bicin reported by Villani *et al.* [7] in which doxorubicin (200 μM) acutely decreased the maximal contractile response to calcium with little change in the apparent affinity of the calcium receptive sites in the isolated atrial preparations for calcium. The low and high daunorubicin concentrations (1 and 100 μM) used in the present experiments were near the range of anthracycline concentrations observed in patients during infuson of these drugs (range = 7 to 70 μM, plasma concentrations reported for doxorubicin) [16]. The mechanisms behind these effects are not yet definable. It may be speculated that changes in membrane fluidity [17] induced as the anthracycline attaches to lipid or protein structures [18] in the cell membrane can lead to a decrease in the number of dihydropyridine binding sites and alter calcium channels. There is evidence that lipid peroxidation does not play an important role in the acute phase of doxorubicin-induced cardiotoxicity [19]. It is likely that the changes in dihydropyridine binding are only related to the "acute" phase of exposure to anthracyclines since the inhibitory effect of daunorubicin *in vitro* was rapidly reversed upon washout of the atrial tissue with fresh Tyrode's solution. In addition, there was no evidence for a long-lasting change in the PN200-110 binding to dihydropyridine-sensitive binding sites in cardiac sarcolemma exposed to cumulative doses of daunorubicin (4 mg/kg) *in vivo*.

In summary, the acute effects of an anthracycline antitumor agent, daunorubicin, on the slow calcium channels in cardiac sarcolemma were examined with the aid of a radiolabeled 1,4-dihydropyridine derivative, (+)-[^{3}H]PN200-110. Daunorubicin noncompetitively reduced the concentration of PN200-110-sensitive binding sites. Daunorubicin may inhibit the positive inotropic and chronotropic effects of calcium or neurotransmitters by the disruption of slow calcium channel function in cardiac tissues.

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Lack of inhibition of mouse catalase activity by cimetidine: an argument against a relevant general effect of cimetidine upon heme metabolic pathways

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Cimetidine, an H_2-histamine receptor antagonist, is used clinically in the treatment of a variety of conditions that respond to an increase in gastric pH, and is generally considered to have no serious side effects or contraindications. However, early studies demonstrated a weak, antiandrogenic effect in rodents that was also observed in humans treated for gastric hypersecretory states [1]. A number of clinical studies have shown that cimetidine alters the effective levels of a large number of widely prescribed pharmaceuticals including propanolol [2], diazepam [3], digitoxin and quinidine [4], and procainamide [5], several of which exhibit very narrow therapeutic indices and possess extreme potential for toxicity.

Cimetidine reduces both *in vitro* and *in vivo* hepatic mixed-function oxidase activity for a wide variety of substrates in the rat [6–8]. The appearance of a typical type II spectral change in rat liver microsomes incubated in the presence of cimetidine suggested a direct interaction of the compound with cytochrome P-450 [7]. Furthermore, several groups have demonstrated the presence of a high affinity binding site for cimetidine on cytochrome P-450 in liver microsomes obtained from both humans and rats [9, 10], with both the imidazole and cyano portions of cimetidine interacting with the hemin iron [11]. A binding site on cytochrome P-450 apparently does not exist for ranitidine, a structurally dissimilar histamine H_2 antagonist which does not inhibit hepatic mixed-function oxidases, thus prompting the suggestion that cimetidine alters the oxidative metabolism of other compounds by exerting a direct inhibitory effect on cytochrome P-450.

However, the results of yet other studies suggest that cimetidine may affect hepatic mixed-function oxidase

activity in a more general manner. Hepatic δ-aminolevulinic acid synthetase (δ-ALAS) activity, heme oxygenase activity, and microsomal P-450 content were all decreased significantly 30 min following injection of rats with cimetidine [12]. Therefore, cimetidine may also affect microsomal cytochrome P-450 levels by altering the level of activity of δ-ALAS, the rate-limiting enzyme for heme biosynthesis [13], and/or the activity of heme oxygenase, the enzyme which controls heme degradation [14].

If cimetidine exerts a *relevant* general effect upon heme biosynthetic or degradative pathways (or both), it is reasonable to expect that such effects should become manifest in heme-containing proteins other than cytochrome P-450 in cimetidine-treated animals. We report here that neither acute nor chronic treatment of C57BL/6J male mice with cimetidine produced any effect upon renal or hepatic levels of activity of the hemoprotein catalase. We also demonstrated that cimetidine had no effect upon the activity of mouse liver homogenates or purified beef liver catalase *in vitro*, nor was there any evidence obtained from spectral studies suggesting a direct interaction of cimetidine with purified beef liver catalase.

Materials and methods

C57BL/6J male mice, 10 months of age, were used throughout this study. Mice were obtained at 1 or 2 months of age from Jackson Laboratories, Bar Harbor, ME, and maintained in an aging colony. Animals were housed in plastic cages, six mice per cage, the bottoms of which were covered by a ¾ in. absorbant layer of Bed-O-Cobs. The environmental room holding the cages was maintained at 22.0 ± 0.5°, 50% r.h., with lights on from 6:00 a.m. to 6:00 p.m. EST. Mice were presented with Purina Rodent Laboratory Chow and tap water *ad lib.*

Cimetidine (greater than 99.9% purity) was provided by Smith, Kline & French Laboratories, Inc., Philadelphia, PA, and was used without further purification. Dimethyl sulfoxide (DMSO) was high performance liquid chromatography grade. All other reagents and chemicals were the best grade available.

Chronic administration of cimetidine. One group of mice was injected intraperitoneally at 12-hr intervals with cimetidine (5 mg/100 g body wt per injection) dissolved in sterile 0.9% NaCl (as 5 mg/ml). A second group of animals was injected intraperitoneally with an equivalent volume of sterile 0.9% NaCl. Four mice from each group were killed by cervical dislocation at varying times following initiation of treatment with cimetidine or saline. The liver and kidneys were perfused *in situ* through the inferior vena cava with 20 ml of ice-cold 0.25 M sucrose. The perfused organs were removed, weighed, homogenized in 9 vol. of 0.05 M Tris–HCl–10% sucrose (pH 7.4 at 25°), and assayed for catalase activity by a modification [15] of the polarographic method described by Goldstein [16], using sodium perborate as substrate. One unit of catalase activity is defined as that enzyme activity which liberates 1 μmol O_2 per min at 30°. Protein content of liver and kidney whole homogenates was determined by the method of Lowry *et al.* [17] using bovine serum albumin as standard.

Acute administration of cimetidine. Mice were injected intraperitoneally with cimetidine [20 mg/100 g body wt dissolved in 0.9% NaCl–DMSO (3:1)]. DMSO was utilized to facilitate preparing high concentrations of cimetidine in order to maintain an equivalent injection volume in acute and chronic experiments. Four mice were killed at varying times following injection with cimetidine, and the liver and kidneys were removed and assayed for catalase activity as described above.

In other experiments, four mice in each of five groups were injected with cimetidine (20 mg/100 g body wt) dissolved in 0.9% NaCl–DMSO (3:1) solvent only and killed at varying time intervals following injection. Livers were removed, rinsed in 50 mM Tris–HCl (pH 7.4 at 25°)–150 mM KCl–10 mM $MgCl_2$, and 10% homogenates were prepared in the same solution. The homogenates were centrifuged at 9,000 *g* for 10 min, and the resulting supernatant fraction was centrifuged at 17,000 *g* for 20 min. The supernatant fractions were assayed for cytochrome P-450 content according to the method of Matsubara *et al.* [18].

In vitro *studies.* (a) Mouse liver catalase. Mice were killed, and their livers were removed and homogenized as described. Liver whole homogenates were diluted both in the absence and presence of Triton-X-100, and were preincubated for 1 min at 30° in the presence of various concentrations of cimetidine dissolved in DMSO, after which the homogenates were assayed polarographically for catalase activity as previously described.

(b) Beef liver catalase (code CTR, Worthington Biochemicals, Freehold, NJ) was dialyzed for 48 hr against four changes of 0.1 M Na_2HPO_4 (pH 8.3) to remove the thymol preservative. Protein in the dialysate was determined by the method of Lowry *et al.* [17]. The catalase sample was diluted to 2×10^6 M assuming a molecular weight of 240,000 daltons for beef liver catalase [19]. The UV-VIS spectrum of beef liver catalase was determined with a Varian 118C spectrophotometer both in the absence and presence of cimetidine at a final concentration of 1.0 mM. Cimetidine was added to the sample cuvette in 4.0 μl DMSO, while an equal volume of DMSO was added to the reference cuvette.

In other studies the effect of cimetidine upon the activity of beef liver catalase was determined *in vitro*. One millilitre of dialyzed catalase solution (diluted to 1.2×10^{-9} M) was preincubated for 1 min at 30°, and various concentrations of cimetidine, dissolved in 10 μl DMSO, were added to the sample for an additional minute. The samples were then assayed for catalase activity as previously described.

Results

Chronic exposure of C57BL/6J male mice to cimetidine had no significant effect upon either hepatic or renal catalase activity (Fig. 1). Hepatic catalase activity was higher than that present in kidney, thereby confirming previous observations [17, 20–23].

Acute treatment of C57BL/6J male mice with high doses of cimetidine for up to 2 hr had no significant effect upon either liver or kidney catalase activity (data not shown) or hepatic cytochrome P-450 content (Table 1). Thus, cimetidine does not appear to bind to catalase with subsequent loss of enzymic activity.

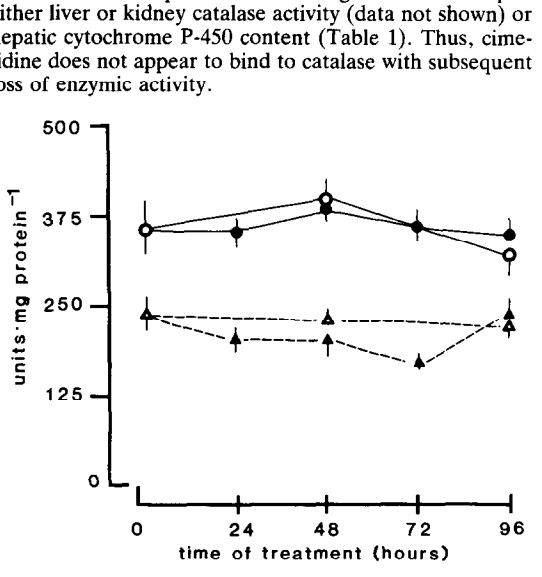

Fig. 1. Catalase activity in liver (solid line) and kidney (dashed line) of C57BL/6J male mice injected at 12-hr intervals with cimetidine. Key: (●) liver, control; (○) liver, cimetidine treated; (▲) kidney, control; and (△) kidney, cimetidine-treated. Vertical bars indicate ± 1 SE of the mean.

Table 1. Cytochrome P-450 content in liver homogenates prepared from C57BL/6J male mice at various time intervals following injection of cimetidine (20 mg/100 g body wt)

Time after injection (min)	Cytochrome P-450 (nmol/mg protein)
Control†	0.245 ± 0.032*
0‡	0.232 ± 0.011
30	0.246 ± 0.012
60	0.226 ± 0.037
120	0.271 ± 0.032

* Mean ± 1 SE.
† Untreated control animals.
‡ Injected and immediately killed.

There exists the possibility in these studies that cimetidine is not present in the tissue(s) of treated animals following either acute or chronic treatment with cimetidine, since the plasma half-life of intravenously administered cimetidine in rats is approximately 1 hr, the drug passing largely unchanged through the kidney [24]. Additionally, dilution of tissue extracts prior to enzyme assay may result in dilution and subsequent loss of binding of cimetidine to hemoprotein molecules. Therefore, we utilized *in vitro* studies to determine unequivocally whether cimetidine exerts any direct effect on catalase activity. When high concentrations of cimetidine were incubated with catalase *in vitro*, there was no effect on the activity of mouse liver catalase as shown by the results of a typical experiment (Table 2). DMSO had no effect on catalase activity, nor was there any significant effect of Triton X-100, a 0.1% solution of which is normally utilized as a detergent to ensure release of all catalase activity in tissue samples.

This seemingly negative effect of cimetidine on mammalian catalase activity was examined more fully utilizing purified beef liver catalase incubated in the presence of a 10^6 molar excess of cimetidine. There was no effect of the compound upon the activity of the enzyme (Table 2), nor was there any spectral evidence for an interaction of cimetidine with highly purified beef liver catalase (Fig. 2).

Discussion

Catalase is a hemoprotein present in high concentration in mammalian liver and kidney [24, 25]. The half-life of this enzyme in both mouse liver and kidney is approximately 48 hr [25, 26]. Thus, if administration of cimetidine to mice significantly and rapidly affects either the synthesis or degradation (or both) of heme, as reported in the rat [12], significant perturbation in the activity of catalase should become manifest. However, the present results show that this prediction was not observed.

Additionally, there was no evidence that high acute doses of cimetidine exerted any effect on liver or kidney catalase activity in mice, nor was there any evidence for interaction

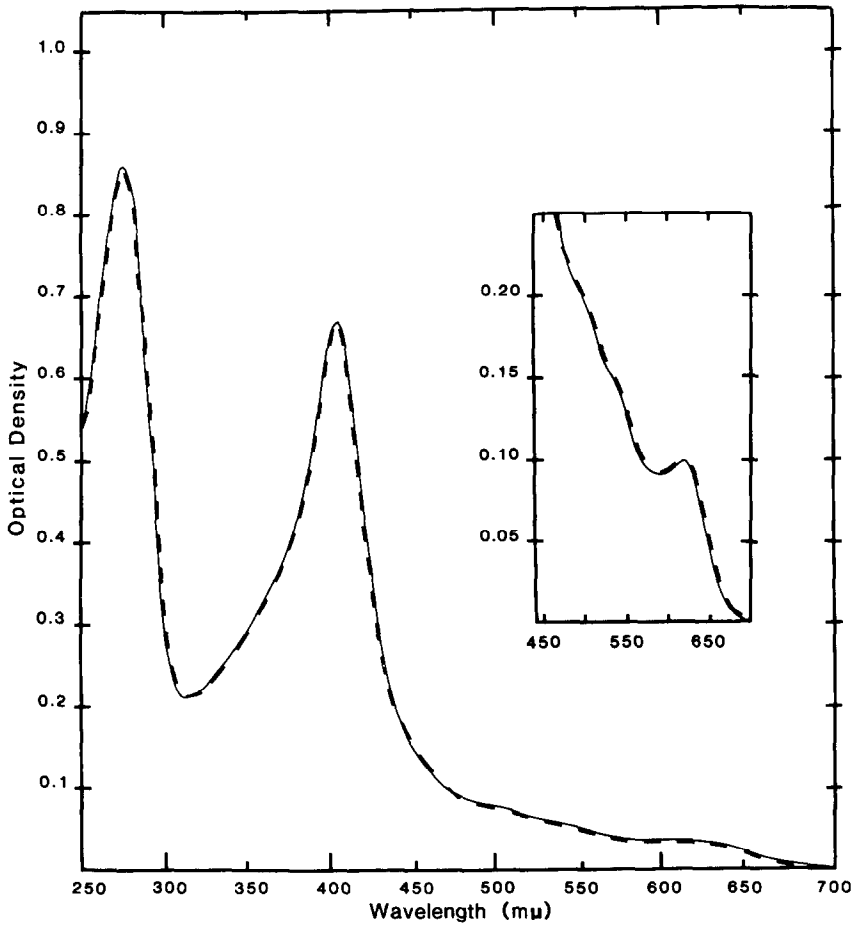

Fig. 2. UV-VIS spectrum of purified beef liver catalase in the absence (dashed line) and presence (solid line) of 2.0 mM cimetidine. The reference cuvette contained buffer and an equal volume of DMSO. Inset: Expansion of the spectrum encompassing the alpha and E regions for beef liver catalase.

Table 2. Catalase activity of mouse liver whole homogenates or beef liver catalase solutions assayed in the presence of various final corrections of cimetidine

Cimetidine (mM)	Mouse liver homogenates: Catalase activity (units/mg protein) With Triton	Mouse liver homogenates: Catalase activity (units/mg protein) Without Triton	μl DMSO in sample	Beef liver* Catalase activity (units/mg protein)
0	312	246	10	31,400
1.0	341	218	2	30,600
2.0	295	274	4	31,500
3.0	315	300	6	25,200
4.0	320	285	8	30,500
5.0	320	309	10	30,800

* All samples in 10 μl DMSO.

of cimetidine with either mouse liver catalase or with purified beef liver catalase *in vitro* as shown by a lack of effect of cimetidine on beef liver catalase activity. Of particular interest, there was no apparent interaction of cimetidine with the sixth ligand position of catalase heme, as demonstrated by a lack of a bathochromic shift, particularly in the alpha (624–626 nm) and E (500–505 nm) bands, where metal to ligand charge transfers would be expected to occur [19]. Thus, cimetidine does not appear to interact directly with mouse renal or hepatic catalase, or with purified beef liver catalase, at least at a site(s) which might result in a loss in enzyme activity or a visible change in the spectrum of the hemoprotein, indicating that this compound does not interact with hemoproteins as a general rule.

The present results obtained with mice demonstrate that cimetidine produces no relevant general effect on heme metabolism, nor does it directly interact with heme-containing proteins in a general fashion. Likewise, there was no evidence that treatment with cimetidine produces a rapid decline in mouse hepatic P-450 content, as previously reported for the rat [11], since the present study demonstrated no effect of cimetidine upon hepatic cytochrome P-450 content in the C57BL/6J male mice. Thus, the findings of the present study are consistent with the hypothesis that cimetidine inhibits the metabolism of other compounds as a consequence of a specific interaction of cimetidine with microsomal cytochrome P-450.

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