Interactions between drugs and chemicals in industrial societies.
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ENZYME INHIBITION BY ENVIRONMENTAL AGENTS

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INTRODUCTION

The inhibition of the hepatic microsomal monooxygenase system activity may be due to complex interferences with microsomal function like binding to heme iron, to substrate binding sites, inactivation of cytochrome P-450 by transformation to cytochrome P-420, inhibition of cytochrome P-450 reduction and diversion of electrons from NADPH (1). Based on in vitro and in vivo data obtained mainly from animal experiments many substances were found to interfere with liver microsomal monooxygenase system (2). However, most of these inhibitors are of no clinical importance and may probably be useful in the search for the possible mechanism of inhibition.

DRUGS OF CLINICAL IMPORTANCE

One of the first substances used in clinical practice and showing non-competitive inhibition of several substrates like aminopyrine, hexobarbital, codeine, acetanilide or 0-nitro-anisole was chloramphenicol (3, 4). This inhibition was also of clinical importance as an interaction with tolbutamide and diphenylhydantoin was observed (5). Giving chloramphenicol, 3 g intravenously, results in an immediate change in half-life of tolbutamide from 5 hours to 8 hours and of diphenylhydantoin from 10.5 hours to 22 hours. Tolbutamide in daily doses of 1.5 g administered together with chloramphenicol 2 g or diphenylhydantoin 250 mg over a period of 6 days raised plasma concentrations of tolbutamide or diphenylhydantoin from 8 to 14 µg/100 mL or from 2 to 10 µg/mL respectively due to a strong inhibition of the metabolism of both drugs. As tolbutamide and diphenylhydantoin both have a narrow therapeutic range, hypoglycaemia and neurological side effects were observed (5).

In recent years much attention has been paid to the H_2-receptor antagonist cimetidine found to be a potent inhibitor of drug metabolism in

man. First an effect of cimetidine on diazepam elimination was observed whereby a prolongation of the half-life and a significant decrease in clearance of diazepam occurred (6). Besides this finding, another important interaction was reported, when the anticoagulatory drug warfarin was given together with cimetidine (7). An increase in plasma warfarin concentrations and prothrombin time was found in patients treated concomitantly with both drugs. Based on the inhibition of warfarin elimination severe bleeding may occur in these patients. In addition, cimetidine also affects the metabolism of many other drugs, mostly beta-blocking agents, as seen in Table 1.

Recently ranitidine, and H_2-antagonist with higher antiacidic potency was introduced into the treatment of duodenal ulcers. Concerning the inhibitory capacity of this drug on the hepatic microsomal enzyme system, many conflicting results in man have been published (8). However, using human microsomal preparations and 7-ethoxycoumarin as substrate, an inhibition of ranitidine on the liver microsomal enzyme system could be demonstrated. Ranitidine binds to cytochrome P-450 but the inhibitory affinity was five-fold less compared to cimetidine (9). In addition, ranitidine inhibited the metabolism of paracetamol in a dose-dependent manner in vitro but to a lesser degree than cimetidine (10). Thus, comparing the potency of both H_2-receptor antagonists, ranitidine seems a weaker inhibitor of drug metabolism. This finding may also explain the discrepancies often found in human studies testing identical drugs. The beta-blocker metoprolol administered together with ranitidine showed a tendency of increased areas under the plasma concentration time curve (AUC) in all studies published but reached statistical significant values only in two studies (11, 12). The beta-blocking effect of metoprolol as assessed by inhibition of exercise-induced tachycardia was not affected by the increase of plasma metoprolol concentrations following ranitidine (13). Conflicting data were also reported concerning the metabolism of warfarin. Investigating warfarin elimination under steady states conditions the mean prothrombin time and warfarin concentrations were not affected (14). On the other hand, a decrease in warfarin clearance was also reported and the inhibition seems roughly equipotent comparing cimetidine and ranitidine on a molar basis (15). In addition, depending on the group of volunteers investigated, certain individuals may be more sensitive to ranitidine (16). This fact could probably be a likely explanation for the conflicting data, as in most studies a slight inhibitory tendency was always present but not always reaching significant differences. The drugs whose metabolism was found to be affected by ranitidine so far are shown in Table 1. Altogether the inhibitory efficacy

of ranitidine is certainly less compared to that of cimetidine based on in vitro and in vivo data and may be therefore only important in certain individual patients.

Table I

Cimetidine		Ranitidine
	inhibits the metabolism of	
Antipyrine		Chlormethiazol
Carbamazepin		Fentanyl
Chlordiazepoxide		Metoprolol
Chlormethiazol		Midazolam
Desmethyldiazepam		Nifedipine
Diazepam		Theophylline
Labetolol		Warfarin
Lorazepam		
Metoprolol		
Morphine		
Oxazepam		
Propranolol		
Warfarin		

SELECTIVE INHIBITION OF METABOLISM

Despite possible differences in the inhibitory potency of certain drugs on the isoenzymes of cytochrome P-450, a differential inhibition of the microsomal enzyme system has so far not been assessed in vivo. Therefore, in the present study three different drugs with known inhibitory potency were given to healthy volunteers: primaquine 45 mg as a single dose for 1 day, sulfaphenazol 1 g daily in two divided doses for 3 days, cimetidine 1 g daily

(3 x 200 mg and an evening dose of 400 mg) and 1.6 g (4 x 400 mg) daily for 4 days. Antipyrine 1200 mg orally and 500 mg tolbutamide intravenously in single doses were given as model drugs assessing liver microsomal enzyme activity in vivo. Plasma and urine samples were collected at certain time intervals from 0-48 hours and the concentrations of both drugs were measured by HPLC. Calculating the elimination of tolbutamide and antipyrine, following a single dose of primaquine, a decrease in antipyrine clearance from 35.2 $\pm$ 6.0 mL/min to 24.2 $\pm$ 6.6 mL/min ($p < 0.01$), but no change in tolbutamide clearance (17.5 $\pm$ 3.9 mL/min or 16.3 $\pm$ 2.4 mL/min) was found. Sulfaphenazol, in contrast, did not change antipyrine clearance, showing values of 36.2 $\pm$ 6.3 mL/min on both occasions while tolbutamide clearance was decreased from 25.2 $\pm$ 7.0 mL/min to 5.9 $\pm$ 1.4 mL/min ($p < 0.01$). Also cimetidine given in a dose of 1 g for 4 days did not show any effect on antipyrine or tolbutamide clearance, having more or less the same values before and after drug administration. However, cimetidine 1.6 g given for 4 days decreased antipyrine clearance from 32.9 $\pm$ 9.9 mL/min to 18.3 $\pm$ 2.4 mL/min ($p < 0.01$) and also tolbutamide clearance from 21.8 $\pm$ 14.8 mL/min to 10.4 $\pm$ 2 2 mL/min ($p < 0.01$). These results found for cimetidine may reflect a dose-dependent inhibition in this group of volunteers as the same volunteers took part in both studies. Also a dose of cimetidine 1.2 g daily (3 x 400 mg) given for 3 weeks showed an identical antipyrine clearance before and after this time period having values of 41.2 $\pm$ 16.2 mL/min or 41.0 $\pm$ 13.5 mL/min respectively. After the cessation of cimetidine and controlling antipyrine clearance 14 days later a nearly identical value of 43.1 $\pm$ 12.1 mL/min was observed. This finding may be explained by a non-effective inhibition of the microsomal enzyme system in this group of volunteers. However, cimetidine may have lost its inhibitory efficacy on the microsomal enzyme system during long-term treatment, whereby both findings were so far not reported in the literature. Based on recent investigations, cimetidine does not influence its own metabolism (16). Administering cimetidine intravenously in a dose of 400 mg before and one, two and three weeks after daily administration of 1.2 g cimetidine, the elimination of cimetidine remained unchanged at every occasion tested. This would suggest that the 40% proportion of cimetidine metabolized in the liver is not affected by cimetidine itself.

The new quinolone derivative enoxacine used in the treatment of lower respiratory tract infections showed a higher incidence of side effects in patients treated concomitantly with theophylline (17). Measuring the plasma theophylline concentrations during a combined treatment with theophylline (2 x

300 mg daily) and enoxacine (2 x 400 mg daily) a doubling of theophylline plasma concentrations was found (18). Investigating the pharmacokinetics of theophylline during the administration of enoxacine, besides the increase in plasma concentrations, a prolongation of the half-life of theophylline from 5.5 hours to 16.5 hours was calculated. As no changes in protein binding and non-renal clearance of theophylline occurred, an inhibitory effect of this new quinolone on theophylline metabolism was the most likely explanation. Therefore, the probable inhibitory capacity of enoxacine on the metabolism of antipyrine was studied. In addition, the widely used antidiabetic drugs chlorpropamide and glibenclamide, both extensively metabolized in the liver and with a narrow therapeutic range were also investigated because of possible drug interactions. Antipyrine elimination was studied in 12 healthy male volunteers and afterwards two groups of 6 volunteers were formed. To each group, 200 mg chlorpropamide or 3.5 mg glibenclamide were administered and later enoxacine was concomitantly given in a dose of 400 mg twice daily for 12 days. After 4 days antipyrine, chlorpropamide and glibenclamide elimination was reinvestigated in random order. Chlorpropamide plasma concentrations were measured at certain time intervals from 0-96 hours using an HPLC method. Glibenclamide was estimated in plasma from 0-24 hours using and HPLC method. Trough levels and plasma concentrations 2 hours after enoxacine administration were measured on days 4 and 5 by HPLC in order to assess the appearance of steady-state concentrations and the compliance of the volunteers.

Following 4 days enoxacine administration, enoxacine concentrations were in the same range as reported previously, showing values of 0.6 µg/mL to 0.8 µg/mL for trough levels and 2 µg/mL to 2.2 µg/mL for 2 the hours plasma concentrations. The antipyrine half-life, plasma clearance and urinary metabolites, excreted as percentage of the dose, and the calculated clearance to metabolites in urine for 48 hours are shown in Table 2. Thereby a clear reduction of antipyrine clearance by about 50% and a decrease in the total amount of metabolites excreted in the urine occurred. This decrease was mainly due to a diminution of 4-hydroxyantipyrine and 3-hydroxymethylantipyrine urinary excretion, while antipyrine and norantipyrine showed no significant changes. The calculated clearance to metabolite in urine was significant for all three metabolites. However, 4-hydroxyantipyrine and 3-hydroxymethylantipyrine values showed a decrease of about 80% but those of norantipyrine were diminished only by about 60%.

TABLE II

Enoxacine 2 x 400 mg daily

	before		during
Antipyrine $t_{1/2}$ (h)	10.8 ± 2.0	*	21.1 ± 7.2
Cl (mL/min)	50.1 ± 2.0	**	25.4 ± 8.2
3-OH (%)	16.7 ± 5.9	**	6.8 ± 3.6
4-OH (%)	28.6 ± 11.4	**	12.1 + 6.4
Nor-A (%)	18.2 ± 6.5	n.s.	15.0 ± 10.0

* $p < 0.01$

** $p < 0.001$

In contrast, no inhibitory effect of enoxacine was seen during the administration of chlorpropamide whereby the maximal concentration, C_{max}, the time in which this maximal concentration was reached, t_{max}, the half-life and the clearance of chlorpropamide were not affected as seen in Table 3. In contrast, the plasma concentration curve of glibenclamide before and after enoxacine administration was quite different showing a reduction in C_{max} from 136 ± 38 ng/mL to 92 ± 34 ng/mL ($p < 0.05$) and the area under the plasma concentration time curve from 0.35 ± 0.09 to 0.26 ± 0.03 µg/mL x hr ($p < 0.01$). Thereby the half-life of glibenclamide and its clearance were not changed (Table 3) suggesting a reduced absorption during administration of enoxacine. The pharmacodynamic response, as measured by the blood sugar concentrations, was not different for both antidiabetic drugs given together with enoxacine. The lowest blood sugar level occurred 3 hours or 6 hours after drug administration following glibenclamide or chlorpropamide respectively, despite that the volunteers were allowed to have breakfast 2 hours after drug administration. In conclusion, enoxacine prolonged the elimination of antipyrine in plasma and urine, whereby 4-hydroxyantipyrine and 3-hdroxymethylantipyrine pathways were selectively inhibited. Chlorpropamide and glibenclamide elimination was not influenced by enoxacine. However, the

area under the plasma concentration time curve and the maximal concentration of glibenclamide reached was decreased suggesting an inhibited absorption during enoxacine administration.

TABLE III

	Enoxacine 2 x 400 mg daily		
	Before		During
Clorpropamid			
C_{max}(µg/mL)	136 ± 38		24 ± 3.4
t_{max}(h)	3.5 ± 2.4		4.2 ± 2.3
$t_{1/2}$(h)	38.8 ± 12.4		37.7 ± 18.1
Cl(mL/min)	3 ± 1.3		3.3 ± 1.4
Glibenclamide			
C_{max}(ng/mL)	136 ± 38	*	92 ± 34
AUC(µg/mL.h)	0.35 ± 0.09	**	0.26 ± 0.03
$t_{1/2}$(h)	0.8 ± 1.1		1.1 ± 0.5
Cl(mL/min)	185 ± 60		232 ± 20

*p < 0.05

**p < 0.01

SUMMARY

In conclusion, based on the different studies presented several drugs can obviously induce a selective inhibition of certain isoenzymes of cytochrome P-450. Also new substances coming onto the market, like the new quinolone enoxacine, should be considered for their potency to inhibit the hepatic microsomal enzyme system in a selective manner.

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DISCUSSION - Enzyme inhibition by environmental agents.

P. du Souich

I would like to ask whether you have conducted studies with glibenclamide given intravenously? Your data on the effects of enoxacine could somehow be explained by a change in the volume of distribution.

E.E. Ohnhaus

I agree that an intravenous study would definitely settle that question, but I believe that our data is quite suggestive of a reduced absorption of glibenclamide during enoxacine administration.

D. Mansuy

Have you studied the nature of the interaction of cimetidine and ranitidine with human liver microsomes? and, have you measured inhibition constants of quinolones in preparations of human liver microsomes?

E.E. Ohnhaus

Not yet, but we plan to do this type of studies.

T. Wieland

A point that is seldom considered in studies about inhibition of drug metabolism is that interference in the rate of hepatic uptake of one drug by another could offer an additional explanation of some effects.

M.M. Reidenberg

It would be of interest to know if either in your studies of cimetidine as an inhibitor or in some of those that you quoted there was data on actual serum cimetidine concentration in the subjects. There are apparently rather large differences in response for small differences in dose, and it is known that there is a large interindividual variation in cimetidine levels in subjects treated with the same dose. (Drayer DE, Romankiewicz J, Lorenzo B, Reidenberg MM, Clin Pharmacol Ther 31 : 45, 1982).

E.E. Ohnhaus

No, in most of the studies, no cimetidine concentrations were measured, but this is an important point, and I think that differences in blood levels may explain some of the discrepancies in the studies with ranitidine.

O. Pelkonen

Do you have data on the variation in the high affinity interaction of cimetidine with human liver microsomes?

E.E. Ohnhaus

Yes. We detected a three to fourfold variation among different subjects.

G.J. Mulder

Can you comment on the inhibitory effect of chloramphenicol? This drug is mainly conjugated in vivo and I wonder what kind of interaction with cytochrome P 450 is established.

E.E. Ohnhaus

Animal experiments have shown that it is bound to cytochrome P 450, but it is not clear whether in a competitive or non-competitive manner. It is interesting that most drugs that act as inhibitors are not highly metabolized.

A.L. de Weck

Does anyone know whether an indirect mechanism, for instance through H_2 receptors on the cell membrane, is involved in the inhibitory effect of cimetidine on drug metabolism?

O. Pelkonen

It does not seem to be so, but what is of interest is that there is some sort of P450 isozyme specificity in the inhibitory effect of cimetidine. I have also a question for Dr. Ohnhaus, do you think that cimetidine affects the enzyme responsible for debrisoquin hydroxylation?

E.E. Ohnhaus

We have not studied that, but we have shown that antipyrine,

phenobarbital and rifampicin can induce spartein metabolism, in extensive but not in poor metabolizers. If we turn that around, it is possible to envisage that some inhibitors of drug metabolism can also affect the isozyme involved.

G.L. Plaa

Is there any evidence to indicate that cimetidine behaves in a manner similar to SKF 525 A, i.e. that either it or its metabolites may act as inducers and that the net effect is inhibition of drug metabolism when given acutely and induction when administered repetitively?

O. Pelkonen

I have data that may answer this question. A number of years ago we studied the effect of rather high doses of cimetidine given for one week to the rat and we detected either no change or a decrease in some of the pathways of drug metabolism evaluated. (Pelkonen & Puurunen, Biochem Pharmacol 29 : 3075-3080, 1980).

J.M. Baeyens

The imidazole structure in cimetidine has been implicated in the inhibition of drug metabolism by this drug. Can you mention other drugs that contain an imidazole group and that are enzyme inhibitors?

E.E. Ohnhaus

Ketoconazole is one of them. On the other hand, famotidine, another H_2-receptor antagonist not having the imidazole structure is definitely much less inhibitor than cimetidine.

S. Erill

Let me add that imidazole itself is an inhibitor of drug metabolism and that it acts in a manner similar to that of SKF 525 A, in the sense that repeated doses lead to enzyme induction.

D. Mansuy

We have data on the effects of miconazole in vivo in rats and while this drug is a good inhibitor of cytochrome P 450

monooxigenases it can also behave as an enzyme inducer.

L.F. Prescott
Is it reasonable to assume that with agents showing this dual effect the inhibition and the induction involve the same isozyme?

D. Mansuy
We have data, and there are data in the literature indicating that when the inhibition is specific, the induction generally involves the same isozyme. When the mode of inhibition is not specific, the situation is far more complex. This is the case of imidazole derivatives, in which the imidazole binds to iron enzymes.