). The calculated V_{max} for control incubations was 50.39±14.94 nmol/mg microsomal protein per h, and the Michaelis constant, K_m, was 4.24±1.81 mM. The inhibition constant K_i was calculated for cimetidine and metiamide from the linear portion of Dixon plots (1/v vs. [I]) or using the relationship:

$$K_i = \frac{K_m([I])}{(K_m - K_{m_i})},$$

where [I] is inhibitor concentration, K_m is the Michaelis constant in the absence of the inhibitor and K_{m_i} is the Michaelis constant in the presence of the inhibitor (19). The inhibition constant for cimetidine was determined to be 0.13±.016 mM and for metiamide was 0.20±.050 mM. Incubation of acetaminophen, cysteine and NADPH with human liver microsomes also resulted in enzymatic formation of a covalent adduct of acetaminophen with cysteine. The apparent affinity constant (K_m) for this reaction was 4.06 mM with a V_{max} of 3.20 nmol/mg protein per h. Cimetidine inhibited this reaction in a partially competitive fashion with a K_i of 1.80 mM (Table I).

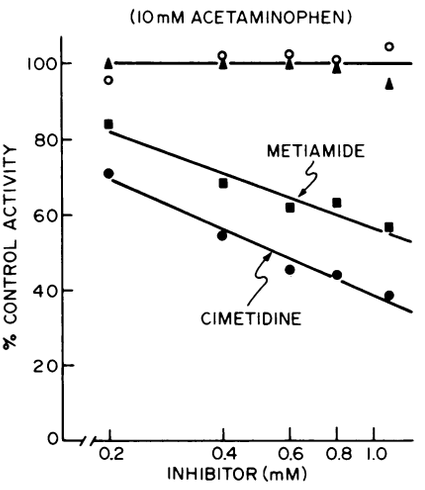

Figure 3. Inhibition of acetaminophen-cysteine adduct formation by H_2-receptor antagonists. In control incubations acetaminophen (10 mM), cysteine (1.0 mM), NADPH (1.0 mM), and hepatic microsomes were incubated in the presence of O_2 for 30 min at 37°C. The acetaminophen-cysteine adduct formed was assayed by HPLC. The results shown in this figure are a representative example of four experiments. When added to microsomal incubations, both cimetidine (filled circles) and metiamide (squares) resulted in a dose-dependent inhibition of the formation of the acetaminophen-cysteine adduct, whereas neither ranitidine (triangles) nor cimetidine sulfoxide (open circles) resulted in any appreciable inhibition.

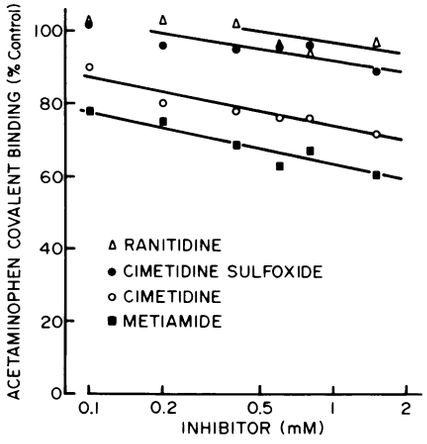

Figure 2. Inhibition of covalent binding of [^{3}H]acetaminophen to hepatic microsomes in vitro. Hepatic microsomes were incubated in the presence of [^{3}H]acetaminophen (0.1 mM), NADPH (1.0 mM), and O_2 at 37°C for 30 min. In control incubations binding was 5.21 pmol acetaminophen/mg microsomal protein per min. The results shown in this figure are a representative example of five experiments. A dose-dependent inhibition of covalent binding of [^{3}H]acetaminophen was observed when either metiamide (squares) or cimetidine (open circles) was added to microsomal incubations. Only minimal inhibition of covalent binding occurred when either ranitidine (triangles) or cimetidine sulfoxide (filled circles) was added to microsomal incubations.

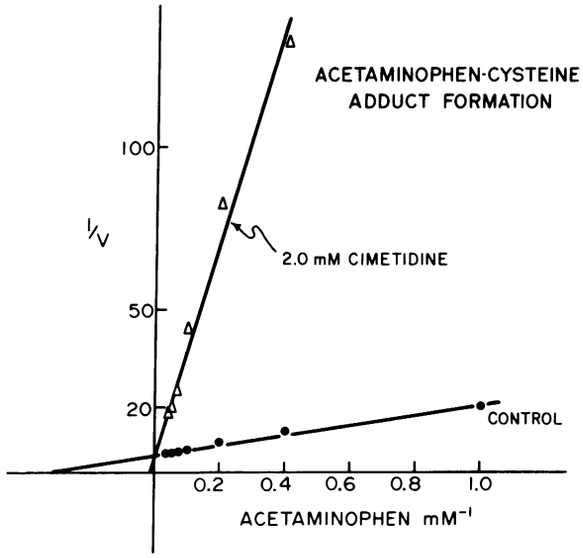

Figure 4. Kinetic analysis of the inhibition of acetaminophen-cysteine adduct formation by cimetidine. This figure shows a representative plot of 1/acetaminophen vs. 1/velocity in the presence (triangles) and absence (filled circles) of 2.0 mM cimetidine. Cimetidine caused competitive inhibition of the formation of acetaminophen-cysteine adduct by hepatic microsomes. V_{max} for control incubations averaged 50.39±14.94 nmol acetaminophen-cysteine/mg protein per h with a K_m of 4.24±1.81 mM.

Acetaminophen glucuronidation. The effect of cimetidine on glucuronidation of acetaminophen in rat microsomes was significantly less than on acetaminophen oxidation. At higher concentrations of cimetidine, minimal competitive inhibition of the formation of acetaminophen glucuronide was observed (Fig. 5). The inhibition constant K_i, for cimetidine in inhibiting glucuronidation was 1.39±0.23 mM, which is significantly higher than the inhibition of oxidation of acetaminophen by cimetidine ($P < 0.0001$). Incubation of acetaminophen with 5.0 mM UDPGA and human microsomes resulted in enzymatic formation of acetaminophen glucuronide with K_m = 5.50 mM and V_{max} = 0.51 nmol/mg protein per h. Cimetidine again competitively inhibited this reaction, with an inhibition constant of 7.90 mM (Table I).

Cimetidine-related inhibition of acetaminophen-induced human lymphocyte toxicity. Incubation of 20.0 mM acetaminophen with human lymphocytes in the presence of microsomes and an NADPH-generating system resulted in a 36.9±9.5% killing of lymphocytes at 24 h as indicated by trypan blue dye exclusion ($n = 5$). At a concentration of 1.7 mM cimetidine only 18.9±5.8% of cells were dead at 24 h, a 49% decrease in toxicity ($P < 0.05$). Using lymphocytes from two subjects, increasing concentrations of cimetidine produced dose-dependent decreases in acetaminophen-related toxicity (Fig. 6). Lactate dehydrogenase released into the culture medium (data not shown) agreed with the data obtained using trypan blue dye exclusion.

Effects of cimetidine upon pharmacokinetics of acetaminophen in normal volunteers. The effects of cimetidine upon pharmacokinetic parameters of acetaminophen are summarized in Table II. As shown there was a 24% reduction in mean plasma clearance of acetaminophen ($P < 0.05$), however, no change was observed in the plasma elimination rate or half-life. Urinary recovery of acetaminophen and its metabolites were not significantly different in subjects while taking cimetidine compared with the base-line period. Recoveries ranged from 65 to 105%. Urinary recovery of the oxidized metabolites was decreased from 9.26±2.08 to 6.38±0.74% but these results did not reach statistical significance. However, the fractional clearance of the oxidized metabolites is a more accurate index of in vivo conversion of acetaminophen to its oxidized metabolites, since clearance = V_{max}/K_m. Cimetidine significantly decreased the fractional clearance of acetaminophen by oxidation to 53% of the control period. Cimetidine also significantly decreased the fractional clearance of acetaminophen by glucuronidation to 76% of the control period. The fractional clearance of acetaminophen sulfate was not significantly changed by cimetidine treatment. These results are summarized in Table III. These data suggest that in man cimetidine inhibits oxidation of acetaminophen and to a lesser extent glucuronidation of acetaminophen.

Discussion

The results described in this study demonstrate that cimetidine inhibits the potentially toxic oxidation of acetaminophen in vitro. By contrast, one of the major routes of elimination of

Table I. Effects of Cimetidine upon Acetaminophen Metabolism In Vitro

Oxidation*	Rat	Human
	n = 6	
K_m (*mM*)	4.24±1.81	4.06
V_{max} (*nmol/mg protein/h*)	50.39±14.94	3.20
K_i (*mM*)	0.13±0.016‡	1.80
Glucuronidation§	*n = 5*	
K_m (*mM*)	5.06±0.45	5.50
V_{max} (*nmol/mg protein/h*)	5.66±1.21	0.51
K_i (*mM*)	1.39±0.23	7.90

* Acetaminophen oxidation was followed by measurement of acetaminophen cysteine adduct formation in the presence or absence of added cimetidine. The enzyme preparation was a 105,000-*g* microsomal pellet from either rat liver or human liver. Data was transformed to double-reciprocol plots and the above constants calculated.
‡ $P < 0.0001$, oxidation vs. glucuronidation.
§ Acetaminophen glucuronidation was followed in the above-mentioned microsomal preparations incubated in the presence or absence of cimetidine by measuring acetaminophen glucuronide.

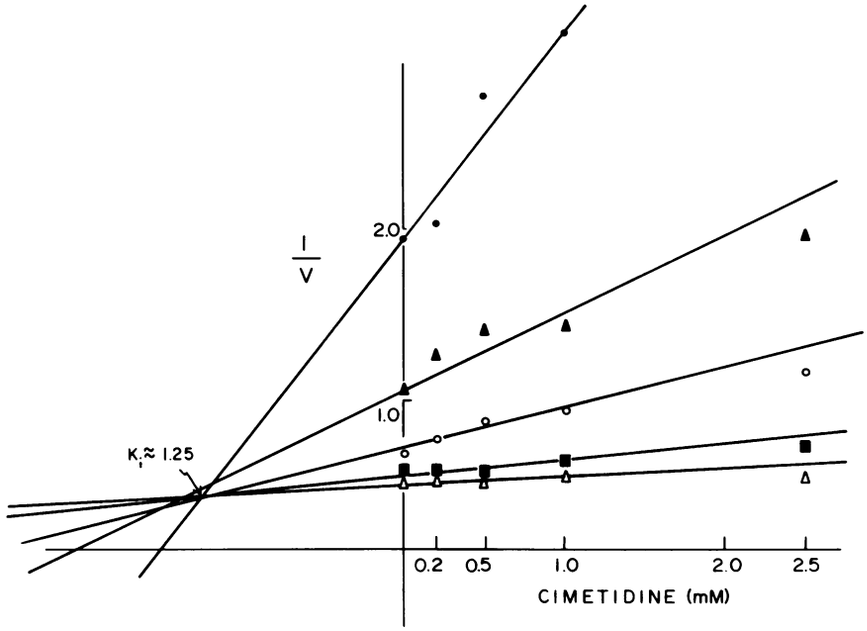

Figure 5. Inhibition of acetaminophen glucuronidation by cimetidine. This figure shows a representative plot of cimetidine vs. 1/velocity for the formation of acetaminophen glucuronide by hepatic microsomes. Microsomes were incubated with 5.0 mM UDPGA and variable concentrations of acetaminophen: 1.0 mM (filled circles), 2.5 mM (filled triangles), 5.0 mM (open circles), 10 mM (squares) and 15 mM (open triangles). In this experiment K_i was 1.25 mM. The mean K_i for five experiments was 1.39±0.23 mM.

acetaminophen, namely glucuronidation, is relatively less affected by cimetidine. Comparison of the inhibitory constants for cimetidine in inhibiting acetaminophen biotransformation suggests that cimetidine is ~5–10 times more potent in inhibiting acetaminophen oxidation than it is in inhibiting acetaminophen glucuronidation. Thus, cimetidine is a relatively selective inhibitor of the potentially toxic oxidation of acetaminophen, especially at lower concentrations of this drug that are likely to be achieved in the liver in vivo (6). The relative lack of inhibition of acetaminophen oxidation seen in vitro in the rat with cimetidine sulfoxide suggests that the parent compound rather than its oxidized metabolite is responsible for the dose-dependent inhibition of cytochrome P-450-mediated oxidation of acetaminophen. Furthermore, in other studies in the rat, metiamide, another imidazole derivative H_2-receptor antagonist, also inhibited acetaminophen oxidation, whereas ranitidine, a furan derivative H_2-antagonist had a much smaller effect (Figs. 2 and

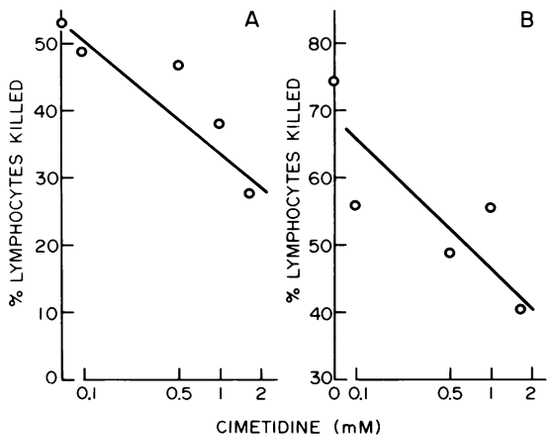

Figure 6. Effect of cimetidine on acetaminophen-mediated killing of human lymphocytes. 1×10^6 lymphocytes from two subjects (*A* and *B*) were incubated for 3 h in the presence of rat liver microsomes, an NADPH-generating system, acetaminophen, and increasing concentrations of cimetidine from 0 to 1.7 mM. Cells were then washed and incubated for an additional 22–24 h at 37°C. Trypan blue was added to the lymphocytes and 200 cells from each tube were scored for viability as measured by trypan blue exclusion. Each point represents the mean of triplicate samples.

*Table II. Effects of Cimetidine upon Pharmacokinetics of Acetaminophen**

	Clearance	Half-life	Vol. dist.‡	Recovery
	ml/min	*h*	*liter*	*24 h*
Control (n = 9)	442±61.2	2.18±0.23	78.1±8.2	81.9±6.4%
Cimetidine (n = 9)	336±38.3§	2.11±0.13	60.6±4.2§	89.5±5.5%

* Healthy volunteers took 1.5 g acetaminophen orally and blood and urine were collected for analysis and calculation of pharmacokinetic parameters. On a second occasion, after taking cimetidine 300 mg four times a day for 3 d, the study was repeated.
‡ Apparent volume of distribution extrapolated from beta phase assuming rapid and complete absorption.
§ $P < 0.05$.

Table III. Effects of Cimetidine upon Metabolism of Acetaminophen in Volunteers

	Sulfate	Glucuronide	Mercapturate	Cysteine	Total oxidized	Total conjugates
Total recovery*, %						
Control	28.2±2.82	59.7±2.55	4.95±1.24	4.30±0.87	9.26±2.08	87.8±2.47
Cimetidine	32.0±2.47	59.7±1.57	3.39±0.46	3.00±0.29	6.38±0.74	91.7±0.77
Fractional clearance (*ml/min*)‡						
Control	127±22.4	265±39.0	19.7±4.20	18.4±4.33	38.1±4.33	392±57.7
Cimetidine	107±13.2	202±26.0§	10.6±1.06§	9.7±1.05‖	20.3±2.01§	310±37.7§
Percentage of Control	84.3	76.2	53.8	52.7	53.3	79.1

* Urinary acetaminophen metabolite concentrations were determined by HPLC on the 24-h urines collected after 1.5 g oral acetaminophen in the control period and after 3 d of cimetidine therapy. ‡ Fractional clearance of acetaminophen by each metabolic pathway was calculated by multiplying the total plasma clearance of acetaminophen by the fractional recovery of each metabolite in the 24-h urine samples. § $P \leq 0.05$. ‖ $P = 0.067$.

3). This finding suggests that the imidazole structure of cimetidine, rather than its H_2-receptor antagonist properties is important in the inhibition of cytochrome P-450-mediated drug oxidation. Previous studies have shown that cimetidine binds much more avidly to oxidized cytochrome P-450 in vitro than does ranitidine or cimetidine sulfoxide, with all drugs yielding a type II spectral change (6). Based on the results of electron paramagnetic resonance studies, Rendic et al. (20) have suggested that both the imidazole and amine nitrogens in cimetidine are possible ligands for the heme portion of the cytochrome. This direct interaction with cytochrome P-450 may provide the molecular basis for how cimetidine inhibits cytochrome P-450-mediated oxidation both in vitro and in vivo.

Cimetidine-related inhibition of the oxidation of acetaminophen to its reactive metabolite is an important toxicological effect. Both pretreatment and posttreatment with cimetidine have been reported to increase survival of animals given lethal doses of acetaminophen (10–12). Cimetidine also appears to inhibit the formation of the reactive metabolite of acetaminophen in vivo. The rate and absolute degree of acetaminophen-induced glutathione depletion were lower in rats pretreated with cimetidine than in those receiving saline. Furthermore, the amount of acetaminophen covalently bound to liver proteins, an index of the potential toxicity of acetaminophen, was lower in cimetidine-pretreated rats (Fig. 1). In the rat cimetidine has a relatively short half-life, which is ~45 min (21). By 120 min after receiving acetaminophen the amount of cimetidine remaining in the plasma would be <20% of the initial levels. This short half-life could explain the continuing decrease in hepatic glutathione depletion observed in our study. Cimetidine also protected human lymphocytes from acetaminophen-related toxicity. This protection was dependent on the dose of cimetidine. Since cimetidine was removed from the lymphocytes at the same time as acetaminophen, NADPH and microsomes were removed, this finding is consistent with the hypothesis that cimetidine inhibits the formation of toxic acetaminophen metabolites.

The results of the present study demonstrate that cimetidine significantly reduces the clearance of acetaminophen by oxidative (potentially toxic) pathways in human volunteers. Previous studies have shown that the elimination of acetaminophen metabolites in urine parallels the elimination of the parent compound from plasma (22). The fractional clearance of each metabolite thus represents the contribution of each separate enzymatic pathway to the overall metabolism of acetaminophen. The actual effects of cimetidine on the formation of the toxic intermediate of acetaminophen are not easily measured in man. After the drug is oxidized, further conjugation with glutathione occurs and ultimately this glutathione adduct is degraded to mercapturic acid and cysteine derivates before final urinary elimination. These additional metabolic steps may compound interpretation of changes in fractional clearance of acetaminophen by oxidation. Glucuronidation of acetaminophen is significantly reduced by cimetidine, but to a lesser extent than oxidation. Although cimetidine does not affect glucuronidation of the benzodiazepines, lorazepam, and oxazepam, in man, the observed inhibition is consistent with the in vitro data on glucuronidation of acetaminophen in rat and human microsomes in the present study. These findings are in contrast to those of Abernethy et al. (12), who reported that clearance of acetaminophen was unchanged after cimetidine. In that study acetaminophen was given intravenously rather than orally as in the present study. Although complete absorption of acetaminophen is assured by intravenous administration of acetaminophen, changes in hepatic blood flow rather than changes in enzyme activity may play a larger role in determining clearance, since the extraction ratio of acetaminophen is intermediate rather than low. Our results do however support the hypothesis that cimetidine is a more potent inhibitor of the potentially toxic oxidation of acetaminophen than of the nontoxic conjugation.

Previous studies have demonstrated that sulfhydryl compounds such as cysteamine, cysteine, *N*-acetylcysteine, α-mercaptoproprionylglycine, propylthiouracil, and others can prevent acetaminophen-induced hepatic necrosis (23–29). Although

some of these compounds may inhibit the formation of the toxic metabolite of acetaminophen, their primary mechanism of action appears to be related to their ability to prevent covalent binding and/or toxicity of the reactive intermediate after its formation. These agents may form covalent adducts with reactive acetaminophen metabolites directly or in reactions mediated by glutathione-*S*-transferase or they may detoxify the metabolite by serving as reducing agents or through other membrane stabilizing effects. Consistent with this hypothesis, we found metiamide, which has a thiol sulfur, to be more effective in preventing in vitro covalent binding than cimetidine, although other studies of oxidative metabolism showed metiamide to be equal to or less potent than cimetidine as an inhibitor. Piperonyl butoxide and metyrapone also prevent acetaminophen-induced damage in animal models (1, 30). These agents are well recognized inhibitors of cytochrome P-450-mediated oxidation, the metabolic pathway through which acetaminophen is converted to a toxic, electrophilic intermediate. However, piperonyl butoxide is toxic and both agents have potentially undesirable side effects (1, 30). Therapy with *N*-acetylcysteine is frequently complicated by nausea and vomiting when this agent is given orally (31). Cimetidine is a remarkably safe drug when used in usual therapeutic doses such as those given in this study and may be given intravenously. Inhibition of drug oxidation is rapid in onset and is reversible after the drug is eliminated by renal clearance (32). Evaluation of the potential benefit of cimetidine or any other agent for acetaminophen hepatotoxicity in man is difficult. Our results do not prove that cimetidine is effective for reducing acetaminophen hepatotoxicity in man, however, inhibition of formation of the toxic metabolite does occur in both man and animals. When considered with the results of previous studies showing that cimetidine reduces the severity of acetaminophen-induced liver injury in animals (10–12), the present study provides a rational basis for assessing possible benefit of cimetidine treatment of acetaminophen overdoses in man. One potential problem that might arise with the use of cimetidine for this purpose is in treatment of patients with multiple drug ingestions. In such a situation cimetidine may prolong the elimination of other drugs such as benzodiazepines and barbiturates (33–34). For these reasons, the use of cimetidine as a treatment of acetaminophen overdoses in man cannot be recommended at this time. Further studies to assess the usefulness of cimetidine as an adjunct to standard treatment of acetaminophen overdoses in man with *N*-acetylcysteine are warranted.

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