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Drug Interactions of H2-Receptor Antagonists Involving Cytochrome P450 (CYPs) Enzymes: from the Laboratory to the Clinic

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This paper reviews the main steps in the research of the interactions of H2-receptor antagonist drugs with cytochrome P450 (CYP) enzymes. Cimetidine, ranitidine, and related compounds are used as examples. The results from in vitro studies are related to the observed clinically significant in vivo drug-drug and drug-chemical interactions. Uses of the in vitro results are discussed for the interpretation and possible prediction of drug-drug interactions, which may be important in developing new drugs. Other approach in the use of the in vitro data is to prevent undesirable and toxic actions of drugs related to the catalytic activity of CYP enzymes. In the case of H2-receptor antagonists, the inhibition of the metabolic reactions due to the binding of the drugs with the enzymes was used to avoid side effects of co-administered drugs. The in vitro metabolic studies using recombinant human as well as animal CYP enzymes are now widely used as model systems for designing new drugs with improved therapeutic properties.

Key words: cimetidine; CYP; cytochrome P450; drug antagonism; drug combinations; histamine H2 receptor antagonists; liver; microsomes; polypharmacy; ranitidine

Cimetidine (Fig. 1) was the first histamine H2-receptor antagonist approved by the Food and Drug Administration, USA, for the treatment of duodenal ulcers, Zollinger-Ellison syndrome, and other gastric hypersecretory states. Later, the drug was also approved for the management of peptic ulcers (1,2). There are also reports that cimetidine, due to its effects on the immune system and as an H2-receptor antagonist, can inhibit growth of carcinogen-induced colonic tumors in rats, as well as the in vitro human colon cancer cell lines (3).

Figure 1. Structures of H2-receptor antagonists.

Since inhibition of cytochrome P450 (CYP) enzymes by cimetidine was reported in the literature (4-7), the occurrence of clinically significant drug-drug interactions were interpreted in the first place as a consequence of the inhibition of in vivo metabolism of drugs co-administered with cimeti- dine (8-11). Other mechanisms, such as the effects on gastric acid production and gastric emptying and inhibition of the renal clearance, were also suggested (12).
In the present paper, the metabolism of H2-receptor antagonists (section Metabolism of H2-receptor antagonists), the in vitro and in vivo studies using CYP enzymes (section Laboratory studies), and the results from clinical studies (section Clinical studies) are reviewed and discussed in the light of both the structural characteristics of the compounds and clinically observed drug-drug interactions. The catalytic function of CYP enzymes (specificity and properties of the enzymes in respect to drugs and chemicals that act as substrates, inducers, and/or inhibitors) are reviewed in a separate paper (21, and references therein).

Metabolism of H2-Receptor Antagonists

Cimetidine and ranitidine (13-20) are examples for the metabolism of H2-receptor antagonists in vivo (Figs. 2 and 3). Cimetidine is metabolized in humans by hydroxylation at the imidazole methyl group and by S-oxidation, and CYP enzymes (Fig. 2) catalyze both reactions. The major part of cimetidine is excreted in urine as unchanged drug (up to 66%); a conjugate with glucuronic acid (cimetidine-N'-glucuronide, about 18%); and as the S-oxide (cimetidine sulphoxide, about 11%). Ranitidine is biotransformed by the following reactions: N-demethylation (desmethyl- ranitidine), N-oxidation (ranitidine N-oxide), and by S-oxidation (ranitidine S-oxide), (Fig. 3). Ranitidine is excreted in man as an unchanged drug (up to 77%) and as the N-demethylated metabolite (about 4%).

Figure 2. Metabolism of cimetidine in humans.

Figure 3. Metabolism of ranitidine in humans.

Laboratory Studies
Binding of H2-Receptor Antagonists – Spectral Studies
The first studies on binding of cimetidine and related drugs with cytochromes P450 were performed using microsomal preparations and partially purified enzymes prepared from different model species: rat, pig, and human (4-7,22-24). The results from these studies may be summarized as follows:
1. Ligand interaction of cimetidine with rat liver microsomal CYPs indicated peaks in difference spectra at 429, 547, and 585 nm, and troughs at 392 and 567 nm (4).
2. Pre-treatment of rats with different inducers of CYPs (phenobarbital, 3-methylcholanthrene or polychlorinated biphenyl, ClophenR-60) produced qualitatively similar optical difference spectra compared with control animals, but of different amplitudes (4).
3. A peak in difference spectra at about 431 nm, and a trough at about 392 indicated ligand type interaction of cimetidine with human CYPs (Fig. 4 and ref. 7).

Figure 4. Optical difference spectra of human liver microsomes in the presence of cimetidine and ranitidine.
A) Difference spectra in the presence of cimetidine. Final concentrations of cimetidine for each curve were 0.08, 0.24, 0.4, 0.72, 1.36 mmol/L. B) Difference spectra in the presence of ranitidine. Final concentrations of ranitidine for each curve were 1.2, 3.2, 8.4 mmol/L. Difference spectra were recorded using tandem cells, each compartment containing 1.25 mL of either 0.1 mol/L Tris/HCl or microsomal suspension in the same buffer. Either cimetidine dissolved in methanol, or ranitidine dissolved in buffer, were added to the compartment of the sample cell containing microsomal suspension, and to the compartment with buffer of the reference cell. The coresponding volume of the solvent was added to other compartments. The microsomal suspension contained 1.25 mg of protein/mL.

4. Similar optical difference spectra were obtained when microsomal preparations from other animal species (rabbit or pig) and partially purified cytochromes P450 from pig liver were used (4-7).
5. Titration of the microsomal suspensions with cimetidine showed a biphasic interaction with CYPs.
6. Depending on the enzyme preparation used, the values of apparent spectral dissociation constants (Ks-values) for cimetidine interactions ranged from about 0.01 to 0.1 mmol/L, and from about 0.1 to 0.75 mmol/L, for Ks1 and Ks2, respectively. The value for Ks1 using ranitidine was 0.34 mmol/L (detected when microsomes prepared from liver of phenobarbital pre-treated pigs were used) and the Ks2-value ranged from 1.4 mmol/L to 6.6 mmol/L (Table 1).

Table 1. Interaction of cimetidine and ranitidine with cytochromes P450 in liver microsomes and purified liver cytochromes P450 (refs. 4-7)

7. Interaction of cimetidine and ranitidine with cytochrome P-450s using microsomes prepared from human liver (Figs. 4A and 4B, and Table 1) showed that the affinity of binding of cimetidine was about 10 times higher than that estimated for ranitidine.
8. Electron spin resonance (e.s.r) studies indicate that cimetidine interacts with CYPs by a ligand type (or type II) binding by sharing of the lone electron pair of the imidazole or the cyano nitrogen atom with heme iron (Fig. 5).

Figure 5. Schematic presentations of cimetidine-nitrogen ligand binding with the heme iron in cytochrome P450s. A) Binding with the imidazole nitrogen. B) Binding with the cyano nitrogen.

The differences between the Ks values obtained when microsomes from differently induced animals were used indicated the interaction of the compounds with different isoforms of cytochromes P450. For example, in comparison to phenobarbital pre-treatment, pre-treatment of animals with 3-methylcholanthrene resulted in only a single binding constant for cimetidine, suggesting the predominant interaction with a single form of the enzyme (Table 1). Currently, these isoforms are recognised as enzymes belonging to different CYP families of enzymes (21,25). In addition to nitrogen compounds (imidazoles), also phosphorus- (phosphines), sulfur- (thiols), and oxygen-(furanes) containing compounds bind to cytochromes P450 as ligands by providing a lone electron pair from a hetero atom to a heme iron (26,27).
A reversible ligand binding of cimetidine with the heme-iron in cytochromes P450 was confirmed by

the ligand exchange reaction using diethyl- phenylphosphine as a competitive ligand. The latter compound was chosen as it performs the ligand exchange reaction with other strong inhibitors of CYPs, such as metyrapone and carbon monoxide (28). However, direct evidence for the ligand type of interaction of cimetidine and related compounds with CYP enzymes was obtained by electron spin resonance measurements of the changes in the ligand field of the iron in cytochromes P450, in the presence of the compounds tested (4-6). The main conclusions drown from these studies were that (6): (a) cimetidine interacted with CYP enzymes through both its imidazole ring and cyano group as presented schematically in Fig. 5, and (b) interaction of cimetidine with the cyano group is more effective for the inhibition of the cytochrome P450 catalyzed O-dealkylation of 7-ethoxy-coumarin than with the imidazole group.

It was also suggested (4-7) that: (a) binding of cimetidine to cytochromes P450 should reduce the metabolism of other drugs when co-administered with cimetidine in vivo, and (b) this effect results from interaction of both the imidazole and the cyano groups in the structure of cimetidine.

It was also shown that cimetidine interacted even more strongly with CYP enzymes if experiments were performed under reducing conditions (29,30).

As is shown in Fig. 1, the structure of ranitidine, another H2-receptor antagonist (31,32), differs from that of cimetidine in the following structural characteristics: (a) the imidazole ring is substituted with a furane ring, and (b) the side chain cyano group is substituted with a nitro group (33).

The optical difference, as well as the e.s.r. spectra indicated that ranitidine interacts in a different manner or with different forms of CYP enzymes when compared with cimetidine (refs. 5-7 and Fig. 4B). Markedly lower affinity (about tent-fold), and higher IC50-values for the inhibition of the ethoxycoumarin deethylase (about 2.4 times greater) were obtained (Table 1). It was suggested that at lower concentrations of ranitidine a ligand exchange reaction occurred with an oxygen atom, whereas at higher concentrations an exchange reaction with nitrogenous or thioether ligand occurred. Comparison with the data obtained for structurally related compounds (furan and tetrahydro- furan) allowed the conclusion that the oxygen atom of the furan ring of ranitidine is not a ligand of the heme iron (5,34).

Considering the results discussed and bearing in mind that the daily doses of ranitidine are about one fifth of those for cimetidine (31), it was suggested that ranitidine will exert a negligible effect on the metabolism of co-administered drugs (5). This suggestion was in agreement with both the in vitro studies on the inhibition of metabolism of other drugs using hepatic microsomal enzymes (31) and the in vivo clinical studies (9,10,35).

The Ks values calculated from the spectral studies and reported by other authors for interaction of cimetidine and ranitidine with human liver microsomes were in good agreement with the values reported in Table 1. For example, the values obtained by Pasanen et al for cimetidine and ranitidine were 0.87 and 5.1 mmol/L, respectively (36). In the latter study, only cimetidine and oxmetidine but not ranitidine, famotidine or nizatidine (Fig. 1) were potent in vitro inhibitors of the CYP activity. The activity in rat liver microsomes measured in this study was aryl-hydro-car- bon-hydroxylase, 7-ethoxycoumarin-O-deethylase, and 7-ethoxyresorufin-O-deethylase. However, when human enzymes were used, the inhibition by cimetidine was not detected. These discrepancies between the animal and the human model study were explained by too low concentrations of cimetidine used in the study, as well as by the inappropriate source of human microsomes (the microsomes were prepared from biopsy samples from alcoholics and diabetics). Of all H2-receptor antagonists studied, only cimetidine inhibited significantly the hepatic elimination of diazepam (by about 45%) in vivo.

Schulz and Schmoldt (37) investigated the interactions of etinitidine with rat liver CYP. This drug is structurally very closely related to cimetidine and differs only in the side-chain structure (Fig.1). The affinity of etinitidine for CYP was 5 times higher that of cimetidine for both low and high affinity binding sites. The results on the inhibition of the benzphetamine N-demethylation by both drugs were comparable. The Ks1- and Ks2-values for cimetidine in this study were slightly higher when compared with the values presented in Table 1. The differences were assigned to the different animal strain used in the latter study.

The interaction of famotidine, another H2-receptor antagonist, was studied by Wang et al (38) using phenobarbital pre-treated rat and human liver microsomes. In comparison to cimetidine, famotidine possesses a thiazole nucleus instead of the imidazole ring, and the cyano-group in the side chain was substituted by aminosulfonyl- group (Fig. 1). The results indicated a low affinity interaction of famotidine with CYP enzymes, and an insignificant effect on in vitro metabolism of 7-ethoxycoumarin and benzphetamine using both rat and human liver microsomes.

In contrast to cimetidine, the H2-antagonist roxatidine, possessing no imidazole group in its chemical structure, gave a completely different type of the spectrum in interaction with liver microsomes. The spectra thus recorded were so called ”reverse type of spectra”. Roxatidine also did not impair the

theofylline metabolism in humans and rats, and was a weak inhibitor of in vitro testosterone hydroxylation, aminopyrine N-demethylation, and aniline hydroxylation (39-41).
Other substituted imidazole derivatives were also investigated. For example, compound VII (4,5-hydroxymethyl-5(4)-methylimidazole) showed only a low affinity binding constant Ks2=3.2 mmol/L) and markedly higher IC50-values (Table 1). Cimetidine S-oxide (Fig. 2) showed the lowest binding affinity of all compounds investigated; ranitidine S-oxide did not interact with the enzyme (Fig. 3, Table 1) (6).
Comparing the binding characteristics of cimetidine and imidazole, the optical and the e.s.r. spectra indicated a similar pattern of binding for both compounds. The high affinity binding constants (Ks1) were of the same order of magnitude for both compounds (i.e., 0.06 mmol/L and 0.04 mmol/L for cimetidine and imidazole, respectively, Table 1). However, imidazole was the more potent inhibitor of CYP activity in vitro as the IC50-value for imidazole was about 10 times lower than the value for cimetidine (Table 1). For detailed discussion on binding properties of other structurally related compounds see ref. 6. Results from studies of other authors also showed imidazole and derivatives to be potent inhibitors of CYP activity (42-45).
In Vitro Metabolic Studies
Cimetidine and ranitidine inhibited debriso- quine 4-hydroxylase and bufuralol 1´-hydroxylase activity in a competitive and non-competitive manner, respectively (46). The differences observed in the inhibitory patterns for the two reactions were attributed to the interaction of the inhibitors with different isoenzymes. The IC50-values for the debrisoquine 4-hydroxylase activity were about the same for both drugs (0.18 mmol/L and 0.20 mmol/L for cimetidine and ranitidine, respectively). However, the IC50-value for the inhibition of the bufuralol 1´-hydroxylase activity by ranitidine was 5 times higher compared with that obtained by cimetidine (1.00 mmol/L and 5.00 mmol/L for cimetidine and ranitidine, respectively). The IC50-values and the differences observed for the inhibition of bufuralol 1´-hydroxylase activity are in agreement with the results obtained for the inhibition of 7-ethoxycoumarin deethylase using microsomes from phenobarbital pre-treated rats presented in Table 1.
The effect of H2-receptor antagonists and related compounds on the formation of the metabolic intermediate-ferrocytochrome P450 complex during the metabolism of tofenacine was investigated by Rekka et al (47). The results of that study supported the earlier conclusion that a cyano-guanidine moiety is essential for the inhibition of cytochrome P450, regardless of the substituents that replace the imidazole part of the cimetidine structure. Furthermore, the binding ability and the inhibitory activity were in good correlation with the lipophilicity of the compounds tested.

Table 2. Inhibition of CYP enzymes by cimetidine in human liver microsomal preparations (A) and in genetically engineered yeast microsomes expressing human enzymes (B) (from ref. 48)

Selectivity in the inhibition of CYPs by cimetidine has been shown in experiments using human microsomal preparations and enzymes produced by methods of genetic engineering (Table 2). These experiments indicated differential selectivity and a high level of variability of the inhibition, depending on the enzyme and the substrates used (48). The results presented in Table 2 might be summarised as follows:
1. Low extent of inhibition of CYP3A4 (by about 11%) as measured by the erythromycin demethyl-ation activity, and a high extent of inhibition of the same enzyme measured by the nifedipine aroma-tization activity (by about 70%). Interestingly enough, both erythromycin and nifedipine are specific substrates of CYP3A4 enzyme (21).
2. Intermediate extent of inhibition of CYP1A2 (by about 23%), CYP2E1 (by about 32%), and CYP2C9 (by 28-44%). The extent was substrate dependent.
3. High extent of inhibition of CYP2D6 (by about 80%).
In Vivo Metabolic Studies
In vivo treatment of rats with cimetidine indicated selectivity of inhibition of CYP activities by the drug. It was reported that the in vitro activity of CYP2C11, the major hepatic CYP enzyme in uninduced adult male rats, was inhibited after in vivo cimetidine administration (49). Pre-treatment with cimetidine in vivo selectively inhibited in vitro activity of testosterone 2a-hydroxylase (specific for CYP2C11) in microsomes from uninduced, and phenobarbital- and dexamethasone-induced rats by 65%, 73%, and 46%, respectively. Selectivity was indicated by the fact that the microsomal activity specific for rat cytochrome CYP2A1, CYP2B1,2 or CYP3A1,2 was not affected. Model reactions used in this study were testosterone 7a-hydroxylation (for CYP2A1), testosterone 16b-hydroxylation and pentoxyresorufin O-de- alkylation (for CYP2B1,2), and testosterone 2b- and 6b-hydroxylation and erythromycin N-de- methyl- ation (for CYP3A1,2). Moreover, the differential inhibitory activity shown

by cimetidine was tested after in vitro addition of cimetidine (50). When cimetidine was added to microsomes from untreated and phenobarbital pre-treated rats, just prior to commencing the reaction (without a preincubation period), the drug acted as a relatively non-selective inhibitor of cytochromes P450 by affecting several enzymes: CYP2C11 (testosterone 2a-hydroxylation), CYP2B1,2 (testosterone 16b- hydroxylation), and CYP3A1,2 (testosterone 2b- and 6b-hydroxylation). However, if hepatic microsomes were pre-incubated with cimetidine (addition of cimetidine in the final concentration of 0.05 mmol/L, for 15 min before the initiation of the reaction in the presence of NADPH, specific activity of CYP2C11 was inhibited but the activities specific for CYP2A1, CYP2B1,2 or CYP3A1,2, were not affected. Thus, the results were comparable to those obtained after administration of the drug in vivo. In another study, performed under the similar experimental conditions, cimetidine inhibited CYP2C6 in addition to CYP2C11 but showed little or no effect on CYP1A1 (51). In the latter study, methoxyresorufin and ethoxy- resorufin O-dealkylation were used as model reactions and animals were induced by b-naphtho- flavone. The formation of a cimetidine metabolite-intermediate complex with rat CYP2C11 and CYP2C6 following both in vivo and in vitro treatment with cimetidine was suggested (51,52).

Effect of pre-treatment of rats with cimetidine or ranitidine on the activity catalysed by CYP2D enzymes in rat liver microsomes in vitro was investigated by Orishiki et al (46). O-demethylation of p-nitroanisole, N-demethylation of aminopyrine or benzphetamine, and 7-ethoxycoumarin O-deethyl-ation were decreased to a greater extent following pre-treatment with cimetidine; a higher decrease of debrisoquine 4-hydroxylation (reflecting the activity of CYP2D1) was obtained after pre-treatment with ranitidine; and pre-treatment with both drugs led to a similar decrease (by about 47%) of bufuralol 1´-hydroxylation activity (reflecting CYP2D activity). Decreased activity of the CYP2D enzymes in rat microsomes after pre-treatment with both drugs was explained by a decreased content of the CYP2D protein. Decreased in vitro estradiol hydroxylation, ethylmorphine demethylation, aromatic and aniline hydroxylation but not deethylation of 7-ethoxycoumarin was observed after pre-treatment of rats with cimetidine. Furthermore, pre- treatment of animals with cimetidine enhanced, in an additive manner, inhibition of estradiol hydroxylation by cimetidine in vitro (53).

After continuous pre-treatment of rats with cimetidine (doses were in the range of 25-150 mg/kg body wt/day for 7 days), the specific CYP content was elevated (54). The elevation was accompanied by a higher specific activity of ethoxyresorufin O-dealkylation, while the activity of other CYP catalysed reactions was decreased (i.e., dealkylation of drugs such as ethoxycou- marin, ethylmorphine, aminopyrine, dimethyl- nitrosamine, and morphine). These results were explained by the suggestion that pre-treatment with cimetidine changed the relative abundance of enzymes that catalysed specific reactions. Thus, a dual role for cimetidine was proposed after continuous pre-treatment by the drug, acting as both inducer and inhibitor of CYP activities. A putative iductive effect of cimetidine was studied also by Wright et al (55), Ioannides et al (56), and Ritter and Franklin (57), and the results suggest that in humans, under therapeutic concentrations, induction of CYP enzymes by cimetidine should not have any clinical relevance.

Clinical Studies

When cimetidine was co-administered with other drugs clinically significant drug-drug interactions were observed and reported (9,10). Side effects resulted in either potentatiation of therapeutic and/or toxic effects of co-administered drugs and other xenobiotics (9,10,58-63) or inhibition of toxicity of the drugs and chemicals that were metabolically activated. As clinical studies indicated a decrease in metabolism of drugs when co-administered with cimetidine (31,63), it was suggested that this, at least in part, resulted from the inhibition of the enzymes responsible for the drugs metabolism. The in vitro results discussed revealed that CYPs are the enzymes that are inhibited by cimetidine and other H2-receptor antagonists.

Table 3. Therapeutic classes that promote clinically significant drug-drug interactions when co-administered with cimetidine: metabolic reactions and human enzymes that might be inhibited by cimetidine are suggested in accordance with data published in ref. 21

For illustration, Table 3 indicates a list of drug classes, with examples, which elicit clinically significant drug-drug interactions when co-administered with cimetidine. The metabolic reactions and human cytochrome P450s that are inhibited by cimetidine are given as well (in accordance with the data reported in ref. 21). Drug-drug interactions resulting in changed pharmacokinetic parameters and/or metabolic profile were observed in humans, when for example, anticoagulants (warfarin; ref. 64), benzodiazepines (diazepam, desmethyldiazepam, and nitrazepam; refs. 65-68), xanthines (caffeine, theophylline; refs. 39-41,69), parasympathomimetics (tacrine; ref. 70), and b- blockers (71-75) were co-administered with cimetidine (see also Table 3). Inhibition of the renal clearance of zidovudine as

well as the increase of the urinary ratio of the metabolite to the parent drug by cimetidine was, however, ascribed to the competition for tubular secretion (12).
In addition to the drug-drug interactions, cimetidine administration also elicited side effects that reflected the impairment of the metabolic pathways of endogenous compounds such as steroid hormones. The side effects observed were hyperprolactinaemia, gynecomastia, impotence, decreased sperm count, and altered hormone concentrations and excretion (76-79). At least some of these side effects could be also linked to the impairment of biosynthesis and/or metabolic transformation of endogenous steroids by the inhibition of CYP enzymes (53,80-84). In contrast to cimetidine, ranitidine was reported to have only a slight effect on steroidogenesis. It was shown that ranitidine had no influence on the ACTH-stimulated corticosterone synthesis and the 11b-hydroxylase activity (80), cortisol serum or urine concentrations, and the ratios of 6b-hydroxycortisol and 17-hydroxycorticosteroids (85).
The potential of other H2-antagonists (Fig. 1) to elicit in vivo drug-drug interactions can be summarized as follows (9,10,86,87):
1. Etinitidine, as a drug of similar structure to cimetidine, also possesses comparable cytochrome P450 inhibitory and drug-drug interaction potential.
2. Famotidine, nizatidine, and roxatidine possess a weak cytochrome P450 inhibitory potential and a low drug-drug interaction potential.
3. Although the results obtained with ranitidine showed a low inhibitory potential, drug-drug interactions were reported in some cases.
However, when considering drug interaction potential, it has to be taken into account that the inhibitory effects of H2-receptor antagonists also depend on the properties of the inhibited enzyme. For example, it has been reported that both ranitidine and cimetidine only slightly affected the pharmacokinetics of tolbutamide, a specific substrate of the CYP2C9 enzyme, without changing its rate of hydroxylation. As weak inhibition of tolbutamide hydroxylation by cimetidine was observed also in vitro (Table 2), it was concluded that both drugs are weak inhibitors of CYP2C9 enzyme (88). On the contrary, in the case of b-blocking drugs propranolol (a substrate of human CYP1A, CYP2D6, and 2C19) and metoprolol (a substrate of human CYP2D6 and 2C19), significant reductions of clearance in association with cimetidine treatment was observed (71-75,86). These observations, as well as those from the in vitro inhibition studies, suggest that cimetidine is a strong inhibitor of the CYP2D6 and 2C19 enzymes in vivo (see also Tables 2 and 3, and ref. 21).
Beneficial effects of the inhibitory activity of cimetidine toward CYP enzymes are based on the fact that the drug may inhibit toxicity of other drugs and chemicals that are metabolically activated by catalytic activity of these enzymes. For example, cimetidine was used for the prevention of hepatotoxicity induced by overdoses with the analgesic and antipyretic drug paracetamol (89-91), a substrate of several CYP enzymes (i.e., CYP1A1,2, CYP2E1, and CYP3A4), which activate the drug by oxidation to the hepatotoxic metabolite N-acetyl-p-benzoquinone imine. All these enzymes are inhibited by cimetidine (Table 2 and ref. 21). Protective effects of cimetidine toward the metabolic formation of reactive intermediates and metabolites were elicited also with other toxicants (21,92-95). As an inhibitor of CYP enzymes cimetidine was used for the prevention of CYP2E1 related hepatotoxicity induced by tetrachloromethane (94); inhibition of cocaine N-demethyl- ation (catalysed by CYP3A4 enzyme) and protection patients from the liver damaging effects of cocaine (95); and reduction of methaeglobinaemia induced by dapsone (a substrate of CYP2E1 and CYP3A4 enzymes, ref. 21) in treated patients improving the therapeutic/toxic ratio of the drug (96).
Potentiation of the acute toxicity of compounds by cimetidine related to the inhibition of the metabolism was also reported. Cimetidine co- administration with the organophospohorous insecticide diazinon resulted in potentiation of the acute toxicity due to an increase in the amount of diazinon in the systemic circulation and the brain of rats, decreased clearance and the inhibition of diazinon elimination, and a significant increase of the half-life of the chemical. Increased toxicity of parathion via inhibition of its metabolism by cimetidine was also reported (97).
Conclusions
In laboratory studies, interactions of H2-receptor antagonists with cytochromes P450 are characterized by both a low affinity and a great variability of the binding properties. In vitro studies led to the conclusion that cimetidine interacts with CYP enzymes with both the imidazole and the CN-group. For the inhibition of the metabolic reactions by cimetidine the cyano group plays the primary role and is more effective than the imidazole group (Fig. 5). Factors that influence interactions of H2-receptor antagonists with CYP enzymes are: pre-incubation of the enzymes with the drugs; pre-treatment of animals in vivo with the drug investigated in vitro; experimental conditions applied during the experiment (oxidative or reductive).
In clinical studies, drug-drug interactions observed after co-administration of H2-receptor antagonists

with other drugs are in good agreement with the in vitro results on their interactions with CYP enzymes, and are affected by the selectivity and variability of the CYP enzymes, importance of the inhibited reaction for overall metabolism and clearance of co-administered drug, doses administered, and duration and the way of treatment.
Results and conclusions from the in vitro and in vivo laboratory studies on interactions of H2-antagonists with CYP enzymes were shown to be the most useful for explanation of the clinically observed drug-drug interactions and for designing new drugs with improved properties.

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This paper is dedicated to a colleague and friend Dr Fredierck DiCarlo, a world-renowned scientists who passed away on September 15, 1997 in Bethesda, Maryland, USA. Dr Di Carlo was the founding Editor and the Executive Editor of the journal Drug Metabolism Reviews (founded in 1970) and co-founder of the International Society for the Study of Xenobiotics (founded in 1980).

References
1 Black JW, Duncan WAM, Durant CJ, Ganellin CR, Parsons E.M. Definition and antagonism of histamine H2-receptors. Nature 1972;236:385-90.
2 Durant GJ, Emmett JC, Ganellin CR, Miles PD, Parsons ME, Prain H.D, Whita GR. Cyanoguanidine- thiourea equivalence in the development of the histamine H2-receptor antagonist, cimetidine. J Med Chem 1977;20:901-6.
3 Adams WJ, Lawson JA, Morris DL. Cimetidine inhibits in vivo growth of human colon cancer and reverses histamine stimulated in vitro and in vivo growth. Gut 1994:35;1632-6.
4 Rendiæ S, Šunjiæ V, Toso R, Kajfež F, Ruf HH. Interaction of cimetidine with liver microsomes. Xenobiotica 1979;9:555-64.
5 Rendiæ S, Alebiæ-Kolbah T, Kajfež F, Ruf HH. Interaction of ranitidine with liver microsomes. Xenobiotica 1982;12:9-17.
6 Rendiæ S, Kajfež F, Ruf HH. Characterization of cimetidine, ranitidine, and related structures' interaction with cytochromes P450. Drug Metab Dispos 1983;11:137-42.
7 Rendiæ S, Ruf HH, Weber P, Kajfež F. Cimetidine and ranitidine: their interaction with human and pig liver microsomes and with purified cytochrome P450. Eur J Drug Metab Pharmacokin 1984;9:195-200.
8 Feely J, Wood AJJ. Effects of cimetidine on the elimination and actions of ethanol. JAMA1982;247:2819-21.
9 Nazario M. The hepatic and renal mechanisms of drug interactions with cimetidine. Drug Int Clin Pharm 1986;20:342-8.
10 Campagna KD. Drug-drug interactions with H2-receptor antagonists. Am Pharm 1991;7:29-35.
11 DiPadova C, Roine R, Frezza M, Gentry RT, Baraona E, Lieber CS. Effects of ranitidine on blood alcohol levels after ethanol ingestion. JAMA 1992;267:83-6.
12 Fletcher CV, Henry WK, Noormohamed SE, Rhame FS, Balfour HH. The effect of cimetidine and ranitidine administration with zidovudine. Pharmacotherapy 1995;15:701-8.
13 Mihaly GW, Drummer OH, Marshall A, Smallwood RA, Louis WJ. High-presure liquid chromatographic determination of ranitidine, a new H2-receptor antagonist in plasma and urine. J Pharm Sci 1980;69:1155-7.
14 Carey PF, Martin LE, Owen PE. Determination of ranitidine and its metabolites in human urine by reversed-phase ion-pair high-performance liquid chromatography. J Chrom Biomed Appl 1981;225:161-8.
15 Taylor DC, Creswell PR, Batrlett DC. The metabolism and elimination of cimetidine, a histamine H2-receptor antagonists, in the rat, dog, and man. Drug Metab Dispos 1978;6:21-30.
16 Mitchell SC, Idle JR, Smith RL. The metabolism of (14C) cimetidine in man. Xenobiotica 1982;12:283-92.
17 Mitchell SC, Idle JR, Smith RL. Reductive metabolism of cimetidine sulfoxide in man. Drug Metab Dispos 1982;10:289-90.
18 Larsson R, Erlanson P, Bodemar G, Walan A, Bertler A, Fransson L, et al. The pharmacokinetics of cimetidine and its sulphoxide metabolite in patient with normal and impared renal function. Br J clin Pharmacol 1982;13:163-70.

19 Kuzel RA, Bhasin SK, Oldham HG, Damani LA, Murphy J, Camilleri P, et al. Investigations into the chirality of the metabolic sulfoxidation of cimetidine. Chirality 1994;6:607-14.
20 Cross DM, Bell JA, Wilson K. Kinetics of ranitidine metabolism in dog and rat isolated hepatocytes. Xenobiotica 1995;25:367-75.
21 Rendiæ S, DiCarlo F. Human cytochrome P450 enzymes: A satus report summarizing their reactions, substrates, inducers and inhibitors. Drug Metab Rev 1997;29:413-580.
22 Puurunen J, Pelkonen O. Cimetidine inhibits microsomal drug metabolism in the rat. Eur J Pharmacol 1979;55:335-336.
23 Pelkonen O, Puurunen J. The effect of cimetidine on in vitro and in vivo microsomal drug metabolism in the rat. Biochem Pharmacol 1980;29:3075-80.
24 Puurunen J, Sotaniemi E, Pelkonen O. Effect of cimetidine on microsomal drug metabolism in man. Eur J Clin Pharmacol 1980;18:185-7.
25 Guengerich FP. Human cytochrome P450 enzymes. In: Ortiz de Montellano PR, editor. Cytochrome P450 – structure, mechanisms and biochemistry. 2nd edition. New York: Plenum Press; 1995. p. 473-535.
26 Halpert JR. Structural basis of selective cytochrome P450 inhibition. Annu Rev Pharmac Toxicol 1995;5:29-53.
27 Ullrich V, Ruf HH, Weber P. Ligand reactions of cytochrome P450. Biochem Soc Trans 1974;3:803-7.
28 Mansuy D, Duppel W, Ruf HH, Ullrich V. Phosphines as ligands to microsomal cytochrome P450. Hoppe- Seyler's Zeitschr Physiol Chem 1974;355:1341-9.
29 Faux SP, Combes RD. Interaction of cimetidine with cytochrome P450 and effect on mixed function oxidase activities of liver microsomes. Hum Exp Toxicol 1993;12:147-52.
30 Faux SP, Combes RD. Interaction of cimetidine with oxidized and prereduced microsomal cytochrome P-450. Drug Metab Dispos 1994;22:180-2.
31 Henry DA, MacDonald IA, Kitchingman G, Bell GD, Langman MJS. Cimetidine and ranitidine: comparison of effects on hepatic drug metabolism. Brit Med J 1980;281:775-7.
32 Helman CA, Ou Tim L. Pharmacology and clinical efficacy of ranitidine, a new H2-receptor antagonist. Pharmacotherapy 1983;3:185-92.
33 Hohnjec M, Rendiæ S, Alebiæ-Kolbah T, Kajfež F, Blaževiæ N, Kuftinec J. Physical and spectroscopic data of ranitidine. Acta Pharm Iugoslav 1981;31:131-42.
34 Ullrich V, Weber P, Wollenberg P. Tetrahydrofurane – an inhibitor for ethanol-induced liver microsomal cytochrome P450. Biochem Biophys Res Commun 1975;64:808-13.
35 Smith SR, Kendall MJ. Ranitidine versus cimetidine, a comparison of their potential to cause clinically important drug interactions. Clin Pharmacokinet 1988;15:44-56.
36 Pasanen M, Arvela P, Pelkonen O, Sotaniemi E, Klotz U. Effect of five structurally diverse H2-receptor antagonists on drug metabolism. Biochem Pharmacol 1986;35:4457-61.
37 Schulz M, Schmoldt A. Interactions of the histamine H2-receptor antagonist etintidine with rat liver cyto- chrome P-450: a comparison with cimetidine. Naunyn- Schmiedeberg's Arch Pharmacol 1987;336:453-8.
38 Wang RW, Miwa GT, Argenbright LS, Lu AYH. In vitro studies on the interaction of famotidine with liver microsomal cytochrome P-450. Biochem Pharmacol 1988;37:3049-53.
39 Ohashi K, Sakamoto K, Sudo T, Tateishi T, Fujimura A, Shiga T, et al. Effects of diltiazem and cimetidine on theophylline oxidative metabolism. J Clin Pharmacol 1993;33:1233-7.
40 Nagai N, Furuhata M, Ogata H. Drug interactions betwee theophylline and H2-antagonists, roxatidine acetate hydrochloride and cimetidine: pharmacokinetic analysis in rats in vivo. Biol Pharm Bull 1995;18:1610-3.
41 Gascon AR, Calvo B, Hernandez RM, Dominguez- Gil A, Pedraz J-L Interspecies scaling of cimetidine- theophylline pharmacokinetic interaction: inter- species scaling in pharmacokinetic interactions. Pharmaceut Res 1994;11:945-50.
42 Wilkinson CF, Hetnarski K, Yellin TO. Imidazole derivatives – a new class of microsomal enzyme inhibitors. Biochem Pharmacol 1972;21:3187-92.
43 Wilkinson CF, Hetnarski K, Cantwell GP, DiCarlo FJ. Structure-activity relationship in the effects of 1-alkylimidazoles on microsomal oxidation in vitro and in vivo. Biochem Pharmacol 1974;23:2377-86.
44 Hajek KK, Cook NI, Novak RF. Mechanism of inhibition of microsomal drug metabolism by imidazole. J Pharmacol Exp Ther 1982;223:97-104.
45 Ahmed S, Smith JH, Nicholls PJ, Whomsley R, Cariuk P. Synthesis and biological evaluation of imidazole based compounds as cytochrome P-450 inhibitors. Drug Des Discov 1995;13:27-41.
46 Orishiki M., Matsuo Y, Nishioka M, Ichikawa Y. In vivo administration of H2-blockers, cimetidine and ranitidine, reduced the contents of the CYPIID (CYP2D) subfamily and their activities in rat liver

microsomes. Int J Biochem 1994;26:751-8.
47 Rekka E., Sterk GJ, Timmerman H, Bast A. Identification of structural characteristics of some, potential H2-receptor antagonists that determine the interaction with rat hepatic P-450. Chem-Biol Inte 1988;67:117-27.
48 Knodell RG, Browne DG, Gwozdz GP, Brian WR., Guengerich FP. Differential inhibition of individual human liver cytochromes P450 by cimetidine. Gastroenterology 1991;101:1680-91.
49 Chang T, Levine M., Bandiera S, Bellward GD. Selective inhibition of rat hepatic microsomal cytochrome P-450. I. Effect of the in vivo administration of cimetidine. J Pharmacol Exp Ther 1992;260:1441-9.
50 Chang T, Levine M, Bellward GD. Selective inhibition of rat hepatic microsomal cytochrome P-450. II. Effect of the in vitro administration of cimetidine. J Pharmacol Exp Ther 1992;260:1450-5.
51 Law EYW, Bandiera SM, Bellward GD, Levine M. Effect of cimetidine on hepatic cytochromes P450 2C and P4501A in adult male rats. In: ISSX Proceedings, Fourth International ISSX Meeting; 1995 Aug 27-31; Seattle (WA) USA. Bethesda: International Society for the Study of Xenobiotics; 1995. p. 153.
52 Levine M, Bellward GD. Effect of cimetidine on hepatic cytochrome P450: evidence for formation of a metabolite-intermediate complex. Drug Metab Dispos 1995;23:1407-11.
53 Boidin MP, Stuurnman A, Erdman W. Inhibition of steroidogenesis by cimetidine in man. A double-blind prospective study, Scan J Gastroenterol 1988;23:41-5.
54 Ioannoni B, Mason SR, Reilly PEB, Winzor DJ. Evidence for induction of hepatic microsomal cytochrome P-450 by cimetidine: binding and kinetic studies. Arch Biochem Biophys 1986;247:372-83.
55 Wright AWE, Winzor DJ, Reilly PEB. Cimetidine: an inhibitor and an inducer of rat liver microsomal cytochrome P-450. Xenobiotica 1991;21:193-203.
56 Ioannides C, Rodrigues AD, Ayrton AD, Barnett CR, Chown J, Parke DV. Induction of the rat hepatic microsomal mixed function oxidases by cimetidine. Toxicol Lett 1989;49:61-8.
57 Ritter JK, Franklin MR. Induction and inhibition of rat hepatic drug metabolism by N-substituted imidazole drugs. Drug Metab Dispos 1987;15:335-43.
58 Henry A, Langman MJS. Adverse effects of antiulcer drugs. Drugs 1981;21:444-59.
59 Bauman JH, Kimelblatt BJ. Cimetidine as an inhibitor of drug metabolism: therapeutic implications and review of the literature. Drug Int Clin Pharm 1982;16:380-6.
60 D´Arcy PF. Drug interactions and reactions update. Drug Int Clin Pharm 1982;16:668-72.
61 Somogyi A, Gugler R. Drug interactions with cimetidine. Clin Pharmacokin 1982;7:23-41.
62 Sorkin EM, Darvey DL. Review of cimetidine drug interactions. Drug Int Clin Pharm 1983;17:110-20.
63 Breen KJ, Bury R, Desmond PV, Mashford ML, Morphet B, Westwood B, et al. Effects of cimetidine and ranitidine on hepatic drug metabolism. Clin Pharmacol Ther 1982;31:297-300.
64 Serlin MJ, Mossman S, Sibeon RG, Breckenridge AM, Williams JRB, Atwood JL, et al. Cimetidine: interaction with oral anticoagulants in man. Lancet 1979;2:317-9.
65 Klotz U, Antilla VJ, Reimann I. Cimetidine/diazepam interaction. Lancet 1979;2:699.
66 Klotz U, Reimann I. Elevation of steady-state diazepam levels by cimetidine. Clin Pharmacol Ther 1981;30:513-7.
67 Klotz U, Reimann I. Influence of cimetidine on the pharmacokinetics of desmethyldiazepam. Eur J Clin Pharmacol 1980;18:517-20.
68 Ochs HR, Greenblatt DJ, Gugler R, Müntefering G, Locniskar A, Abernethy DR. Cimetidine impairs nitrazepam clearance. Clin Pharmacol Ther 1983;34:227-30.
69 May DC, Jarboe CH, VanBakel AB, Williams MW. Effects of cimetidine on caffeine disposition in smokers and nonsmokers. Clin Pharmacol Ther 1982;31:656-61.
70 Forgue ST, Reece PA, Sedman AJ, deVries TM. Inhibition of tacrine oral clearance by cimetidine, Clin Pharmacol Therap 1996;59:444-9.
71 Reimann IW, Klotz U, Siems B, Frölich JC. Cimetidine increases steady state plasam levels of propranolol. Brit J Clin Pharmacol 1981;12:785-90;
72 Reimann IW, Klotz U, Frölich JC. Effets of cimetidine and ranitidine on steady-state propranolol kinetics and dynamics. Clin Pharmacol Ther 1982;32:749-57.
73 Rey E, Jammet P, d'Athis P, deLauture D, Christoforov B, Weber S, et al. Effect of cimetidine on the pharmacokinetics of the new b-blocker betaxolol. Drug Res 1987;37:953-6.
74 Toon S, Davidson EM, Garstang FM, Batra H, Bowes RJ, Rowland M. The racemic metoprolol H2-antagonist interaction. Clin Pharmacol Ther 1988;43:283-9.
75 Ishida R, Fujita S, Suzuki T. Bunitrolol metabolism and its inhibition by cimetidine. J Pharm Pharmacol 1988;40:64-5.

76 DelleFave GF, Tamburano G, DeMagistris L, Natoli C, Santro ML, Carratu R, et al. Ginaecomastia with cimetidine. Lancet 1977;1:1319.
77 Winters SJ, Banks JL, Loriaux DL. Cimetidine is an antiandrogen in rats. Gastroenterology 1979;76:504-8.
78 Mignon M, Vallot Th, Mayeur S, Bonfils S. Ranitidine and cimetidine in Zollinger-Ellison syndrome. Brit J Clin Pharmacol 1980;1:173-4.
79 Hoie EB, Swigart SA, Nelson RM, Leuschen MP. Development of secondary sex characteristics in male rats after fetal and perinatal cimetidine exposure. J Pharm Sci 1994;83:107-9.
80 Kenyon CJ, Fraser R, Birnie GG, Connell JMC, Lever AF. Dose related in vitro effects of ranitidine and cimetidine on basal and ACTH-stimulated steroido- genesis. Gut 1986;27:1143-6.
81 Galbraith RA, Jellinck PH. Decreased estrogen hydroxylation in male rat liver following cimetidine treatment. Biochem Pharmacol 1989;8:313-9.
82 Galbraith RA, Jellinck PH. Differential effects of cimetidine, ranitidine, and famotidine on hepatic metabolism of estrogen and testosterone in male rat. Biochem Pharmacol 1989;38:2046-9.
83 Galbraith RA, Michnowitz JJ. The effect of cimetidine on the oxidative metabolism of estradiol. N Engl J Med 1989;321:269-74.
84 Rodriguez LAG, Jick H. Risk gynaecomastia associated with cimetidine, omeprazole, and other antiulcer drugs. Brit Med J 1994;308:503-6.
85 Mönig H, Hoffman K, Ohnhaus EE, Schulte HM. Ranitidine treatment and cortisol metabolism in man. Eur J Drug Metab Pharm 1992;17:9-12.
86 Krishna DR, Klotz U. Newer H2-receptor antagonists, clinical pharmacokinetics and drug interaction potential. Clin Pharmacokin 1988;15:205-15.
87 Berardi RR, Tankanow RM, Nostrant TT. Comparison of famotidine with cimetidine and ranitidine. Clin Pharmacokin 1988;7:271-84.
88 Toon S, Holt BL, Mullins FGP, Kahn A. Effects of cimetidine, ranitidine, and omeprazole on tolbutamide pharmacokinetics. J Pharm Pharmacol 1995; 47: 85-88.
89 Abernethy DR, Greenblatt DJ, Divoll M, Ameer B, Shader RI. Differential effect of cimetidine on drug oxidation (antipyrine and diazepam) vs. conjugation (acetaminophen and lorazepam): prevention of acetaminophen toxicity by cimetidine. J Pharmacol Exp Ther 1983;224:508-13.
90 Vermeulen NPE, Bessems JGM, Van der Straat R. Molecular aspects of paracetamol-induced hepato- toxicity and its mechanism-based prevention. Drug Metab Rev 1992;24:367-407.
91 Burkhart KK, Janco N., Kulig KW, Rumack BH. Cimetidine as adjunctive treatement for acetamino-phen overdose. Hum Exp Toxicol 1995;14:299-304.
92 Hazinski TA, France M, Kennedy KA, Hansen TN. Cimetidine reduces hyperoxic lung injury in lambs. J Appl Physiol 1989;67:2586-92.
93 Paller MS, Jacob HS. Cytochrome P-450 mediates tissue-damaging hydroxyl radical formation during reoxygenation of the kidney. Proc Nat Acad Sci USA 1994;91:7002-6.
94 Mera E, Muriel P, Castillo C, Mourell M. Cimetidine prevents and partially reverses CCl4-induced liver cirrhosis. J Appl Toxicol 1994;14:87-90.
95 Pasanen M, Pellinen P, Stenbäck F, Juvonen RO, Raunio H, Pelkonen O. The role of CYP enzymes in cocaine-induced liver damage. Arch Toxicol 1995;69:287-90.
96 Rhodes LE, Tingle MD, Park BK, Chu P, Verbov JL, Friedman PS. Cimetidine improves the terapeutic/toxic ratio of dapsone in patients on chronic dapsone therapy. Brit J Dermatol 1995;132:257-62.
97 Wu HX, Evreux-Gros Cl, Descotes J. Influence of cimetidine on the toxicity and toxicokinetics of diazinon in the rat. Hum Exp Toxicol 1996;15:391-5.

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