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EFFECT OF FIVE STRUCTURALLY DIVERSE H_2-RECEPTOR ANTAGONISTS ON DRUG METABOLISM

MARKKU PASANEN, PENTTI ARVELA, OLAVI PELKONEN, EERO SOTANIEMI and ULRICH KLOTZ*

Departments of Pharmacology and Internal Medicine, University of Oulu, SF-90220 Oulu, Finland, and *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, D-7000 Stuttgart, Federal Republic of Germany

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Abstract—Some H_2-receptor antagonists can interact with the biotransformation of other drugs. This is due to their binding to cytochrome P-450. We tested the *in vitro* effects of 5 different H_2-receptor antagonists cimetidine (C), oxmetidine (O), ranitidine (R), famotidine (F) and nizatidine (N) on arylhydrocarbon-hydroxylase, 7-ethoxycoumarin-*O*-deethylase and 7-ethoxy-resorufin-*O*-deethylase activity using liver microsomes from man as well as from untreated, phenobarbital and 3-methylcholantrene treated rats. In addition their binding to human microsomal cytochrome P-450 was evaluated. The *in vivo* effects of these antagonists were investigated on the hepatic elimination of diazepam in healthy volunteers. *In vitro* O was found to be the most effective inhibitor of the enzyme activities studied. C showed a clear inhibitory effect only with rat liver microsomes whereas the remaining drugs were more than 10 times less potent. The binding affinities of these antagonists showed a similar tendency: the K_s-values for O, C and R were 0.2, 0.9 and 5.1 mM, respectively; for F and N no binding up to 4 mM could be observed. However, in man, only C inhibited the hepatic elimination of diazepam by about 45% while R, O, N and F did not affect the pharmacokinetics of diazepam. Thus, it could be concluded from our studies that one cannot extrapolate *in vitro* data of the inhibitory potency of H_2-receptor antagonists in every case to human *in vivo* drug metabolism.

H_2-receptor antagonists are world-wide among the most widely used drugs. Cimetidine and ranitidine have gained rapidly an established place in the treatment of gastrointestinal ulcer disease [1, 2] and in higher dosage in the Zollinger Ellison Syndrome [3]. Since in the Western world peptic ulcer afflicts approximately 12% of the adult population and H_2-receptor blocking agents are drugs of first choice, it is not too surprising that this succesful long-term treatment has already initiated clinical trials with additional compounds, such as oxmetidine, famotidine or nizatidine. It should be realized that at the same time many patients receive a variety of other drugs. Thus, it is important to investigate the interaction potential of these H_2-receptor antagonists, especially since it is well-known that cimetidine can inhibit the hepatic elimination of many drugs by binding to the cytochrome P-450 system [4].

The suppression of acid secretion by H_2-receptor antagonists is mediated by their corresponding receptors in the parietal cells of the stomach and differences in the ring structure and side chains (see Fig. 1) determine the potency of these drugs and consequently their therapeutic dosage.

In respect to the effect of H_2-receptor antagonists on hepatic elimination it would be interesting to know whether such structural differences affect also their inhibitory potential in terms of drug metabolism. We therefore studied the effect of five different H_2-blockers on drug metabolism by an interdisciplinary approach involving human and animal studies in combination with *in vivo* and *in vitro* techniques. Hepatic elimination of diazepam, a drug often administered concomitantly with H_2-blocker, was used as a probe characterizing *in vivo* drug metabolism in man. *In vitro* metabolism was determined with human and rat liver microsomes by measuring under normal and induced conditions (e.g. with phenobarbital or 3-methylcholanthrene) the activities of three marker enzymes for different cytochrome P-450-dependent profiles, e.g. aryl-hydrocarbon-hydroxylase (AHH), 7-ethoxycoumarin-*O*-deethylase (ECDE), 7-ethoxyresorufin-*O*-deethylase (ERDE). In addition, binding characteristics of the five H_2-blockers to human microsomal cytochrome P-450 were evaluated. From these comprehensive investigations it should be possible to gain some information on the structure/activity relationships of this important class of drugs and whether animal or *in vitro* data can be extrapolated to the clinical situation in man.

MATERIALS AND METHODS

Clinical studies. After giving their written informed consent, groups of 4–9 healthy drug-free volunteers received in random cross-over order on two occasions (control and pretreatment trial), separated by an interval of at least 2 weeks, either 0.1 mg/kg diazepam i.v. or, after an overnight fast, 10 mg orally (only in the nizatidine study). Prior to the test dose subjects received cimetidine

* To whom correspondence should be addressed.

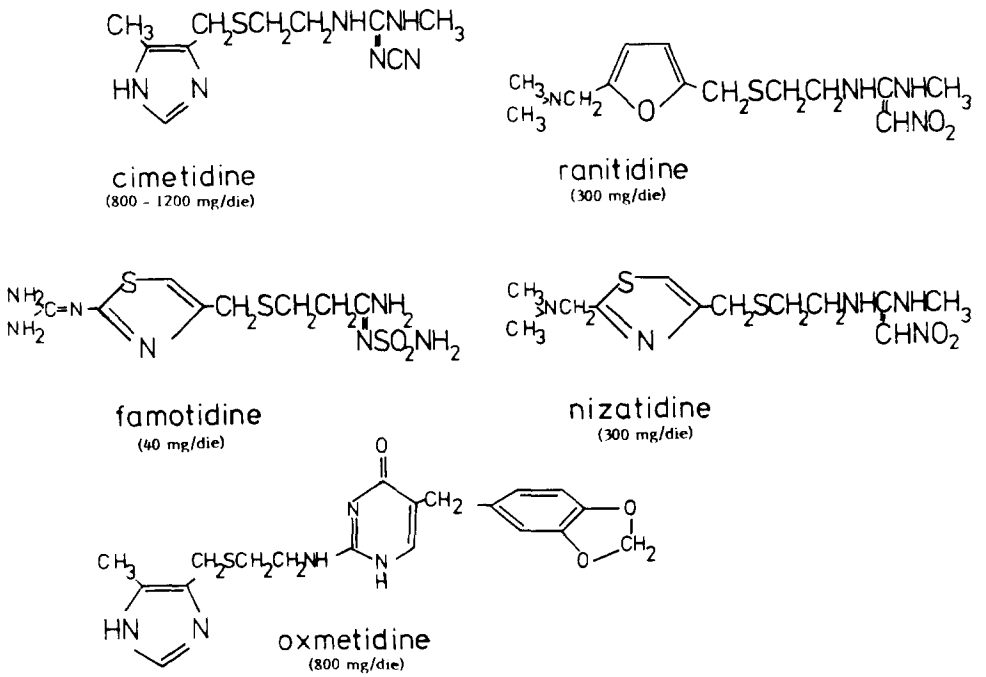

Fig. 1. Chemical structures of the five H_2-receptor antagonists tested and their corresponding clinical doses.

(5 × 200 mg/die) and for 5 days ranitidine (2 × 150 mg/die), oxmetidine (2 × 400 mg/die) and nizatidine (300 mg nocte/die) or famotidine (40 mg nocte/die). With the exception of the cimetidine experiment this therapeutic dosage regimen were continued for 72 hr during the pharmacokinetic evaluation of diazepam. Following the test dose of diazepam multiple blood samples were drawn up to 84 or 96 hr. These samples were centrifuged and the plasma stored frozen until analysis of diazepam and its major metabolite desmethyldiazepam by a specific and sensitive GLC-ECD assay [5]. The kinetic data for diazepam were individually analyzed according to a two compartment open model by the iterative curve fitting computer program SAAM 25 [6]. The pharmacokinetic results were compared by the paired Student's *t*-test and the mean ± SD are reported.

Binding studies. Fresh human liver was immediately chilled, cut into pieces and kept at −75° until processed. Liver samples were homogenized in 4 vol. (w/v) of 0.1 M phosphate buffer (pH 7.4). Microsomes obtained by differential centrifugation were resuspended in the buffer to a concentration corresponding to 100 mg liver/ml. Difference spectra were recorded by a Shimadzu MPS 3000 spectrophotometer using 2.5 ml of microsomal suspension as described recently [7]. The K_s-values were determined graphically.

Liver preparations. Male rats of the Wistar strain weighing about 200–250 g were treated with phenobarbital (PB; 0.5 g/l in drinking water for 1 week) or 3-methylcholanthrene (MC; 25 mg/kg dissolved in corn oil, three daily i.p. doses) and decapitated 24 hr after the last treatment. Control animals were untreated. Liver microsomes were prepared by the standard ultracentrifugation schedule. The microsomal pellets were dissolved in 0.1 M potassium phosphate buffer pH 7.4 so that 1 ml contained microsomes from 1 g of liver.

Human liver samples for studies *in vitro* were obtained in connection with diagnostic procedures from alcoholics, who had some suspicions of liver damage. The main part of the biopsy was used for histological examination and the left-over part (5–24 mg) forwarded to the Department of Pharmacology, where it was stored at −70° until assayed. On the day of the assays, the sample was allowed to thaw in ice and it was homogenized in 100 vol. of 0.1 M potassium phosphate buffer, pH 7.4.

Determination of xenobiotic metabolizing enzymes. Liver AHH activity was determined by a fluorometric method [8] and ECDE was assayed by the method of Greenlee and Poland [9]. With rat liver microsomes, about 200 to 500 μg of microsomal protein were used. With human liver biopsy homogenates, about 1 mg tissue wet weight was used. The final concentrations of the substrates were 75 μM benzo(*a*)pyrene and 500 μM 7-ethoxycoumarin. ERDE activity was measured according to the modified end-point fluorometric method of Burke and Mayer [10] as described previously [11].

Inhibition of enzymes by H_2-antagonists. H_2-antagonists concentration–inhibition curves with rat liver microsomes as an enzyme source were determined over the concentration range from 40 μM to 10 mM. Because we had only limited human biopsy homogenates available, the construction of complete inhibition curves was not possible. Consequently, inhibition studies *in vitro* were done with all H_2-antagonists at the concentration of 0.63 mM, which was selected on the basis of rat liver experiments

giving the best resolution between different inhibitors. Protein determinations were carried out with bovine serum albumin as a standard according to the method of Lowry *et al.* [12].

RESULTS

Effect of H_2-receptor antagonists on the pharmacokinetics of diazepam

Following the single test dose of diazepam the plasma levels declined bi-exponentially. The clinically relevant pharmacokinetic parameters have been summarized in Table 1. From the five H_2-receptor antagonists tested only cimetidine significantly ($P < 0.03$) impaired the hepatic elimination of diazepam as could be seen from the increase in its elimination half-life ($t_{1/2}$) or the decrease in its total plasma clearance (CL).

Binding of H_2-receptor antagonist to human liver microsomes

The calculated spectral dissociation constants (K_s) of the five tested agents are summarized in Table 2. Whereas cimetidine (K_s = 0.9 mM) and especially oxmetidine (0.2 mM) exhibited a strong ligand interaction with cytochrome P-450, ranitidine gave a much weaker spectrum at higher concentrations (K_s = 5.1 mM) and nizatidine and famotidine did not show any effects up to 4 mM. It must be stressed that due to the weakness of ranitidine-induced spectral changes, the K_s value is only an approximation.

Influence of H_2-receptor antagonists on in vitro *drug metabolism*

With rat liver microsomal enzymes, experiments with different H_2-antagonists gave a rather clear picture (Fig. 2). First of all, different compounds fell into two categories, oxmetidine and cimetidine resulting in a more pronounced inhibition than ranitidine, famotidine or nizatidine. Furthermore, oxmetidine was by far more potent than cimetidine, especially in microsomes from phenobarbital- or 3-methylcholanthrene-pretreated animals. These two pretreatments decreased clearly the inhibitory effect of cimetidine and 3-methylcholanthrene pretreatment almost abolished it. This finding might be interpreted as showing that the respective isozymes are not sensitive to cimetidine.

Table 2. Spectral dissociation constants (K_s) of H_2-receptor antagonists with human liver microsomes

Drug	Type	Max λ (nm)	Min λ (nm)	K_s
Cimetidine	II	429	395	0.87 mM
Ranitidine	II	427	395	5.1 mM
Famotidine	—	—	—	no effect up to 4 mM
Nizatidine	—	—	—	no effect up to 4 mM
Oxmetidine	II	429	395	0.2 mM

With human liver homogenates, oxmetidine was the most potent inhibitory for AHH, ECDE and ERDE activities, leaving 10%, 46% and 38% of the control activities, respectively, at concentration of 0.63 mM tested (Table 3). Nizatidine and famotidine had almost equal inhibitory capacity resulting in about 35%, 70% and 80% of the original AHH, ECDE and ERDE activities, respectively. In the light of earlier studies a surprising finding was the inability of cimetidine to inhibit significantly any of the enzyme activities. Ranitidine was also almost devoid of inhibitory effect at the concentration used.

DISCUSSION

The present clinical data and experimental *in vivo* and *in vitro* investigations have indicated that cimetidine exerts its significant inhibitor potency in three applied systems testing drug metabolism: it impairs hepatic elimination of diazepam in man (see also ref. 13), binds strongly to human cytochrome P-450 and inhibits all three rat microsomal marker enzymes tested. The probe reactions used (e.g. AHH, ECDE, ERDE) probably reflect the activity of a narrow range of cytochrome P-450 isozymes. However, the inducibility of ERDE (MC-specific), AHH and ECDE (PB, MC) is somewhat different. Surprising results were obtained with human liver microsomes. In earlier studies cimetidine has been shown to

Table 1. Pharmacokinetic parameters (mean ± SD) of diazepam in healthy volunteers

	$t_{1/2}$ (hr)†	CL (ml/min)‡	Reference
Control (N = 6)	33.5 ± 10.2	19.9 ± 8.2	17
+ cimetidine	51.3 ± 13.3*	11.4 ± 4.2*	
Control (N = 4)	22.5 ± 3.5	12.0 ± 0.9	18
+ ranitidine	23.3 ± 2.6	12.5 ± 3.1	
Control (N = 8)	45.6 ± 17.5	19.2 ± 5.0	19
+ famotidine	39.0 ± 11.4	21.9 ± 4.3	
Control (N = 8)	45.2 ± 11.8	13.6 ± 1.8	21
+ oxmetidine	50.5 ± 18.0	12.8 ± 3.5	
Control (N = 9)	35.3 ± 24.2	28.2 ± 12.0	
+ nizatidine	37.3 ± 18.3	26.7 ± 10.4	

* $P < 0.03$.

† Calculated from the terminal slope.

‡ Plasma clearance calculated from (iv)dose/AUC and based on complete bioavailability of diazepam (see ref. 5) in the case of the nizatidine study.

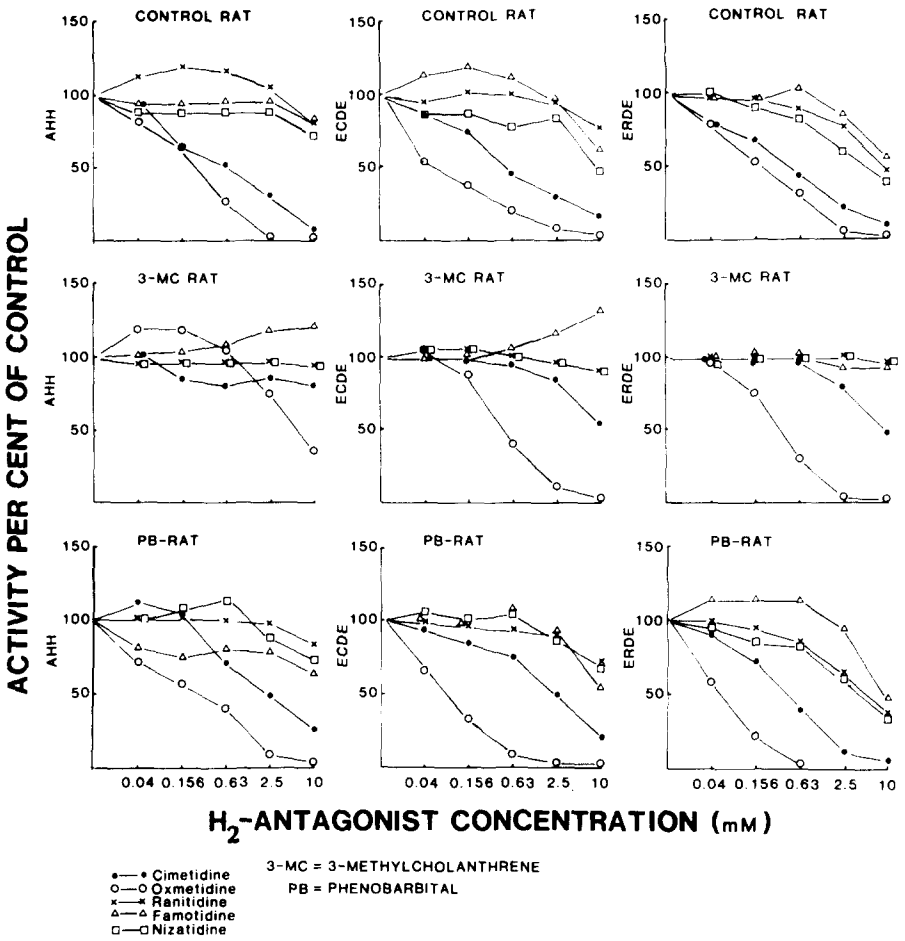

Fig. 2. Percentage of inhibition of AHH, ECDE and ERDE activities in rat liver microsomes from control, 3-methylcholanthrene-(3-MC) and phenobarbital-(PB) pretreated animals by five different H_2-receptor antagonists.

inhibit different cytochrome P-450-mediated activities in microsomes of both species [14]. In this study we could not detect significant inhibition with human microsomes. This inability may be due to two reasons. (1) The concentration selected was clearly smaller than concentrations at which cimetidine has earlier been shown to inhibit drug metabolism. We must point out that the concentration was selected on the basis of rat liver experiments in which it gave the best discrimination between different H_2-

Table 3. Inhibition of AHH, ECDE and ERDE activities in human liver homogenates by H_2-receptor antagonists (0.63 mM)

Control activity	Activity % of control (x ± SD) C	O	N	F	R
AHH (nmol/g/min)					
4.5 ± 2.4	97.2 ± 6.8	10.0 ± 3.4	35.8 ± 6.6	34.6 ± 7.8	86.2 ± 8.9
N = 9	N = 5	N = 5	N = 5	N = 5	N = 5
ECDE (pmol/g/min)					
336 ± 152	100.4 ± 47	46.3 ± 5.4	76.0 ± 8.3	70.7 ± 5.9	95.0 ± 7.5
N = 8	N = 5	N = 4	N = 4	N = 4	N = 4
ERDE (pmol/g/min)					
236 ± 198	89.7 ± 14.0	38.5 ± 18.4	77.8 ± 18.2	83.3 ± 10.6	76.7 ± 28.6
N = 5	N = 4	N = 4	N = 4	N = 4	N = 4

antagonists. (2) Human liver samples were from alcoholics and diabetics and these conditions might have been affecting the P-450 isoenzyme pool in the liver. As rat liver studies clearly demonstrated the pretreatments with PB or MC decreased or totally abolished the inhibitory potency of cimetidine. Thus, one can easily hypothesise that alcohol or diabetes would change the composition of P-450 isozyme pool so that only cimetidine-insensitive isozymes are present. Recently it has been shown with human hepatic microsomes isolated from wedge biopsies that the inhibitory potencies of H_2-receptor antagonists seems to depend on the individual isozyme pattern [15].

On the other hand ranitidine, famotidine and nizatidine appear to be very weak inhibitors of *in vitro* drug metabolism, which is in accordance to their low binding affinities to cytochrome P-450 and to their non-existing potency in man to impair the hepatic elimination of the probe drug diazepam. Confirmatory *in vivo* results were recently reported for famotidine [13] and ranitidine [16] which would suggest that our relatively low baseline level of CL (see Table 1) is not the cause for the observed non-interaction. Total plasma CL was used as an indicator for hepatic metabolism. Since diazepam is a so-called low clearance drug and it was previously shown that H_2-blockers do not affect the plasma protein binding of diazepam [17–19] this is a valid assumption.

Somewhat more complicated is the situation in the case of oxmetidine where such nice parallelism could not be observed. Both *in vitro* experiments (binding and metabolism studies) indicate impairments in microsomal metabolism, even more pronounced than those seen with cimetidine. In similar *in vitro* studies Bast *et al.* showed also ligand (type II) interactions of cimetidine and oxmetidine with rat-hepatic cytochrome P-450. Both H_2-blockers produced a concentration dependent inhibitory effect of the metabolic intermediate-cytochrome P-450 complex formation [20]. In contrast, hepatic elimination of diazepam (see Table 1) was not significantly changed. It could be argued that control values indicate already a relatively slow elimination of diazepam and subsequently an impairment is harder to detect. However, since also the pharmacokinetics of theophylline, antipyrine or propranolol were not altered by oxmetidine [21] it is more likely that concentrations of oxmetidine in human plasma or liver for yet unknown reasons (e.g. incomplete absorption, low bioavailability) did not reach the necessary levels to exert their inhibitory potential which could be clearly documented *in vitro*. Similarly, in the isolated perfused rat liver model oxmetidine appeared not to affect antipyrine pharmacokinetics [22]. Thus, one could conclude that an extrapolation or prediction from experimental *in vitro* data to the clinical situation cannot be performed in every case.

However, our and older [23] studies suggest that agents with an imidazole ring, such as cimetidine and oxmetidine, exert a significant affinity to the cytochrome P-450 and consequently this structural feature might be of importance for a hepatic interaction potential.

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