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Isolation of Human Hepatic Microsomes and Their Inhibition by Cimetidine and Ranitidine

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Summary. Human hepatic microsomes were isolated from wedge biopsies of the liver from 13 patients undergoing abdominal surgery. Ultrasonic homogenisation was used to increase the yield of microsomal monooxygenase activity (7-ethoxycoumarin O-deethylase, NADPH-cytochrome c reductase), resulting in a 30% higher total enzyme activity per g liver than preparation by other techniques. In 4 individual microsomal preparations the influence of cimetidine and ranitidine on Michaelis-Menten kinetics of O-deethylation and of reductase activity were studied. Without the H_2-receptor blocking drugs, enzyme kinetics of O-deethylation with a K_m of 51.0 ± 16.4 μM ($n = 3$) were obtained using Lineweaver-Burke plots. Both, cimetidine and ranitidine inhibited the O-deethylation; cimetidine had a five-fold higher inhibitory affinity (K_i 1.01 and 3.94 mM) to the monooxygenase than ranitidine (K_i 4.96 and 17.70 mM) in the uninduced liver. However, in liver from a patient with induced enzyme activity ($K_m = 478.0$ μM), the K_i of ranitidine was similar to that of cimetidine (K_i ran 3.57 versus K_i cim 2.49 mM). The reductase activity was not inhibited by ranitidine and only marginally so by cimetidine.

The results suggest that in human hepatic microsomes oxidative drug metabolism is inhibited by both H_2-receptor antagonists. However, the inhibitory potency of the compounds seems to depend on the individual isozyme pattern of the hepatic microsomes. Thus, while cimetidine is an relatively nonspecific enzyme inhibitor, ranitidine might more selectively inhibit induced drug metabolizing enzymes.

Key words: cimetidine, ranitidine, liver microsomes; enzyme inhibition and induction, monooxygenase activity, microsomal drug metabolism, human liver

Cimetidine and ranitidine are potent inhibitors of gastric hydrochloric acid secretion and are widely used in the treatment of duodenal ulcer disease, reflux oesophagitis and erosive lesions of the stomach (Zeldis et al. 1983). Their mechanism of action is based on their ability to bind to the H_2-receptors (histamine) in the gastric mucosa, thereby antagonizing the acid-liberating activity of histamine.

While their therapeutic usefulness has been proven in many clinical trials, the rate and type of undesirable side-effects has not been established. However, cimetidine treatment has been associated with antiandrogenic effects, leukopenia, anicteric hepatitis, interstitial nephritis, mental depression and allergic reactions (Mc Carthy 1983). Cimetidine has also been shown to impair the metabolic hepatic clearance of drugs which are oxidatively metabolized by the cytochrome P-450-dependent microsomal enzyme system of the liver (Somogyi et al. 1982). The inhibition of oxidative drug metabolism is due to competitive binding of cimetidine to the active site of the haemoprotein-containing enzyme (Rendic et al. 1983). Ranitidine, although as effective in reduction of gastric acid secretion as cimetidine, does not seem to have the same inhibitory effect on hepatic drug metabolism. (Henry et al. 1980; Zeldis et al. 1983).

However, it has been found to cause inhibition of microsomal enzymes of rat liver (Rendic et al. 1982) and of human liver (Puurunen et al. 1980). The type and the extent of interaction of these H_2-receptor antagonists with human hepatic microsomes has not been clearly elucidated. In the present study microsomes were isolated from human liver and the effects of cimetidine and ranitidine on monooxygenase enzyme kinetics were examined.

Materials and Methods

Subjects

Thirteen patients were included in the investigation, divided into two groups. Group 1 was used to verify the microsomal isolation procedure; it comprised 7 female patients undergoing cholecystectomy because of cholelithiasis. All had normal or near normal liver histology (2 with mild fatty liver, 2 with minor pericholangitis) and unremarkable serum liver biochemistry (2 had a slight elevation of alkaline phosphatase, one had a serum bilirubin of 1.9 mg/dl). The age range was 27 to 82 years (49.0 ± 17.1y, $\bar{x} \pm SD$), and none was taking enzyme inducing drugs or abused drugs or ethanol.

Group 2 was used to study the kinetic properties of microsomal enzymes and the effects of cimetidine

Table 1. Clinical details of the patients[a]

Patient	Sex	Age [years]	Diagnosis and type of operation	Liver histology	Ethanol/smoking	Drugs
I	f	66	Malignant duodenal stenosis/ Duodenal resection	No metastasis	neg./neg.	Antihypertensives, Oxyfedrine, Allopurinol
II	f	50	Cholecystolithiasis/ Cholecystectomy	Normal liver	neg./neg.	Laxatives
III	f	64	Cholecystolithiasis, Diabetes mellitus/ Cholecystectomy	Severe fatty liver	unknown	Methyl-Digoxin Antihypertensives
IV	f	49	Cholecystolithiasis/ Cholecystectomy	Slight fatty liver	50 g per day/neg.	no drugs
V	m	74	Cholecystolithiasis/ Cholecystectomy	Slight fatty liver	80 g per day/neg.	Fosfestrole, Laxatives, Antihypertensives
VI	m	75	Cardia-Carcinoma/ Insertion of tube	Liver metastasis	neg./neg.	Digoxin, Amitryptyline, Chlordiazepoxide, Prenylamine
VII	f	22	Cholecystolithiasis/ Cholecystectomy	Normal liver	unknown	Analgesics, Spasmolytics

f = female, m = male, neg. = no ethanol abuse/no smoking
All patients had general anesthesia with thiopental except Patients III and VI.
The liver specimen from Patient VI used for the preparation of microsomes was free from malignancy.
[a] Liver samples from these patients were used to measure enzyme kinetics

and ranitidine on microsomal function. It consisted of 6 patients whose clinical data are contained in Table 1.

Liver biopsy material was obtained by surgical wedge biopsy, yielding 0.5–2.0 g wet weight of liver per patient. The liver was kept in ice-cold saline and was divided into equal pieces of about 30 mg wet weight blotted, weighed, wrapped in aluminium foil and frozen at −20 °C. This procedure was completed in less than 1 h.

Methods

Isolation of Microsomes. The tissue was kept frozen for 5–10 days. It was then thawed, cut with a scalpel into small fragments, immersed in 0.1 M Tris-HCl-buffer (pH = 7.6) to obtain 40 mg liver/1 ml buffer, and homogenized using a glass-glass Dounce homogenizer (5 ml volume) with a tight fitting pestle by applying 25–30 strokes. Since human hepatic tissue frequently contains fibrous strands, ultrasonic homogenisation was also performed. The homogenate was transfered into Eppendorf vials (1.5 ml volume) and sonicated using a microtip probe (Branson Sonifier B-12, Branson Sonic Power Comp., Dabury, Connecticut, USA) for 20 s at 30 W.

To obtain the 9000-*g* supernatant the sonicated homogenate was centrifuged at 9000*g* for 10 min in a refrigerated Sorvall Centrifuge (Modell RC-3). The supernatant was ultracentrifuged at 104000 *g* for 1 h in a Beckman-Ultracentrifuge, the supernatant discarded, the pellet rehomogenized in 1.15% ice-cold KCl-solution and sedimented at 104000 *g* for 30 min. The washed microsomal pellet was resuspended in 0.1 M Tris-HCl-buffer (pH = 7.6) using a Dounce homogenizer to give a final concentration of 1 or 2% (equivalent to 10 or 20 mg wet weight/ml buffer), for the binding spectrum of 25% (equivalent to 250 mg wet weight/ml buffer) microsomal suspension.

Enzyme Assays

7-Ethoxycoumarin O-Deethylase (EOD). The final incubation volume (0.1 ml) contained about 1 mg liver wet weight, 500 µM 7-ethoxycoumarin and 200 µM NADPH in 0.1 M Tris-HCl-buffer. Increase in fluorescence was measured at 380 nm excitation and 450 nm emission (Ulrich et al. 1972), with a millimolar extinction coefficient of umbelliferone of 117.5, using a spectrofluorometer (Aminco-Bowman SPF 5). The enzyme reaction was proportional with time up to 30 min, and with a protein concentration of the microsomal suspension from 0.1–0.6 mg/ml, equivalent to 10–50 mg wet weight/ml. With the 9000-*g* supernatant, the linearity was between 0.1–2.0 mg protein/ml.

NADPH-Cytochrome C Reductase. The final incubation volume (0.6 ml) contained about 1 mg liver wet weight, 50 µM oxidized cytochrome c, 980 µM KCN, and 160 µM NADPH in 0.05 M K_2HPO_4-KH_2PO_4 buffer, pH 7.4 (Williams et al. 1962). The enzyme

reaction was linear with time only up to 1 min and with a protein concentration of 9000-*g* supernatant from 0.15–3.50 mg/ml (equivalent to 2.5–50.0 mg wet weight/ml).

P-Nitroanisole O-Demethylase (PNA). The final incubation volume (0.25 ml) contained about 1 mg liver wet weight, 10 mM $MgCl_2$, 0.20 mM p-nitroanisole, and 0.40 mM NADPH in 0.1 M Tris-HCl-buffer, pH 7.6 (Netter et al. 1964). The enzyme reaction was linear with time up to 30 min and with a protein concentration of the microsomal suspension from 0.06 to 0.60 mg/ml (equivalent to 5.0 to 50.0 mg liver wet weight/ml). With 9000-*g* supernatant the linearity was between 0.35 to 2.0 mg protein/ml, equivalent to 5.0 to 30.0 mg wet weight/ml.

For the reductase and PNA measurements an Aminco-DW 2 spectrophotometer was used.

Protein determination was carried out according to Lowry et al. (1951), using 0.1 M Tris-HCl-buffer (ph 7.6) as blank.

Enzyme Kinetics and Inhibition Study

The substrate and cofactor concentrations were saturating for the enzyme reactions, as shown by the Michaelis-Menten kinetics. Michaelis-Menten kinetics were performed for EOD with 6 substrate concentrations ranging from 24 to 480 µM, and for the reductase with 8 substrate concentrations ranging from 4–50 µM, using the microsomal suspension as enzyme source (10 mg wet weight/ml). For each set of substrate concentrations, 3 different concentrations of inhibitors were added and the enzyme activities assayed. For determination of K_m and V_{max}, Lineweaver-Burke plots were performed and the kinetic parameters determined using the intercept of the regression line with the activity and concentration axes.

EOD. Diluted cimetidine hydrochloride stock solution (0.396 M in bidestilled water made acid with HCl to pH 4.5–6.0) was added in 2 µl to the incubation mixture to obtain the following final concentrations: 0.38, 1.52 and 3.81 mM. Ranitidine (0.57 M and 0.71 M stock solution) was diluted with acidified (HCl) bidestilled water (pH 4.5–6.0) and added in 2 µl to the incubation to obtain final concentrations of 1.1, 2.19 and 5.48 mM (Patients I and II) and 1.71, 3.43 and 6.85 mM (Patient III).

NADPH-Cytochrome c Reductase. The cimetidine solution (10 µl) was added to give final concentrations of 0.32, 1.28 and 3.20 mM. Ranitidine solution (10 µl added) gave final concentrations of 2.3, 5.75 and 11.49 mM in the incubation volume.

The freshly prepared microsomal enzymes exhibited a time dependent decrease in activity while kept on ice; 5 h after the completion of the preparation, a 25–30% loss acitivity was observed for the EOD. The reductase activity was also unstable with time. Therefore, to account for loss due to the delay in making measurements, an uninhibited enzyme assay was performed in close temporal proximity (less than 60 min) to each inhibitor concentration, to obtain the inhibitor-related, time-independent percentage depression of enzyme activity. The degree of drug-related enzyme inhibition was expressed as the percentage reduction from fresh microsomal enzyme activity. Activities were plotted as Lineweaver-Burke diagrams and the kinetic data were plotted using a curve-fitting program for linear regression (Hewlett-Packard calculator). The slopes of the Lineweaver-Burke plots were taken as the degree of inhibition and were plotted against the concentration of the inhibitor (Bisswanger 1979) to obtain the inhibitor constants (Hoensch et al. 1982).

Binding Studies

An Aminco-DW2 spectrophotometer in the split beam mode was used to record the binding spectra in a 25% microsomal suspension (equivalent to 250 mg wet weight/ml in 0.1 M K_2HPO_4-KH_2PO_4 buffer, pH 7.4) and a protein concentration of 3.1 mg/ml (cimetidine) and 2.1 mg/ml (ranitidine). The final inhibitor concentration in the sample cuvette was 12.77 mM for cimetidine and 22.98 mM for ranitidine; the reference cuvette contained an equal volume of the vehicle.

Results

Preparation of Microsomal Enzymes

The effect of various intensities of ultrasound on microsomal EOD activity is shown in Fig. 1. The specific enzyme activity remained unchanged at low sonication energy, suggesting the presence of intact microsomal vesicles. With increasing duration of low energy sonication, however, the total activity was enhanced, which indicated a higher yield of microsomal membranes from the cells and tissue. Therefore, the sonication procedure augments the efficiency of extraction of microsomes from human liver, which displays a higher tissue consistency than the soft liver of the rat. As for EOD, the microsomal activity per g liver was significantly increased (by about 30%) for the PNA and the reductase when ultrasound homogenisation was used. However, when sonification at 30 W was extended to longer than 20 s, a reduction in total and specific activity was observed, suggesting destruction of the microsomal membranes.

Freezing at −20 °C for 5 to 20 days caused a 40% loss of EOD and PNA activity per g liver, while the reductase activities remained unchanged. The fall in

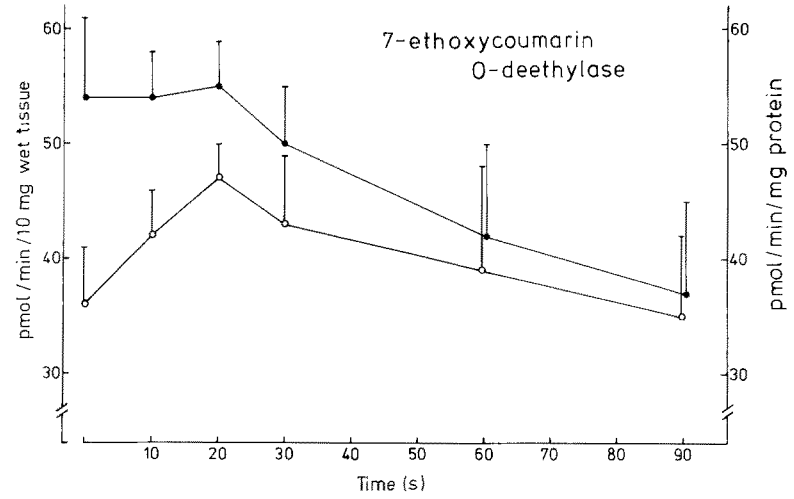

Fig. 1. Effect of sonication on activity of 7-ethoxycoumarin O-deethylase ($\bar{x} \pm SD$ of 4 experiments). Enzyme activity was measured in 9000 g supernatant (10 mg wet tissue/ml) and is expressed per 10 mg wet weight (○) and per mg protein (•). Thirty Watt was applied for various length of time using a microtip probe in a 1.0–1.5 ml volume

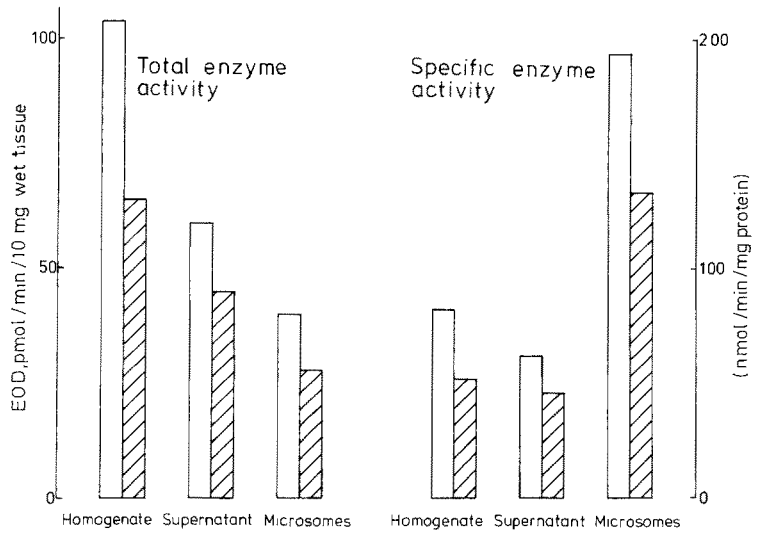

Fig. 2. Changes in total and specific activities during the preparation of microsomes from human liver. □ 7-ethoxycoumarin O-deethylase as pmol/min/10 mg wet tissue (*left panel*) and pmol/min/mg protein (*right panel*). ▨ NADPH-cytochrome c reductase: nmol/min/10 mg wet tissue (*left*), nmol/min/mg protein (*right*). The liver biopsy from one patient was used to prepare the homogenate, 9000-*g* supernatant (100 mg wet weight/ml) and microsomal suspension (20 mg wet tissue/ml). The mean of duplicate assays is shown

enzyme activity occurred during the first 5 days. Determination of EOD in liver tissue frozen for 5, 7, 10, 13, 17 and 20 days did not reveal any significant difference between the time intervals, using analysis of variance for the comparison (coefficients of variation of means were 11 and 14% for microsomes and supernatant, respectively). There was no significant difference in EOD in 9000-*g* supernatant when needle biopsy material was frozen at −20 °C (39.0 ± 22.1, $n = 10$) or −80 °C (39.4 ± 27.5, $n = 14$). Furthermore, others (Farrel et al. 1979) have shown that freezing of human liver material at −20 °C did not result in loss of benzo(a)pyrene hydroxylase activity following storage for up to 2 month.

During the isolation procedure, 40% of the total activity was lost while preparing the supernatant and 60% at the microsomal step (Fig. 2); for the reductase activity similar percentage losses were found. However, the specific activities of both enzymes rose by about three-fold. This indicates that the isolation procedure produced microsomes with high specific monooxygenase activities.

To obtain information on the reproducibility of the determination of enzyme activity and on the variability introduced by the isolation procedure, the liver specimen from one patient was divided into 7 pieces (each 20–50 mg wet weight) and microsomal isolation and enzyme assays were performed separately on each sample. The coefficient of variation of EOD activity was 9 and 10% for specific and total activity, respectively, of reductase activity it was 6 and 10%, and of protein content 12%.

Enzyme Kinetics and the Effects of the Inhibitors

The specific enzyme activities in 7 patients with normal liver function obtained at saturating concentrations of substrates and cofactors are listed in Table 2. The dependence of the EOD and reductase activity on substrate and cofactor concentrations were studied using Michaelis-Menten kinetics. The NADPH-concentration of 200 μM for the EOD assay and of 160 μM for the reductase assay proved to be saturating. The results of the kinetic studies with 5 patients

are summarised in Table 3. The affinity constant (K_m) of 7-ethoxycoumarin from 3 patients (II, III, IV) with normal liver was 51.04 ± 16.43 μM, and in Patient I the affinity of the microsomal enzyme for the substrate was much lower.

The K_i-values shown in Table 3 indicate that ranitidine was a weaker inhibitor of monooxygenase activity than cimetidine. The inhibitory affinity of cimetidine for the enzyme was about 5-times stronger than that of ranitidine, as reflected by the lower K_i of cimetidine. However, ranitidine had an inhibitory affinity only slightly less than of cimetidine towards EOD activity in the liver of Patient I (Fig. 3). There was an overlap between the inhibitor constants of cimetidine (2.48 ± 1.46 mM, $n = 3$) and ranitidine (8.74 ± 7.79 mM, $n = 3$). The Lineweaver-Burke inhibitor plots did not permit a conclusion about the specific type of inhibition, and the scatter around the regression lines was too great to exclude either competitive or noncompetitive inhibition (Fig. 3 A, B).

The inhibitory effect of both drugs on reductase activity was much less pronounced than on EOD activity, as reflected by the high K_i-values. Cimetidine appeared to produce a minor degree of inhibition while randitidine had no discernible effect. Cimetidine bound to hepatic microsomes resulted in a typical Type II binding spectrum (Fig. 4), which indicates that it was attached to the haem iron of the cytochrome P-450. There was also a spectral interaction of the microsomal membranes with ranitidine (Fig. 4), which resulted in an atypical spectral pattern.

Table 2. Specific monooxygenase activities of normal human liver

	9000-*g* Supernatant	Microsomes
7-Ethoxycoumarin O-deethylase (pmol/min/mg protein)	90.8 ± 57.1 (7)	286 ± 241 (5)
p-Nitroanisole O-demethylase (pmol/min/mg protein)	579 ± 121 (5)	1417 ± 598 (4)
NADPH-cytochrome c reductase (nmol/min/mg protein)	45.1 ± 10.6 (7)	162 ± 34.5 (5)
Protein content (mg/10 mg wet weight)	0.84 ± 0.21 (7)	0.17 ± 0.04 (5)

7 female patients (age: 49.0 ± 17.1 years) had a surgical wedge biopsy of the liver during cholecystectomy for cholelithiasis. Liver histology and serum biochemistry were unremarkable; no enzyme inducing drugs were being taken. Numbers of individual determinations are given in brackets

Discussion

Isolation and characterisation of human hepatic microsomes is a prerequisite for studying the inhibitory properties of H_2-receptor antagonists. The enzymatic activity of the microsomes prepared from human liver was comparable to that reported by others (Pelkonen et al. 1974; Ahmad et al. 1977; Kapitulnik et al. 1977; Schöne et al. 1972).

A major modification in the preparation of microsomes was the application of ultrasound. This was done to improve the yield of microsomal protein. It is at variance with procedures described by others (Boobis et al. 1980; Bahr et al. 1980). Low energy sonication was shown to increase the total EOD activity by about 30% (Fig. 1), indicating a higher degree of microsomal recovery than the other procedures. Indeed, a higher yield of microsomal protein per g liver (17 ± 4 mg) was obtained than reported previously (13.4 ± 0.7 mg/g, Ahmad et al. 1977; 15.2 mg/g, Pelkonen et al. 1974; Boobis et al. 1980; Bahr et al. 1980).

A detergent effect on the microsomal membrane was not observed at low energy levels. However, the enzyme activities fell with increased sonication time, suggesting destruction of the membranous ultrastructure of the enzymes. Ultrasound may be especially useful for fibrotic tissue, when conventional homogenisation fails to release a sufficient amount of subcellular particles. The ultrasound homogenisation technique has also been used for the determina-

Table 3. Kinetic parameters of microsomal enzyme activities in human liver of patients: inhibitory effects of cimetidine and ranitidine

	O-deethylation of 7-ethoxycoumarin (EOD)				Reductase
	Pat. I	Pat. II	Pat. III	Pat. IV	Pat. V
K_m [μM]	478	40.9	42.2	70.0[a]	12.2
V_{max} [nmol/min/mg protein]	1.52	0.39	0.54	0.13[a]	193
K_i, cim [mM]	2.49	3.94	1.01	n.d.	10.23
K_i, ran [mM]	3.57	17.7	4.96	n.d.	225

Michaelis-Menten kinetics were measured using microsomes from 5 patients: K_m = substrate concentration at half maximal velocity, V_{max} = maximal velocity, K_i = inhibitor concentration at half maximal inhibition of enzyme activity for cimetidine (cim) and for ranitidine (ran). EOD: 7-ethoxycoumarin O-deethylase, reductase: NADPH-cytochrome c reductase. [a] activity determined in 9000-*g* supernatant; n.d. = not determined

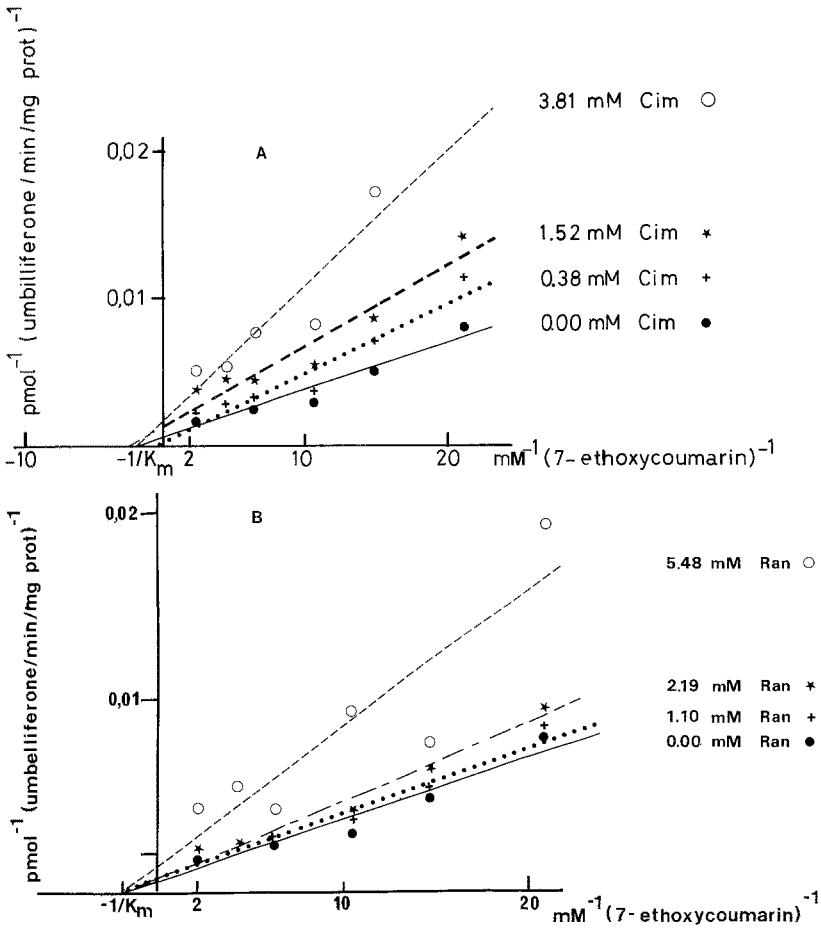

Fig. 3. Inhibition of microsomal O-dealkylation of 7-ethoxycoumarin (Patient I) by various concentrations of cimetidine (A) and ranitidine (B). Regression r was 0.90 to 0.98. The Lineweaver-Burke kinetics are shown individually for the uninhibited enzyme and for each of the inhibitor concentrations

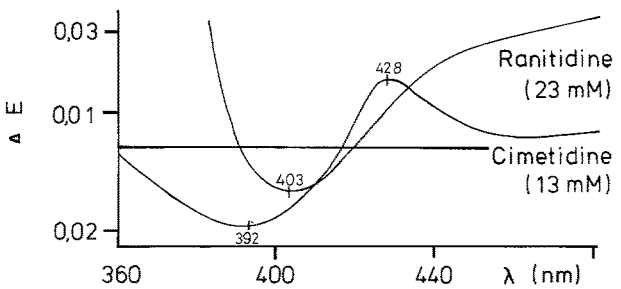

Fig. 4. Substrate binding difference spectra of cimetidine and ranitidine in human liver microsomes.
Protein concentration I: 3.125 mg/ml (Patient VII) Cimetidine
II: 2.075 mg/ml (Patient VI) Ranitidine

tion of monooxygenation activity in human hepatic needle biopsies (Hoensch et al. 1979). Ultrasound appears to be a useful tool to improve the recovery of microsomal monooxygenase enzymes from human liver.

The hepatic microsomes from human liver displayed Michealis-Menten kinetics for the monooxygenase (EOD) activities. A K_m of $51.0 \pm 16.4\,\mu M$ was found in 3 patients (Table 3). Biphasic EOD kinetics with two K_m's were reported by Boobis et al. (1981; 1.8 and 205 μM), and by Woodhouse et al. (1984; 9.4 and 111 μM) for EOD activity in human hepatic microsomes. The EOD reaction is catalysed by different forms of cytochrome P-450 (Imai 1979). The failure here to find biphasic EOD kinetics may have been due to the fact that, owing to shortage of liver tissue, only 6 substrate concentrations between 24 and 480 μM were used, whereas in the reports mentioned above the wider range of 1 and 1000 μM was employed (Boobis et al. 1981; Woodhouse et al. 1984).

Genetic individuality in the monooxygenase system has been shown for a variety of compounds (Nebert et al. 1982). Looking at the properties of the individual microsomes (Table 3), it was clear that data from Patient I differed from the others. Her EOD activity was much higher, possibly due to enzyme induction. The monooxygenase enzyme system of her liver presumably had low affinity and high capacity sites which were not present in the other livers. The reason for the deviation is unclear: enzyme induction or a greater contribution of a certain isozyme might have been involved. Allopurinol with which she was treated usually inhibits drug metabolism (Vesell et al. 1973) but could have been a potential inducer in this patient. It is known that some inhibitors given chronically can exert an inducing action (Park et al. 1981).

The potency of a drug or other chemical compound to inhibit an enzyme reaction is best characterized by its K_i value. This parameter reflects the inhibitory affinity of a xenobiotic for the enzyme: the lower the K_i the higher is the inhibitory activity.

The K_m value characterizes the affinity of the substrate for the enzyme protein: a low K_m means high affinity. Enzymatic studies in vitro can clearly delineate drug effects on monooxygenase activities, since other variables, such as blood perfusion, plasma protein binding and hormonal influences, are eliminated. These studies, however, must be complemented by in vivo investigations. The half maximal enzyme inhibitory concentrations (K_i) of cimetidine were between 1-18 mM while the therapeutic plasma concentration ranges between 1 and 6 μM. However, drug concentrations at the enzymatic sites might be much higher than the plasma concentration due to the greater binding capacity of the smooth endoplasmic proteins.

Cimetidine is a potent inhibitor of hepatic oxidative drug metabolism in vivo (Somogyi et al. 1982) and in vitro (Knodell et al. 1982; Speeg et al. 1982; Pelkonen et al. 1980). The results show that human hepatic microsomal enzymes (EOD) were also inhibited by cimetidine, although the affinity of the inhibitor for the enzyme was less (K_m 2.48 ± 1.46 mM) than that reported for rat liver microsomes (0.22 mM according to Rendic et al., 1979). The inhibitory effect of cimetidine was close to that reported for aminopyrine N-demethylase inhibition in rats which ranged from 1-10 mM (Pelkonen et al. 1980). With human hepatic microsomes cimetidine exhibited a Type II binding spectrum (Fig. 4), which is consistent with the type of binding reported for rat liver (Knodell et al. 1982). Cimetidine contains an imidazole ring structure, which is probably responsible for its attachment to the haem of cytochrome P-450 (Hajek et al. 1982). NADPH cytochrome c reductase, which supplies the electrons for the cytochrome P-450 cycle, was not inhibited by imidazole. In agreement with this finding the human hepatic reductase activity was only marginally affected by cimetidine (Table 3).

Ranitidine is a new H_2-receptor antagonist of similar potency to cimetidine in terms of inhibition gastric acid secretion, but with apparently less interaction with oxidative drug metabolism (Zeldis et al. 1983). It consists of a furan ring, is less lipophilic than cimetidine and has a lower spectral binding affinity for rat liver microsomes (Bell et al. 1981).

With human hepatic microsomes, inhibitor constants of ranitidine between 3.6 and 17.7 mM for EOD activity (Table 3, Fig. 3 B), which are in the range of those reported for inhibition of aminopyrene demethylation (Speeg et al. 1982) and p-nitroanisole demethylation (Rendic et al. 1982) by ranitidine in rat liver microsomes. A binding spectrum with ranitidine was obtained, but it could not be assigned to any of the known types (Fig. 4). Thus, ranitidine is capable of binding to and inhibiting human hepatic microsomal drug metabolism using 7-ethoxycoumarin as the substrate. Its effect in man however, is smaller than in rats and ranitidine might possess a higher affinity for some types of isozymes while leaving others unaffected. At an in-vitro concentration of 2.9 mM, ranitidine did not inhibit oxidative metabolism of meperidine and pentobarbital by human hepatic microsomes, while cimetidine at that concentration was a strong inhibitor (Knodell et al. 1982). No effect at all was seen when ranitidine was added to microsomes catalyzing the reduction of cytochrome c (NADPH cytochrome c reductase activity), as shown in Table 3.

In two patients ranitidine had a 5th of the inhibitory activity on EOD of cimetidine, and in one patient, presumably with induced EOD activity, the K_i-value was comparable to that of cimetidine. Enzyme induction can enhance the affinity of inhibitors to monooxygenase enzymes by producing changes in the binding sites, in the spin state of cytochrome P-450 or in the isozyme pattern (Hajek et al. 1982). Thus, ranitidine could probably cause the same strong inhibitory action as cimetidine under this condition.

Ranitidine in therapeutic doses does not impair the metabolic clearance of oxidatively metabolized drugs, as exemplyfied by antipyrine, theophylline (Breen et al. 1983), diazepam (Klotz et al. 1983) and propranolol (Heagerty et al. 1982). It does however, delay the elimination of metoprolol in man (Spahn et al. 1982).

Ranitidine is oxidatively metabolized in the liver and exhibits first-pass kinetics (Zeldis et al. 1983; Garg et al. 1983). Its metabolism most probably occurs by a microsomal cytochrome P-450-associated enzyme system. This evidence and the in vitro inhibition of human microsomal metabolism suggests that in addition to metoprolol other drugs whose elimination depends on the activity of the monooxygenase system might reach higher blood and tissue concentrations in patients receiving ranitidine. In such subjects side effects of those drugs might occur. The present results also suggest that binding and inhibition take place with both compounds, but that the extent of the interaction depends on the individual enzymatic make up of the microsomes.

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