Enzyme Inhibition and Induction - PK/PD Impact

Ulrich Klotz\textsuperscript{a} and Kari T. Kivisto\textsuperscript{b}

\textsuperscript{a}Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany
\textsuperscript{b}Department of Clinical Pharmacology, University of Helsinki, Finland

ABSTRACT

Disposition and action of many drugs depend on their (extra-) hepatic metabolism which is accomplished by several cytochrome P450 (CYP) enzymes and/or conjugating activities. The CYP3a subfamily is the most abundant in the liver and in the small intestine. At these metabolic sites high clearance drugs sustain presystemic elimination. Ingredients of grapefruit juice have no influence on the systemic clearance of various drugs whereas intestinal drug metabolism may be decreased and consequently oral bioavailability increases. Midazolam represents a probe of CYP3A4. Its oral availability is higher during treatment with fluconazole, itraconazole, ketoconazole, diltiazem, verapamil, erythromycin or grapefruit juice ingestion. On the other side midazolam metabolism is induced by rifampicin. Thus, switching from inhibition to induction causes an about 400 fold change in the AUC of oral midazolam which has a significant impact on the sedative action of this benzodiazepine. Using verapamil as a model drug it was shown that rifampicin will induce, not only hepatic but also intestinal drug metabolism. During oral coadministration of both drugs, steady state concentrations of verapamil decreased more than 30 fold and the response was nearly abolished. These examples show that inhibition or induction of drug metabolizing enzymes will have an impact on PK and both contribute to variability in drug response.

Key words: inhibition, induction, drug metabolism, pharmacokinetics, pharmacodynamics

Correspondence: Prof. Dr. Ulrich Klotz, Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstrasse 112, D-70376 Stuttgart, Germany, Tel: +49-711-81-01-37-02, Fax: +49-711-85-92-95, Email: ulrich.klotz@ikp-stuttgart.de

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INTRODUCTION

Most drugs undergo biotransformation before excretion by renal, biliary or other routes. The liver, which contains a number of drug metabolising systems, is the major site of metabolism. Among those enzymes the cytochrome (CYP) P450 superfamily enzymes represent the most important class [1,2]. More recently it has been revealed that these enzymes are also present at extrahepatic sites, especially in the small intestine [3].

During the past numerous in vitro and in vivo studies have been performed indicating that the activity of the various CYP can be modified, i.e. drugs, environmental factors or food constituents can inhibit or induce these enzymes. Impairment or acceleration of drug metabolism will lead to pharmacokinetic (PK) changes which may have also pharmacodynamic (PD) consequences [4,5].

This article will concentrate on in vivo studies in man where interactions between different compounds have been investigated. In these interaction studies often probe drugs have been used to characterize indirectly the in vitro activity of certain CYP [6,7]. The pharmacokinetic modifications due to inhibiting or inducing agents can be best visualized by changes in the plasma level-time profiles (AUC measurements) or by the calculation of systemic (CL) and oral (CL\textsubscript{0}) clearance of the affected drugs [8].

We will present some recent examples from literature and our own work to illustrate the clinical impact of the observed PK- and/or PD-changes, rather than provide a comprehensive overview of the involved problems.

GRAPEFRUIT JUICE AS INHIBITOR

The inhibitory potential of grapefruit juice was serendipitously discovered during an interaction study with ethanol (EtOH) and Ca-channel blockers to mask the typical flavor of EtOH [9]. Since then, with an increasing number of drugs, such as felodipine, nifedipine, nitrendipine, nisoldipine, terfenadine, cyclosporine and midazolam, elevated plasma concentrations following oral administration have been observed when grapefruit juice was concomitantly ingested. Most studies focused on drugs that are CYP3A substrates, since this subfamily is particularly inhibited, especially in the intestinal epithelium. Consequently, the affected drugs showed a substantial increase in their relative oral bioavailability (F) ranging from a mean value of about 40% to over 250% [10, 11]. In the case of terfenadine [12,13] and midazolam [14] these PK-changes were associated with more pronounced PD-effects. Recently, it was suggested that 6',7'-dihydroxybergamottin is the putative inhibiting agent in the grapefruit juice [15]. The unpredictability of the magnitude of the individual increase in F would suggest to reduce the oral dosage of the affected CYP3A-substrates.

EtOH AS INDUCER

EtOH is consumed worldwide in tremendous amounts and it is an effective inducer of hepatic drug metabolism, especially involving pathways mediated by CYP2E1. Therefore individuals consuming chronically EtOH have an accelerated metabolism of CYP2E1
substrates, such as chlorzoxazone (CZX), paracetamol, halothane, enfurane, methoxyflurane, sevoflurane and many organic solvents [16]. The metabolic ratio of CZX, a probe of CYP2E1, increased significantly from 0.43 in non-induced subjects to 1.2 in EtOH-induced patients and it normalized within 1 week after withdrawal of EtOH (17).

The hepatotoxic potential of paracetamol resides in its reactive metabolite N-acetyl-p-benzo-chinonimine which is formed by CYP2E1 [18]. In two recent reviews, 67 and 94 cases of paracetamol-induced hepatotoxicity associated with regular intake of EtOH were presented indicating the risk of combined use of both agents [19,20]. Likewise, the hepatotoxic and nephrotoxic potential of fluorinated volatile anaesthetics depends on the CYP2E1-mediated formation of reactive metabolites and inorganic fluoride. Therefore it is conceivable that induction of CYP2E1 by EtOH may increase the toxicity of these drugs [16].

INHIBITION OF DRUG METABOLISM BY CIMETIDINE

Due to its imidazole ring structure cimetidine binds to the heme part of several CYP [21]. Therefore it is not surprising that this H₂-receptor antagonist inhibits the metabolism of many drugs (Table 1) which are substrates of different CYP. CL of these substances is decreased on average by 20 to 50% by cimetidine [22,23]. With some drugs (e.g. diazepam, theophylline, carbamazepine, phenytoin, lidocaine, warfarin), the increased plasma concentrations were associated with a more pronounced PD response [22-24]. Therefore it appears prudent to reduce the dose of these drugs or to use ranitidine which does not inhibit drug metabolism.

Table 1.
Drugs, whose hepatic elimination is impaired by cimetidine

<table>
<thead>
<tr>
<th>Acenocoumarol</th>
<th>Cyclosporine</th>
<th>5-Fluorouracil</th>
<th>Moricizine</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alprazolam</td>
<td>Dapsone</td>
<td>Flurazepam</td>
<td>Nicardipine</td>
<td>Quinidine</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Desipramine</td>
<td>Flurbiprofen</td>
<td>Nicotin</td>
<td>Quinine</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Desmethylbupivacaine</td>
<td>Glibenclamide</td>
<td>Nifedipine</td>
<td>Sparteine</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>Diazepam</td>
<td>Glipizide</td>
<td>Nimodipine</td>
<td>Sulindac</td>
</tr>
<tr>
<td>Bromazepam</td>
<td>Diltiazem</td>
<td>Imlorazepam</td>
<td>Nitrazepam</td>
<td>Tacrine</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>Dibenzyl</td>
<td>Indomethacin</td>
<td>Nitrendipine</td>
<td>Theobromine</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Dextromethorphan</td>
<td>Lidocaine</td>
<td>Nortriptyline</td>
<td>Theophylline</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Dextroamphetamine</td>
<td>Mebendazole</td>
<td>Paroxetine</td>
<td>Tolbutamide</td>
</tr>
<tr>
<td>Carbocystine</td>
<td>Encaïne</td>
<td>Metformine</td>
<td>Pentoxifylline</td>
<td>Triamteren</td>
</tr>
<tr>
<td>Chlor Diazepoxide</td>
<td>Enoxacin</td>
<td>Methadone</td>
<td>Pethidine</td>
<td>Triazolam</td>
</tr>
<tr>
<td>Chloromethiazole</td>
<td>Estradiol</td>
<td>Metoprolol</td>
<td>Phenytoin</td>
<td>Valproic acid</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Ethanol</td>
<td>Metronidazole</td>
<td>Pindolol</td>
<td>Verapamil</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Felodipine</td>
<td>Midazolam</td>
<td>Piroxicam</td>
<td>Warfarin</td>
</tr>
<tr>
<td>Clobazam</td>
<td>Flecainide</td>
<td></td>
<td>Prednisone</td>
<td></td>
</tr>
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</table>
RIFAMPICIN AND VERAPAMIL

Drug metabolism in the liver and gut wall can be induced by rifampicin and especially substrates of CYP3A4 (e.g. cyclosporine, midazolam) are cleared much faster during coadministration of rifampicin and consequently F of these drugs is dramatically reduced [25,26].

In other studies verapamil was chosen as a model drug, because several CYP (CYP3A4, CYP1A2, CYP2C) are involved in its metabolism and stereoselective aspects can be investigated with this racemic drug. In young [27] and elderly [28] subjects it was found that 12 days’ co-treatment with rifampicin (600 mg once daily) and verapamil (120 mg twice daily) resulted in a more than 30-fold increase (p < 0.001) in the oral CL of the cardioactive S-verapamil, whereas systemic CL following an iv-dose of 10 mg was increased only about 1.3-fold. F of both enantiomers was dropped almost to zero and consequently the effect of oral verapamil on atrioventricular conduction was nearly abolished (p < 0.01). Induction by rifampicin caused a considerable reduction of stereoselectivity after both iv- and po-administration. As rifampicin altered the PK and PD of verapamil in both populations to a much greater extent after po-dosing compared with iv-administration, it was concluded that - independent of the age - prehepatic (gut wall) metabolism of verapamil was preferentially induced by rifampicin [27,28].

INHIBITION AND INDUCTION OF THE METABOLISM OF MIDAZOLAM

Midazolam represents a well-established probe for CYP3A4 [6,7] and its PK/PD-properties and relationships have been described thoroughly [29]. There is a marked interindividual variation in both CYP3A4 activity in the liver and pharmacokinetics of CYP3A4 substrates. Furthermore, CYP3A4 is readily inhibited or induced by a number of drugs, resulting in many clinically significant drug interactions. The erythromycin breath test is currently the best validated measure of CYP3A4 activity in the liver in vivo. The results of this test have been shown to correlate with the clearance of intravenously administered midazolam [30], and the clearance of intravenous midazolam seems to provide a good estimate of the activity of CYP3A4 in the liver. It should be noted that assays of CYP3A4 activity in the liver are not suitable for accurate assessment of intestinal CYP3A4 activity. This is an important issue because many CYP3A4 substrates undergo significant first-pass metabolism not only in the liver but also in the gut. The first-pass metabolism of CYP3A4 substrates, i.e. the combined CYP3A4 activity in the liver and gut, can, however, be predicted by administering the probe orally.

After oral administration, midazolam undergoes extensive first-pass metabolism in the intestinal wall and the liver and, therefore, its concentrations in plasma are markedly altered by inhibition and induction of CYP3A4. The effects of various drugs on the AUC of oral midazolam are illustrated in Figure 1 [31-36]. In these studies, a single dose of midazolam was given orally after a 2- to 6-day pretreatment with the inhibitor. Most of these interactions are clinically significant, that is, the sedative effect of midazolam is greater and lasts longer during co-administration of a potent CYP3A4 inhibitor. This holds also true for triazolam, the pharmacokinetics of which are also very susceptible to inhibition (and induction) of CYP3A4.
Plasma concentrations of numerous drugs are reduced to a clinically significant extent by co-administration of rifampicin. In a recent study, in which a single dose (15 mg) of midazolam was given orally after a 5-day pretreatment with rifampicin (600 mg once daily) or placebo, rifampicin decreased the AUC of midazolam by 96% compared with placebo [37]. As a result, the pharmacodynamic effects of midazolam, measured by several psychomotor tests, were practically abolished. It is therefore reasonable to assume that orally administered midazolam (and triazolam) are ineffective as hypnotic agents during concomitant treatment with rifampicin and other potent CYP3A4 inducers such as phenytoin and carbamazepine.

**Figure 1:** The effect of various drugs on the AUC of oral midazolam compared with placebo. Keto, ketoconazole; Itra, itraconazole; Eryt, erythromycin; Dilt, diltiazem; Clar, clarithromycin; Vera, verapamil; Roxi, roxithromycin; Azit, azithromycin; Plac, placebo. N.S., not significantly different from placebo. Data from references [31-36].

To summarise, midazolam is a sensitive probe of CYP3A4 activity in vivo, since its pharmacokinetics are very susceptible to inhibition and induction of CYP3A4. By using orally administered midazolam (or triazolam) as a probe, the potential of drugs to affect CYP3A4 activity in vivo can be assessed. For example, the pharmacokinetics of midazolam were unaffected by azithromycin [35], strongly suggesting that azithromycin does not cause clinically significant interactions with CYP3A4 substrates.

**PROTON PUMP INHIBITORS (PPI) AS INHIBITORS OR INDUCERS**

Omeprazole can induce (CYP1A subfamily) and inhibit (CYP2C19) drug metabolism [38,39]. It is controversial whether this is of clinical significance and whether other PPI, such as pantoprazole or lansoprazole share the same interaction potential [22,40]. Therefore
we recently tested in a randomized, placebo-controlled, 4-way cross-over study in 20 healthy, non-smoking and drug-free volunteers (extensive metabolizers of CYP2C19, *H. pylori* negative) whether 10 days' treatment with omeprazole (40mg/day), pantoprazole (80mg/day) or lansoprazole (60mg/day) affected the steady state disposition of theophylline (350 mg bid given as a slow release preparation), a common probe of CYP1A2. During all 4 parts of the study plasma concentration time profiles following the last theophylline dose were nearly identical and the 90% C.I. for the ratios for bioequivalence criteria (e.g. AUC; peak; mean and trough C_{ss}; % PTF) fluctuated around unity. Therefore it appears very unlikely that the 3 PPI, tested at a sufficiently high dosage, have a significant effect on the CYP1A2 activity [41].

**CONCLUSIONS**

According to the examples given drug metabolizing enzymes can be more or less specifically inhibited or induced by a variety of compounds ingested in form of food constituents or drugs (Table 2). The affected (pre-)systemic elimination will influence the disposition (PK) of many therapeutic agents and consequently their action (PD) can be affected as well. The interactions may necessitate dosage modifications, and sometimes selection of alternative drugs with a lower potential for drug interactions should be considered. In summary, enzyme inhibition and induction can significantly contribute to variability in human drug response.

**Table 2.**

Typical inhibitors and inducers of some CYP-enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitory reference compounds</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>α-naphtoflavone</td>
<td>methylcholanthrene</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>furafylline, fluvoxamine</td>
<td>smoking, omeprazole</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>methoxsalen, pilocarpine</td>
<td>phenobarbital</td>
</tr>
<tr>
<td>CYP2C8/9/18</td>
<td>sulfaphenazole</td>
<td>phenobarbital, rifampicin</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>fluconazole, omeprazole</td>
<td>phenobarbital, rifampicin</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>quinidine</td>
<td>not inducible</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>diethylidithiocarbamate</td>
<td>ethanol, INH</td>
</tr>
<tr>
<td>CYP3A(4)</td>
<td>ketoconazole, itraconazole</td>
<td>rifampicin, dexamethasone, anticonvulsants</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT: The valuable secretarial help of Mrs. H. Kühler and Mrs. G. Wilder is highly appreciated.

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Discussion: Enzyme inhibition of polymorphic drug metabolism

E.M. Sellers:
Perhaps we need to give a little bit more attention to how we characterise variations in activity. We tend to use ratios of parent drug to metabolite, which from a psychometric point of view are very unsatisfactory. We should probably be focusing much more on fractional clearances and absolute recoveries by particular pathways, because these give a much more accurate estimate of what is the real activity of the enzyme.

U. Klotz:
We discovered in this interaction study that there were quite dramatic differences by comparing the ratio technique with using area under the curves. But it is much more tedious to measure a whole time profile during the dosing intervals than just a single measurement. For routine use the ratio technique is much more convenient, but it is much more variable.

P. Rolan:
The extent of the variability of the caffeine metabolic ratio is surprising, because that test has been suggested as a screening tool and if its variability is that large, it means that you could fail to find an effect just due to the variability. I wonder whether part of the problem was again selecting the ratio, because there are at least five metabolic caffeine ratios.

U. Klotz:
We used the ratio (MR) which, according also to Geoffrey Tucker and other experts, is independent of the renal excretion [MR = (AFMU+1X+1U)/1,7U]

G.T. Tucker:
The issue of what index of enzyme activity you use is really quite important. From a theoretical point of view certainly I would defend the metabolic ratio over urine metabolite recovery as an index, because the latter depends not just on the pathway you are interested in but on parallel pathways also. Theoretically, the urinary drug to metabolite ratio for a simple system is a function of the partial clearance by the enzyme you are interested in, in the denominator, and in the numerator you have the renal clearance of the parent drug. Thus, renal clearance is the potential source of noise in using these ratios as markers of enzyme activity. The kinetic bases of the indices that we use should be considered more carefully.

N.L. Benowitz:
In the verapamil studies when you look at inhibition, is there a general relationship between hepatic CYP3A4 activity and intestinal activity, or are these regulated separately?

U. Klotz:
For a long time it was thought that CYP3A4 in the intestinal wall and in the liver were using the same substrates. The same inhibitors and the same inducers could affect both sites, and there might be some kind of co-regulation. But more recently, some evidence has appeared showing that both enzymes are probably regulated in a slightly different way.
P. du Souich:  
I would like to reiterate again that it is very difficult to interpret the effect of a drug on theophylline kinetics, because at least four enzymes are contributing to its clearance. When you modify one of these enzymes you do not know exactly how the alternative pathways will respond. We do not know if they will remain exactly the same, and follow predictable first-order kinetics or not. Another practical point I would like to make from the examples you have shown, should we think about developing drugs for alternative routes of administration, for example, sublingual, transcutaneous or even inhaled drugs, to try to avoid these effects of intestinal and liver variability?

U. Klotz:  
This could be one alternative to get round intestinal metabolism, although comparative studies with different routes of administration are required.

A.J.J. Wood:  
We need to be careful just extrapolating from studies done with one route to another route and assuming that the effects will be exactly the same. I suspect that the effects are not identical.

J. Urquhart:  
If you are going to use other routes, you need more potent drugs and there are not very many of them.

U. Klotz:  
Bioavailability then becomes an issue, because to develop a new formulation you have to complete new studies in terms of efficacy and toxicity. Then, it is probably much easier to perform 2 or 3 interaction studies, instead.