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Full Papers

Cimetidine induces hepatic heme oxygenase activity without altering hepatic heme catabolism

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Summary. Cimetidine inhibits oxidative drug metabolism; it is not known whether this drug alters the catabolic fate of hepatic heme. We therefore investigated hepatic heme turnover both by a ^{14}CO breath test and directly by labeling the heme pool. Neither acute (150 mg/kg i.p.) nor chronic (150 mg/kg i.p. bid for 3 days) cimetidine administration significantly affected hepatic heme turnover. Chronic, but not acute, cimetidine significantly ($p < 0.025$) increased heme oxygenase activity. Cimetidine inhibited heme oxygenase activity in vitro at concentrations achieved in vivo.
Key words. Cimetidine; heme; heme oxygenase; cytochromes; breath test.

Introduction

Cimetidine has been shown to inhibit the microsomal monooxygenase system both in vivo and in vitro[1–3]. This has been ascribed to a reversible high-affinity binding of the drug to microsomal cytochrome P-450[3,4]. It is not known whether this binding leads to destruction of the cytochrome and whether turnover of heme is altered by cimetidine.

Materials

Male Sprague-Dawley rats, b.wt 175–225 g, were obtained from Charles River Breeding Laboratories, Wilmington, MA. 5-^{14}C-delta-amino-levulinic acid (ALA, sp.A. 48.9 mCi/mmole) was obtained from Research Products International, Elk Grove, Ill. Methanol HPLC grade and D-4 (dibutylamine-phosphate) were from Waters, Milford, Mass. Cimetidine and cimetidine sulfoxide

were gifts from Smith, Kline and French Corp., Philadelphia, PA. N:methyl-N'-(2-imidazol-4-yl)-propyl)-cyanoguanidine, used as internal standard in the HPLC assay of cimetidine, was a gift from Smith, Kline and French, Welwyn Garden City, United Kingdom. All other reagents were analytical grade purchased from different sources.

Methods

^{14}CO Breath test. To study hepatic heme turnover in vivo, a recently described modification[5] of the ^{14}CO breath test originally described by Bissell[6] was used. Rats with a chronically implanted jugular vein catheter were constantly infused with 5-^{14}C-delta-aminolevulinic acid (300,000 dpm/h). After a 60-h equilibration period the animals were injected once with either cimetidine (150 mg/kg) or an appropriate volume of the solvent. In these acute experiments, ^{14}CO production was measured for 24 h following cimetidine administration. In the chronic experiments, after the 60-h equilibration period, the animals received cimetidine (150 mg/kg) twice daily for three consecutive days. In these experiments, ^{14}CO production was measured for a total of 120 h after initiation of drug administration.

Preparation of microsomes and microsomal enzyme assays. Microsomes from treated and control rats were prepared 24 h after the last drug administration by differential centrifugation[7]. Cytochrome P-450 content of the microsomal preparations was quantitated by the reduced CO binding spectrum[8]. Microsomal heme oxygenase activity was determined according to Tenhunen et al.[9]. The effects of cimetidine and its main metabolite, cimetidine sulfoxide, on heme oxygenase activity in vitro were determined by adding the respective agents to the microsomal incubation mixture at final concentrations of 10^{-5} to 5×10^{-3} M. Effects of the agents were expressed as the ratio of the activity of the 'treated' over the activity of the untreated microsome fraction.

Decay of microsomal heme was determined after labeling the hepatic heme pool with 10 µCi of 5-^{14}C-delta-aminolevulinic acid[6,10]. Heme was extracted from a liver homogenate into diethyl ether under acidic conditions[11]. Twenty-four hours after giving the radiolabeled heme precursor, a laparotomy was performed under ether anesthesia and a biopsy weighing 200–300 mg was removed from the right lateral lobe. Another 24 h later the animals were sacrificed and the remaining liver removed. After extraction of the heme, it was bleached with hydrogen peroxide (30% v/v) and its radioactivity assessed by liquid scintillation counting. Budgetsolve was used as the scintillator and quenching was determined by the external channel ratio method. Extraction efficiency, determined by adding a known amount of exogeneous hemoglobin[6], averaged 92%.

Other biochemical assays. Microsomal protein concentration was measured by a modification of the Lowry assay[12] using bovine serum albumin as the standard.

Microsomal concentration of cimetidine and its main metabolite, cimetidine sulfoxide, were measured by high-pressure liquid chromatography. The drugs were extracted from serum and liver homogenate 1–24 h after cimetidine administration into ethyl acetate: isopropanol in an alkaline milieu[13].

N:methyl-N'-(2-(imidazol-4-yl)-propyl)-cyanoguanidine was added as an internal standard. The mobile phase consisted of methanol:water (1:9) containing D-4 as a mobile phase modifier. The stationary phase was a C-18 reverse phase cartridge in a Waters RCSS radial compression device. The effluent was monitored at 228 nm by a Waters M-480 UV monitor. Cimetidine, cimetidine sulfoxide and the internal standard had retention times of 6.5, 2.9 and 3.7 min, respectively. The coefficient of variation for the cimetidine and cimetidine sulfoxide assay were 4.8 and 5.1%, respectively. The lower limit of detection was 25 ng.

Statistical analysis. All results are expressed as mean ± 1 SD. Means of two groups were tested by Student's t-test after testing the equality of variances with an F-test[14]. If the variances were unequal, a modified t-test was employed[15]. $p < 0.05$ was considered statistically significant.

Results

Twelve hours after the beginning of the infusion of 5-^{14}C-delta-aminolevulinic acid, ^{14}CO-excretion in breath approached a steady state. No significant difference in ^{14}CO-excretion could be seen after either acute or chronic cimetidine administration (fig. 1). Since up to 40% of hepatic heme could be broken down by pathways not involving the formation of CO[10,16], heme breakdown was assessed directly after a pulse of 10 µCi of 5-^{14}C-delta-aminolevulinic acid. The results of these experiments are given in table 1. Neither acute nor chronic cimetidine administration significantly altered the breakdown of microsomal heme.

The effect of acute and chronic cimetidine administration on microsomal cytochrome P-450 content is shown in

Table 1. Effect of acute and chronic cimetidine administration on hepatic heme turnover. Hepatic heme was labeled in vivo by 5-^{14}C-delta-aminolevulinic acid; the amount of labeled heme in microsomes was determined after heme extraction and related to mg microsomal (dpm/mg)

	Acute		Chronic	
	24 h	48 h	24 h	48 h
Control	1592 ± 261	797 ± 219	1632 ± 405	895 ± 294
Cimetidine	1594 ± 424	857 ± 246	1620 ± 306	825 ± 159

Mean ± SD are given (n = 3).

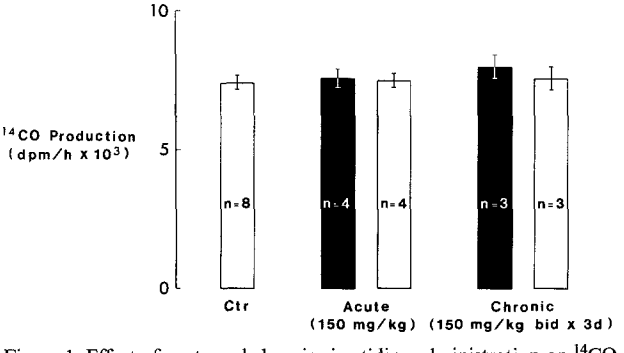

Figure 1. Effect of acute and chronic cimetidine administration on ^{14}CO production in rats chronically infused with 5-^{14}C-delta-aminolevulinic acid. The mean ± 1 SD of the CO production, measured in hourly intervals over a 24-h period are shown. Controls are shown as white, cimetidine-treated animals as black bars. Neither treatment modality significantly affected ^{14}CO production.

figure 2. Neither treatment modality significantly altered microsomal cytochrome P-450 content measured 24 h after the last cimetidine administration. The effects of acute and chronic cimetidine administration on heme oxygenase activity in vivo are displayed in figure 3. Acute cimetidine administration did not alter heme oxygenase activity. By contrast, chronic cimetidine administration increased activity of this enzyme by 87% ($p < 0.025$).

The effect of incubation of untreated microsomes with different concentrations of cimetidine is shown in figure 4. Beginning at 5×10^{-4} M, dose-dependent inhibition of heme oxygenase activity could be observed. The main metabolite of cimetidine, cimetidine sulfoxide, had no effect on heme oxygenase activity in vitro up to the maximal concentration studied (5×10^{-3} M).

The time course of cimetidine concentration in serum and liver is given in table 2. Twenty-four hours after the last administration of cimetidine neither the parent compound (table 2) nor cimetidine sulfoxide (data not shown) could be observed in either serum or liver (table 2).

Discussion

Our studies have shown that cimetidine does not alter turnover of hepatic heme[5]. Somewhat surprisingly, in spite of the absence of an effect on hepatic heme turnover, cimetidine significantly increased the activity of hepatic heme oxygenase, the key enzyme in hepatic heme catabolism[9].

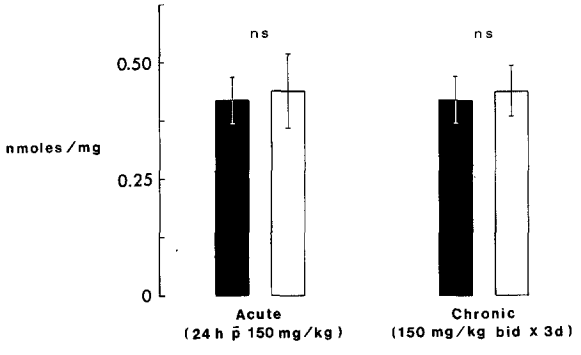

Figure 2. Effect of acute and chronic cimetidine administration (black bars) on microsomal cytochrome P-450 content. Controls are shown as white bars. The microsomes were prepared 24 h after the last drug administration. Mean ± 1 SD are given. Neither treatment resulted in a significant difference in hepatic cytochrome P-450 content.

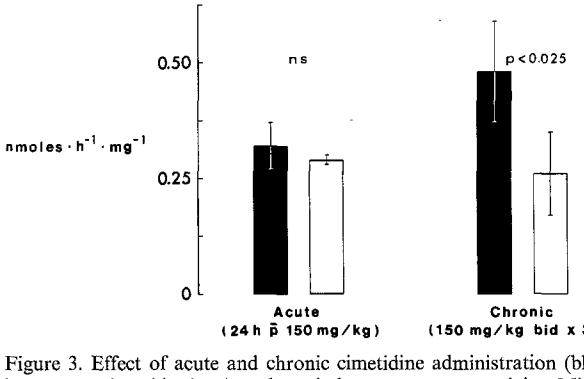

Figure 3. Effect of acute and chronic cimetidine administration (black bars; controls: white bars) on hepatic heme oxygenase activity. Microsomes were prepared 24 h after the last drug administration.

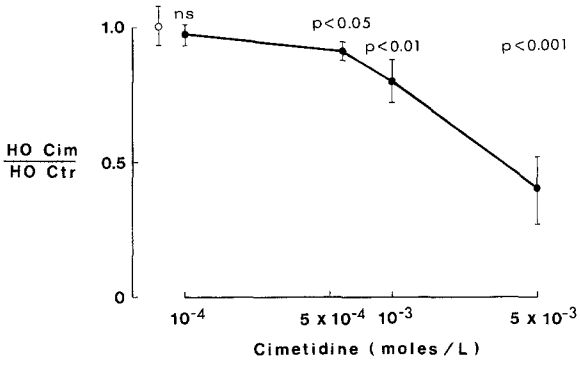

Figure 4. Effect of cimetidine on microsomal heme oxygenase (HO) activity in vitro. The ratio of the activity of microsomes incubated in the presence of the indicated cimetidine concentration over its control activity are displayed as a function of the cimetidine concentration. Starting at cimetidine 5×10^{-4} M a significant and dose-dependent inhibition of enzyme activity was observed.

Cimetidine inhibits oxidative drug metabolism both in vivo[2,17-19] and in vitro[3,4,19] by eliciting a type II binding spectrum in vitro[3,4]. Whether binding of cimetidine leads to destruction of hepatic cytochrome P-450 heme is unknown.

The ^{14}CO breath test assesses heme oxygenase-dependent nonerythrogenic heme breakdown[5,6]. It can detect effects of minor stimulation of heme oxygenase such as those induced by fasting [5]; no alteration of ^{14}CO production was detectable after either acute or chronic cimetidine administration (fig. 1). This suggests that the drug does not induce heme oxygenase-dependent breakdown of heme.

Pathways of hepatic heme breakdown not involving formation of CO may be quantitatively as important as the heme oxygenase-dependent heme catatolism[10,16]. We therefore directly assessed heme turnover by administration of 5-^{14}C-delta-aminolevulinic acid[6,10]; this showed that cimetidine administration did not affect total hepatic heme turnover (table 1).

Acute cimetidine administration affected neither hepatic cytochrome P-450 content (fig. 2) nor heme oxygenase activity (fig. 3). In agreement with earlier work by other investigators, chronic cimetidine administration did not induce an increase in microsomal cytochrome P-450 content[18]. However, chronic cimetidine administration significantly increased hepatic heme oxygenase activity (fig. 3).

Heme oxygenase activity usually parallels heme turnover[6,9,21,22]. An exception to this rule has been found when heme oxygenase was induced by heavy metal or

Table 2. Concentrations of cimetidine in serum (μg/ml) and liver (μg/g) after i.p. administration of cimetidine (150 mg/kg)

Time	Serum	Liver
1 h	5.1 ± 1.2	7.8 ± 3.2
2 h	7.2 ± 2.1	8.4 ± 3.6
3 h	2.3 ± 0.8	3.2 ± 1.2
4 h	0.6 ± 0.2	0.9 ± 0.2
5 h	0.2 ± 0.1	0.3 ± 0.2
6 h	0.1 ± 0.1	0.2 ± 0.1
12 h	n.d.	Trace
24 h	n.d.	n.d.

n.d., not detectable. The lower limit of detection of the assay is 25 ng. Mean ± SD are given (n = 3).

metalloporphyrin administration. Induction of heme oxygenase by heavy metals is usually associated with destruction of cytochrome P-450; this can be prevented by simultaneous administration of SKF 525A. This protective effect was ascribed to formation of a complex of SKF 525A with cytochrome P-450[23]. A similar protective effect of metyrapone has recently been described in hepatocyte culture[24]. It is conceivable that cimetidine similarly protects cytochrome P-450 from increased heme oxygenase activity.

Our finding that cimetidine administered chronically induces heme oxygenase is in apparent contrast to the findings of Marcus and co-workers[20]. These authors found reduced activity of this enzyme 30 min after cimetidine administration. This apparent discrepancy is easily resolved when one considers the effect of cimetidine on heme oxygenase activity in vitro (fig. 6). Inhibitory concentrations of cimetidine are easily achieved in the liver (table 2). It is therefore likely that Marcus et al. were seeing a direct inhibitory effect of cimetidine and not depression of enzyme content.

The inhibitory effect of cimetidine on heme oxygenase may explain the lack of an effect of cimetidine on heme turnover in spite of an increase in heme oxygenase activity. The direct inhibitory action of cimetidine may lead to derepression of a feed-back loop and thereby to induction of the enzyme. Inhibitory concentrations of cimetidine are achieved for about 4 h after a single dose of cimetidine (table 2).

In conclusion, cimetidine does not alter hepatic heme catabolism. However, it increases heme oxygenase activity after chronic administration. The lack of an increase in heme breakdown in spite of the increased activity of this enzyme could be due to formation of a protective complex of cimetidine with cytochrome P-450, as postulated for other inhibitors of oxidative drug metabolism[23,24]. Alternatively, it could be due to the inhibitory activity of cimetidine on heme oxygenase activity demonstrated in vitro.

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Short Communications

Starvation-induced changes in the autoradiographic localisation of valine uptake by rat small intestine

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Summary. We report here the effects of a 72-h fast on the localisation of Na-dependent [^{3}H]-valine uptake by rat small intestine. Starvation results in the earlier appearance of valine transport during cell migration and an enhanced accumulation of the amino acid at the villus tip.
Key words. Intestine; adaptation; absorption.

During the course of a day, the intestinal epithelium is exposed to wide fluctuations in the nutrient composition of the intraluminal fluid. Enterocytes making up this cell layer must therefore be capable of a rapid adaptation of their transport properties if homeostasis is to be maintained. Starvation represents an attractive model for the study of the mechanisms involved in intestinal adaptation since the condition is easy to produce in experimental animals and the effects on nutrient uptake have a rapid onset[3]. While much is known concerning changes in villus morphology following the removal of food[4], no information is available concerning the effects of starvation on the development of transport activity during cell migration along the villus. In order to address this problem, we have performed autoradiography on intestine from either fed or fasted rats to determine the cellular location of amino acid uptake. Valine was chosen as a probe for the study since this is an essential amino acid in this animal.