ELSEVIER

Journal of Chromatography B, 678 (1996) 93–103

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Review

Prediction of CYP2D6-mediated polymorphic drug metabolism (sparteine type) based on in vitro investigations

Georg Engel*, Ute Hofmann, Heyo K. Kroemer

Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstraße 112, D-70376 Stuttgart, Germany

Abstract

Discovery of genetic polymorphism in drug metabolism has contributed a great deal to understanding the variability in dose–concentration relationships introduced by genetic factors, thereby elucidating the mechanisms responsible for unexpected drug reactions. This knowledge should find its way into clinical practice in order to make therapy more efficient and safe. Moreover, genetic factors in drug metabolism should be taken into account during drug development. Therefore, in vitro methods for identifying the metabolic pattern of new compounds during early stages of drug development should be improved. This review summarizes in vitro methods available to identify genetic polymorphism in drug oxidation, in particular the CYP2D6-related polymorphism.

Keywords: Reviews; Drug oxidation; CYP2D6; Cytochrome P450; Sparteine; Dextromethorphan; Dextrophan; Bufuralol; Debrisoquine

Contents

* Corresponding author.

0378-4347/96/$15.00
SSDI 0378-4347(95)00234-0

List of abbreviations

CL_{int}	Intrinsic clearance
CYP	Cytochrome P-450
EM	Extensive metabolizer
K_m	Michaelis constant
LKM	Liver kidney microsome
PM	Poor metabolizer
V_{max}	Maximum velocity of metabolite formation

1. Introduction

Variability in drug response is often caused by interindividual differences in drug metabolism. One major reason for variable drug metabolism in humans is polymorphic expression of drug metabolizing enzymes such as N-acetyltransferases, pseudocholinesterases and cytochrome P-450 enzymes [1]. The latter enzymes are encoded by a supergene family and play an important role in phase I metabolism. The nomenclature used to designate a specific cytochrome P-450 (e.g. CYP2D6) has been reviewed by Nelson et al. [2]. A three-digit term is assigned to each enzyme in which the enzyme family, the subfamily and the particular member of the subfamily are represented by the first, second and third digit, respectively. So far, a genetic polymorphism has been described for two enzymes of the P-450 family in man, namely CYP2C19 [3] and CYP2D6 [4,5]. Polymorphism of the latter enzyme has been subject to intense research efforts both on the clinical and molecular level.

Today it is known that 5–10% of a Caucasian population lack functional CYP2D6. As a consequence metabolism of about 30 drugs, among them cardiovascular agents, antidepressants and neuroleptics [6,7] is grossly reduced in patients. This subpopulation is designated as poor metabolizer (PM) in contrast to the remainder of the population, who are termed extensive metabolizers (EM).

The metabolic capacity of an individual can be characterized by the so-called urinary metabolic ratio (MR), which is the amount of a drug excreted in a defined time period divided by the amount of metabolites in urine [e.g. amount(sparteine)/ amount(2,3-didehydrosparteine + 5,6-didehydrosparteine)] or, in the case of debrisoquine (percentage of a dose excreted as debrisoquine)/(percentage of the dose excreted as 4-hydroxydebrisoquine). A high metabolic ratio indicates a poor metabolizer patient. Impaired CYP2D6-mediated metabolism in poor metabolizers may require dosage reduction once compounds are mainly metabolized by CYP2D6. If such individualization is neglected and standard doses are administered to PMs, an increased incidence of side effects is observed. For example, propafenone more often causes side effects in PMs compared to EMs [8] and neuropathy induced by perhexiline is more frequent in PMs than in EMs [9], accordingly debrisoquine polymorphism was detected by the drug-induced hypotension in PMs [5]. In summary, involvement of CYP2D6 in metabolism of a given compound may lead to large interindividual variability in the dose–concentration and hence dose–effect relationship. Thus knowledge of whether CYP2D6 is involved in the metabolism of a drug is a pivotal question, in particular when compounds with narrow therapeutic indices or new drugs are concerned. Therefore, it is important to know whether a drug is metabolized by CYP2D6 or not. A new drug in development should be investigated with regard to polymorphism in metabolizing enzymes.

The involvement of CYP2D6 in drug metabolism can be investigated using in vivo and in vitro approaches. In vivo a drug can be administered to poor metabolizers and extensive metabolizers of sparteine/debrisoquine in the so-called panel

approach and the disposition and/or excretion of metabolites in these two groups can be compared. Use of suitable in vitro systems for CYP2D6-mediated metabolism may replace the in vivo approach. This is of particular advantage during early stages of drug development since involvement of CYP2D6 in the metabolism of a new compound can be detected before its first administration to man. Here we review the in vitro approaches used to elucidate the involvement of CYP2D6 in drug metabolism.

2. Enzyme kinetics

Biotransformation of drugs by enzymes can be described by means of enzyme kinetics. Enzyme kinetics are characterized by the Michaelis–Menten equation, were V_{max} is the maximum rate of metabolite formation from a substrate (S) and K_m is the Michaelis constant:

$$V = \text{rate of metabolism} = \frac{V_{max} \cdot [S]}{K_{max} + [S]} \quad (1)$$

The clearance of a drug eliminated by metabolism from a physiological liquid can be described by the rate of metabolism over the mean substrate concentration during a time interval.

$$CL = \text{clearance} = \frac{\text{rate of metabolism}}{[S]} \quad (2)$$

Substitution of Eq. 1 into Eq. 2 results in Eq. 3 which describes the clearance of a drug by metabolizing enzymes by means of enzyme kinetics.

$$CL = \frac{V_{max}}{K_m + [S]} \quad (3)$$

In the case of substrate concentration being lower than K_m ($[S] << K_m$), the efficacy of an enzyme for one metabolic step can be characterized by the intrinsic clearance (ml/min) of the respective reaction:

$$CL_{int} = \frac{V_{max}}{K_m} \quad (4)$$

Since the scenario of $[S] << K_m$ applies to many therapeutically used compounds, CL_{int} is often used to describe clearance by biotransformation in vivo. CL_{int} is of value since the relative contribution of one individual pathway to total metabolism can be assessed by this parameter.

3. Systems to study drug metabolism in vitro

At least from a quantitative point of view liver is the most important organ involved in drug metabolism in humans. Thus in vitro investigations are commonly utilizing human hepatic tissue. The most important advantage of using human material is direct applicability without necessity of adjustment for interspecies differences. Human liver tissue, however, is difficult to obtain, and ethical considerations apply. The most common source of human liver tissue is surgical waste, obtained by partial hepatectomy, wedge biopsy and organ transplantation. However, since availability of human liver in general is limited, many investigations have been done with liver tissue from animals, especially from rats.

Different tools are used to investigate drug metabolism in liver tissue. Liver slices and hepatocytes provide the whole spectrum of enzymes responsible for hepatic metabolism, including phase I and phase II enzymes. Use of these systems is limited since both liver slices and hepatocytes have to be prepared from fresh human liver, and long-term storage remains to be a problem [10].

In contrast, liver tissue can be deep-frozen immediately after surgery and stored at −80°C. Such samples can be used to prepare cell homogenates or subcellular fractions of human liver by differential centrifugation. Centrifugation at 9000 *g* provides a supernatant which contains the cytoplasm and the endoplasmic reticulum. Liver microsomes are obtained subsequently by centrifugation at 100 000 *g* and contain the membranes of endoplasmic reticulum [11]. The advantage of this system is that liver tissue can be stored for a long time at −80°C and microsomal fractions are readily prepared once needed.

The microsomal fraction contains the various P-450 enzymes in their natural abundance. For some problems in metabolism it is mandatory, however, to use single enzymes (e.g. once assessing involvement of one enzyme in certain metabolic pathways). Such single P-450 enzymes can be obtained by biochemical procedures or more popular using genetically engineered cells [12].

Advantages and disadvantages of the different in vitro systems available are discussed in the following paragraphs.

3.1. Liver slices

Precision-cut liver slices have been used to investigate drug metabolism in vitro utilizing human and animal tissue. Liver slices provide all cell types representative for liver tissue (hepatocytes, Kupffer cells, endothelial cells) and tissues are still in their in situ topology. This system should therefore reflect enzymatic activity of the whole organ. However, only few papers have been published based on investigations done with slices from human liver and the system is therefore not thoroughly validated [13–15]. No publications estimating CYP2D6 activity in human liver slices have been reported so far.

3.2. Hepatocytes

Hepatocytes are obtained by perfusion of fresh liver with collagenase and are subsequently cultured in appropriate media. Co-culture of hepatocytes with collagen and fibroblasts allows long-term culture. Cryopreservation of human hepatocytes has been used, but after thawing only a part of the cells survive and retain their functional capacity [16,17]. A problem in long-term culture is a shift in the relative P-450 content in hepatocytes compared to native liver which hampers successful predictions [18–20].

Human hepatocytes have not been systematically used to evaluate CYP2D6 function in vitro. A study of Maurice et al. [21], which was primarily targeted to the evaluation of imidazole derivatives and rifampicin on hepatocyte function, also monitored debrisoquine 4-hydroxylase. As expected from previous in vivo experiments [22] CYP2D6 was not inducible by rifampicin, whereas CYP3A4 which was monitored by erythromycin demethylase and cyclosporin oxidase, was induced by clotrimazol and inhibited by ketoconazole.

3.3. Human liver microsomes

Michaelis–Menten kinetics for a number of substrates have been determined in human liver microsomes obtained from EMs and compared with data from PMs. Microsomes obtained from PM livers show lower turn-over numbers for substrates cosegregating with sparteine/debrisoquine than microsomes from EMs. 1′-Hydroxylation of (+)-bufuralol [23] shows a decrease in V_{max} and an increase in K_m in liver microsomes from PMs compared to EMs. A similar observation was made for the O-demethylation of codeine [24].

3.4. Rat liver microsomes

The value of Dark Agouti rats as a model for sparteine/debrisoquine polymorphism has been thoroughly investigated. Female Dark Agouti rats lack CYP2D1 which is the rat cytochrome analogous to human CYP2D6. It was found that debrisoquine hydroxylation was reduced in female Dark Agouti rats compared to Lewis rats [25] or Fischer rats [26]; however, phenacetin O-deethylation was also reduced in Dark Agouti rats, indicating that enzymes other than CYP2D6 may be impaired in these rats as well [25]. Reduced biotransformation in female Dark Agouti rats was shown for (+)-bufuralol 1′-hydroxylation [27,28], sparteine oxidation [29], dextromethorphan O-demethylation [30,31] and codeine O-demethylation [32].

3.5. Stable expressed enzymes

Genetic engineering enables the expression of cDNA encoding for proteins in suitable cell systems such as yeast, COS cells (cells from a monkey kidney cell line named CV1, treated with a mutant of the virus SV40; CV1 origin SV40) or human lymphoblastoid cells, and there-

fore allows the investigation of single P-450s. A variety of investigations describes catalytic characteristics of stably expressed CYP2D6.

The mutagenic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone was investigated in human lymphoblastoid cells stably expressing CYP2D6 (2D6/Hol) or cell lines expressing CYP1A2, CYP2E1 and CYP2A3 [33]. Using a transcriptional cassette system containing two CYP2D6 cDNAs, higher CYP2D6 levels have been expressed in human lymphoblast cells (h2D6v2) [34]. (+)-Bufuralol 1-hydroxylase activity in microsomes from these cells was higher than in microsomes prepared from EM-liver.

Biotransformation of the antipsychotic drug clozapine was investigated in microsomes prepared from V79 chinese hamster lung fibroblasts stably expressing CYP2D6 [35]. In this system the main metabolites of clozapine, i.e. N-demethylclozapine and clozapine-N-oxide, were not found. In contrast, these two main metabolites were identified in presence of the microsomal fraction of human liver, whereas the genetically engineered cells formed metabolites which were different from N-demethylclozapine and the N-oxide but not definitely identified. The same system was used to elucidate the involvement of CYP2D6 in biotransformation of tropisetron and ondansetron [36].

CYP2D6 expressed in yeast has debrisoquine 4-hydroxylase activity [37] and shows characteristics of the enzyme. This system was used to investigate the importance of CYP2D6 in the biotransformation of methylenedioxymethamphetamine (ecstasy) [38]. It was shown, that this drug is converted to dihydroxymethylamphetamine by CYP2D6.

A cDNA coding for CYP2D1, the rat isoform to CYP2D6, has been expressed in COS cells [39], which are then able to form 1′-hydroxy bufuralol.

4. Experimental approach

All experiments described in the following paragraphs refer to human liver microsomes. In principle a similar approach should be possible for hepatocytes and liver slices but has not been validated yet.

4.1. Analytical requirements

Any analytical method used for in vitro investigations has to meet two requirements. (1) It must be sensitive enough to enable determination of picomolar amounts of metabolites formed in the in vitro systems. (2) Since determination of the enzyme kinetics requires relatively high concentrations of the drug as substrate, such an excess concentration of the parent compound should not interfere with the assay for the metabolites.

Prototype reactions to monitor CYP2D6 activity in vivo and in vitro are debrisoquine 4-hydroxylation [40], bufuralol 1′-hydroxylation [40–42], dextromethorphan O-demethylation [40,43,44] and sparteine oxidation [45] (Fig. 1). For all of these prototype reactions, analytical methods exist which exhibit a sensitivity that allows determination of low amounts of metabolites formed in in vitro experiments.

The metabolites of sparteine, 2,3-dehydrosparteine and 5,6-dehydrosparteine, can be determined after alkaline extraction into dichloromethane by GC with a nitrogen selective detector

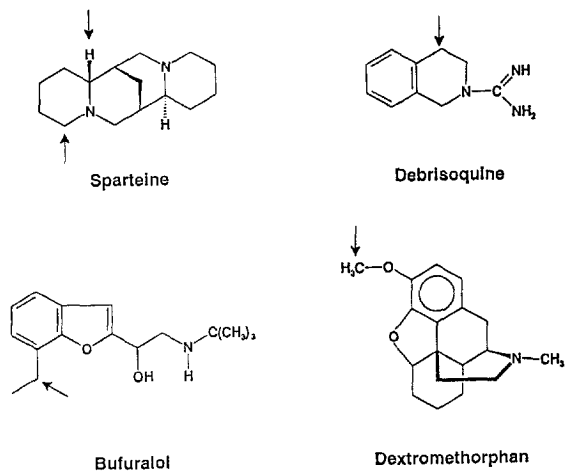

Fig. 1. Prototype substrates used to characterize CYP2D6-dependent polymorphic metabolic reactions. Arrows indicate the sites of oxidation.

[45] (Fig. 2), or with mass spectrometric detection [46]. For the other prototype reactions, i.e. debrisoquine 4-hydroxylation, bufuralol 1′-hydroxylation and dextromethorphan O-demethylation, HPLC methods with fluorescence detection are usually employed. Fluorescence detection is significantly more specific than UV absorbance detection [47] (Fig. 3) and allows the determination of the respective metabolites in the presence of numerous detergents, antibodies or specific inhibitors [48]. All substrates and their respective metabolites are basic compounds and ion suppression reversed-phase HPLC is not applicable, as the required alkaline pH would lead to deterioration of the stationary phase. Therefore, other HPLC techniques are applied. For analysis of dextrorphane, triethylamine was added to the mobile phase [43], for monitoring of bufuralol 1′-hydroxylation, normal-phase HPLC was employed [41]. The most common method, applicable for all three prototype reactions uses inorganic ion-pair reversed-phase HPLC with perchlorate as the counter-ion. The metabolites can be determined directly in incubate mixtures after protein precipitation with perchloric acid [40] (Fig. 4). This procedure shows better sensitivity than extraction procedures described for bufuralol [41,42].

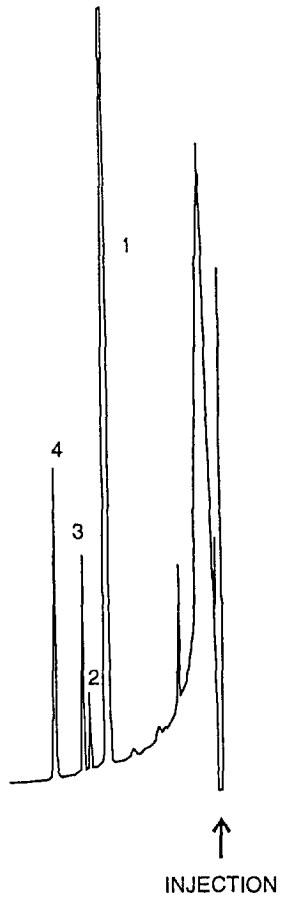

Fig. 2. Separation of sparteine, 2- and 5-dehydrosparteine and 17-ethylsparteine by gas chromatography with nitrogen selective detector. Peaks: 1 = sparteine; 2 = 5-dehydrosparteine; 3 = 2-dehydrosparteine; 4 = 17-ethylsparteine. From Osikowska-Evers and Eichelbaum [45].

4.2. Affinity of a substrate to CYP2D6 and inhibition experiments

Screening drugs for involvement of CYP2D6 in their metabolism can be performed by inhibition experiments. If a drug does not inhibit such a reaction over a wide concentration range it does not bind to CYP2D6 and is hence not metabolized by CYP2D6. In contrast, a drug that inhibits CYP2D6 binds to this enzyme which does, however, not necessarily imply CYP2D6-dependent metabolism of this compound. For example, quinidine is a potent inhibitor of CYP2D6, but is metabolized by cytochrome P-450 enzymes other than CYP2D6 [49,50]. Using such inhibition approach, 25 of 80 alkaloids investigated [51] and a number of anticancer drugs [52] have been shown to inhibit bufuralol-1′-hydroxylase. In another study 64 drugs were investigated with regard to inhibition of sparteine metabolism; 40 of them were found to inhibit sparteine biotransformation [53]. Likewise a number of drugs were found to inhibit sparteine oxidation in the 9000 *g* supernatant of human liver and some of them are now described to be metabolized by CYP2D6 [54].

4.2.1. Inhibition by drugs

Some drugs have turned out to be highly specific inhibitors of CYP2D6. Quinidine is a potent inhibitor of sparteine and debrisoquine biotransformation in vivo [55]. It is a highly

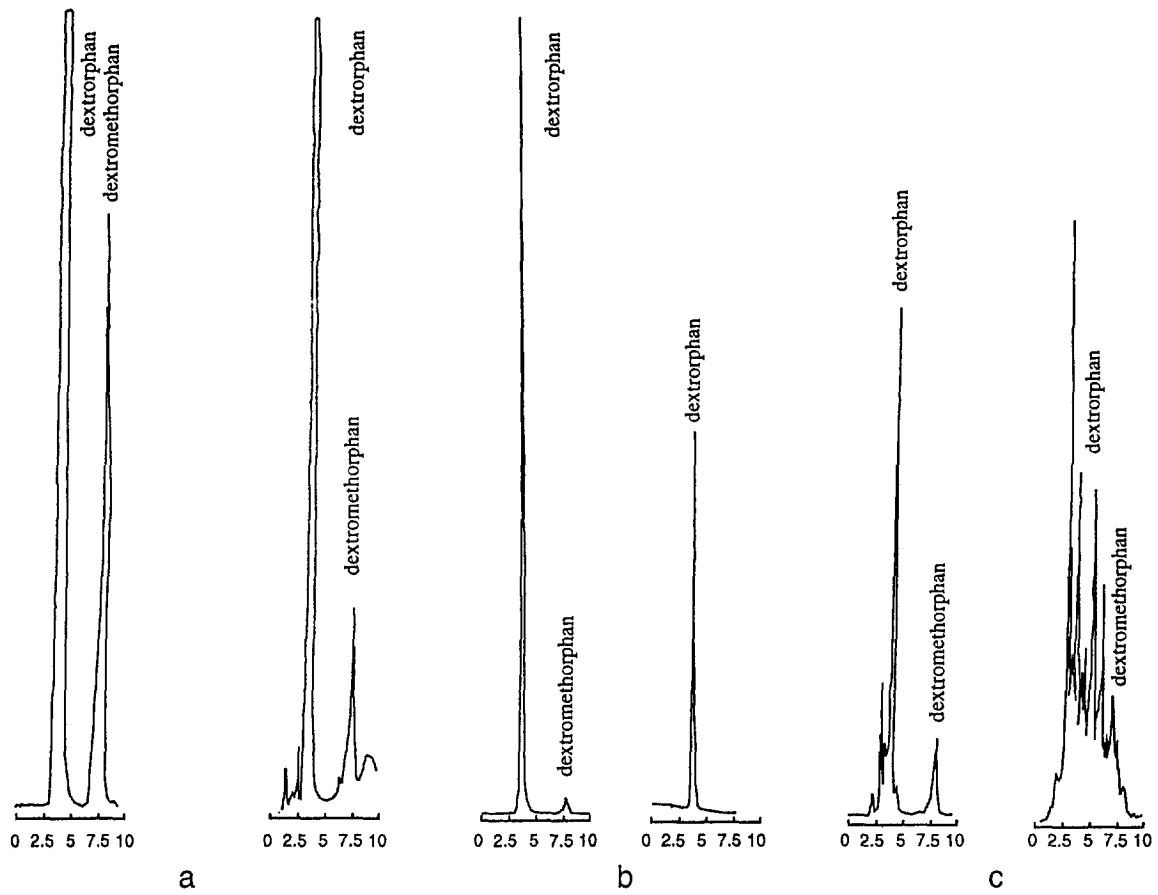

Fig. 3. (a) Chromatogram from drug-free urine spiked with 2.6 μg/ml of dextromethorphan and 25.2 μg/ml of dextrorphan. Fluorescence detection (left), UV absorbance detection (right). (b) Chromatogram from drug-free urine spiked with 0.02 μg/ml of dextromethorphan and 1 μg/ml of dextrorphan. Dextromethorphan could not be detected by UV absorbance detection (right). (c) Chromatogram from a patient from dextromethorphan administration. Fluorescence detection (left) showed less interference UV absorbance detection. From Lam and Rodriguez [47].

selective inhibitor, and thus inhibition of a drug's biotransformation in vitro by quinidine with a K_i in the nanomolar range almost proves the involvement of CYP2D6. A number of inhibition experiments have been done using quinidine [23,54,56,57].

In inhibition experiments, the K_i of the inhibitors used and the Michaelis constant of the drug metabolized have to be taken into account. No inhibition will be observed in vitro if the concentration of the inhibitor is clearly below K_i. Furthermore, the extent of inhibition will be low if the substrate is used in concentrations below K_m. When upscaling is performed from in vitro inhibition experiments to the in vivo situation, the K_i value of the inhibitor, the K_m value of the drug and the concentrations reached in vivo have to be taken into account. If the K_i or K_m are high compared to the concentrations reached in vivo, the inhibition will be negligible and there will be probably no clinically significant interaction.

Concerning inhibitors of drug metabolism in rats, it has to be taken into account that quinidine and quinine exhibit different inhibitory potencies in male Wistar rats compared to humans. In humans quinidine is the more potent

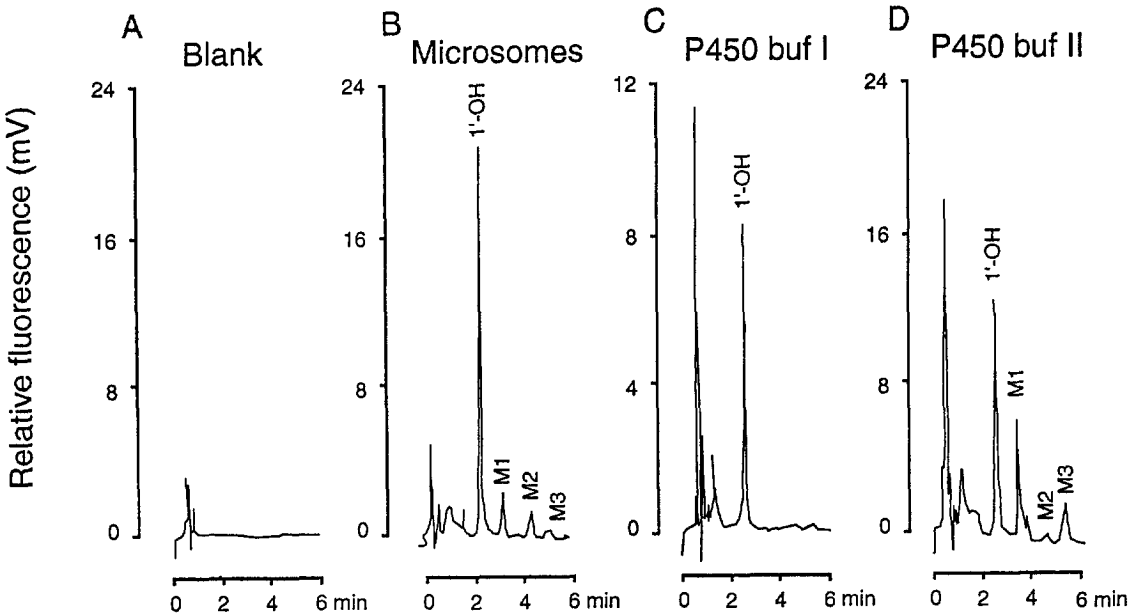

Fig. 4. Chromatograms of (+)-bufuralol metabolites. (A) Microsomal incubation without substrate. (B) Microsomal incubation with (+)-bufuralol. 1'-OH = 1'-OH-bufuralol; M1, M2, M3 = further unidentified metabolites. (C) Metabolite formed by reconstituted cytochrome P-450$_{buf\ I}$. (D) Metabolites formed by reconstituted cytochrome P-450$_{buf\ II}$. From Kronbach et al. [40].

inhibitor of CYP2D6 compared with quinine (K_i 0.6 μM vs. 13 μM), whereas in rats quinidine has a lower affinity for CYP2D6 (K_i 50 μM vs. 1.7 μM) [58].

4.2.2. Inhibition by antibodies

Antibodies directed against an enzyme can be used to inhibit the biotransformation of a drug metabolized by this enzyme. Inhibition by a well characterized antibody then indicates the involvement of the respective enzyme in a drug's metabolism. As an example of this approach, LKM 1 antibodies (liver kidney microsomes) bind to CYP2D6 and inhibit this enzyme [59–61]. These antibodies have been used to inhibit in vitro the metabolism of propafenone [62], mexiletine [63], imipramine [64] and dextromethorphan [65].

Antibodies raised against purified rat debrisoquine 4-hydroxylase inhibited in human liver microsomes in vitro the oxidation of debrisoquine, sparteine, encainide and propranolol [66,67], drugs which are known substrates of CYP2D6. They did not inhibit reactions known to be catalyzed by cytochrome P-450 enzymes different from CYP2D6 [68].

4.3. Contribution of CYP2D6 to the biotransformation of a drug

A drug may be metabolized by a variety of CYPs, among them CYP2D6. The fraction of the total clearance in vivo, which is metabolized by CYP2D6 decides, whether polymorphism affects disposition and hence the dosage of a drug has to be changed depending on phenotype. The contribution of one enzyme to the overall clearance in vivo corresponds to the relative intrinsic clearance of this reaction in vitro.

The intrinsic clearance in vitro can be correlated with the metabolic ratio of a drug in vivo. For example, the intrinsic clearance of CYP2D6-mediated 2,3-didehydrosparteine formation determined in human liver microsomes showed a high negative correlation with the MR of sparteine in the same patients [69]. Therefore CL_{int} is highly predictive for the in vivo metabolic clearance.

If one enzyme catalyzes a particular metabolic step, its contribution to total biotransformation can be determined from Michaelis–Menten kinetics of the corresponding metabolite. If more than one enzyme is involved, some important points have to be considered.

The contribution of a high-affinity low-capacity component in the presence of a low-affinity high-capacity component to clearance in vivo may be underestimated in in vitro investigations. The maximum velocity of metabolite formation by the high-affinity component is low, and the amount of metabolites formed in vitro by the high-affinity component is small and hence may not be detected in in vitro investigations. Nevertheless, the intrinsic clearance of the high-affinity component could be high and the enzyme hence plays a substantial role in a drug's metabolism. For example, bufuralol is hydroxylated in humans by two related enzymes with different Michaelis constants. The high-affinity component shows stereoselectivity for the biotransformation of the (+)-enantiomer with a (−)/(+) ratio of 0.15. The K_m is 31 μM and 54 μM for the (−) and the (+)-enantiomer, respectively. The high-affinity component is inhibited by quinidine with a K_i of 0.08 μM. The low-affinity component is not stereoselective [(−)/(+) ratio = 1.03] and shows a K_m for (−)- and (+)-bufuralol of 314 μM and 245 μM, respectively. The K_i for the inhibition of this component by quinidine is 80 μM [48,70]. Early studies identified only one enzyme [71] and hence failed to phenotype liver samples in vitro [41].

In order to identify the contribution of CYP2D6 to the biotransformation of a drug the following methods have been employed.

4.3.1. Correlation of metabolism of a model drug compared to a new drug

In some studies metabolite formation in vitro from a drug under investigation has been correlated with that of a known CYP2D6 substrate in microsomes from the same livers. A positive correlation indicates the involvement of CYP2D6 in the metabolic reaction. For example, 5-hydroxylation of the antiarrhythmic drug propafenone correlated with the 1′-hydroxylation of (+)-bufuralol, indicating the involvement of CYP2D6 in this metabolic step, whereas the N-dealkylation did not correlate with (+)-bufuralol 1′-hydroxylation in human liver microsomes [62]. The formation of mexiletine metabolites in vitro correlated significantly with the high-affinity component of the O-demethylation of dextromethorphan which is CYP2D6-mediated [72]. These in vitro results were confirmed by in vivo studies showing a correlation between mexiletine metabolism and debrisoquine metabolism in healthy volunteers [72]. In all these investigations, the metabolizing capacity of microsomes from PM livers was clearly reduced for CYP2D6 substrates.

Relative contribution of CYP2D6 to a particular pathway can also be assessed by inhibitors and antibodies. For example, the antiCYP2D6 antibody LKM1 completely blunted 5-hydroxylation of propafenone, indicating no other enzymes to be involved in formation of 5-hydroxypropafenone [62]. In contrast about 25% of tropisetron hydroxylation activity was still preserved in presence of LKM1 antibodies, indicating contribution of other enzymes. Likewise, the CYP2D6 inhibitor quinidine reduced tropisetron hydroxylation at low substrate concentrations completely. At higher substrate concentration inhibition was 75%. The remaining activity is readily explained by affinity of tropisetron to CYP3A [73].

5. Conclusions

In vitro assays allow to identify involvement of CYP2D6 in metabolism of a drug. The technique allows to quantify the contribution of CYP2D6 to overall clearance of the drug. However, as with all in vitro investigations, some caution is mandatory to avoid misleading results. The identification of the contribution of CYP2D6 to drug metabolism is best validated by an integrative approach which consists of the combined use of correlation of metabolite formation with biotransformation of model drugs and inhibition experiments with drugs or antibodies.

Once the limitations and pitfalls are taken into

account, and in vitro experiments are conducted and evaluated in a proper manner, such approaches can contribute a great deal to understand drug metabolism, disposition and hence action before administration to man.

Acknowledgements

Our own work cited in this review has been generously supported by the Robert Bosch Stiftung, Stuttgart, Germany.

References

[1] F.J. Gonzalez and J.R. Idle, Clin. Pharmacokinet., 26 (1994) 59.
[2] D.R. Nelson, T. Kamataki, D.J. Waxman, F.P. Guengerich, R.W. Estabrook, R. Feyereisen, F.J. Gonzalez, M.J. Coon, I.C. Gunsalus, O. Gotoh, K. Okuda and D.W. Nebert, DNA Cell Biol., 12 (1993) 1.
[3] A. Küpfer, P. Desmond, S. Schenker and R. Branch, Pharmacologist, 21 (1979) 173.
[4] M. Eichelbaum, N. Spannbrucker, B. Steincke and H.J. Dengler, Eur. J. Clin. Pharmacol., 16 (1979) 183.
[5] A. Mahgoub, J.R. Idle, L.G. Dring, R. Lancaster and R.L. Smith, Lancet, 2 (1977) 584.
[6] M. Eichelbaum and A.S. Gross, Pharmacol. Ther., 46 (1990) 377.
[7] K. Brosen, Clin. Pharmacokin., 18 (1990) 220.
[8] L.A. Siddoway, K.A. Thompson, C.B. McAllister, T. Wang, G.R. Wilkinson, D.M. Roden and R.L. Woosley, Circulation, 75 (1987) 785.
[9] R.R. Shah, N.S. Oates, J.R. Idle, R.L. Smith and J.D. Lockhart, Br. Med. J. Clin. Res., 284 (1982) 295.
[10] V. Rogiers, W. Sonck, E. Shephard and A. Vercurysee, Human Cells in in Vitro Pharmaco-Toxicology, VUB Press, Brussels, 1993.
[11] P.J. Meier, H.K. Mueller, B. Dick and U.A. Meyer, Gastroenterology, 85 (1983) 682.
[12] J. Doehmer, S. Dogra, T. Friedberg, S. Monier, M. Adesnik, H.R. Glatt and F. Oesch, Proc. Natl. Acad. Sci. USA, 85 (1988) 5769.
[13] J.W. Harris, A. Rahman, B.R. Kim, F.P. Guengerich and J.M. Collins, Cancer Res., 54 (1994) 4026.
[14] A.E.M. Vickers, V. Fischer, S. Connors, R.L. Fisher, J.P. Baldeck, G. Maurer and K. Brendel, Drug Metab. Dispos., 20 (1992) 802.
[15] A.E.M. Vickers, S. Conners, M. Zollinger, W.A. Biggi, A. Larrauri, J.P.W. Vogelaar and K. Brendel, Drug Metab. Dispos., 21 (1993) 454.
[16] A. Guillouzo and C. Guguen-Guillouzo, in G. Yolles and A. Cordier (Editors), In Vitro Methods in Toxicology, Academic Press, London, 1992.
[17] A. Guillouzo, F. Morel, D. Ratanasavanh, C. Chesné and C. Guguen-Guillouzo, Toxicol. Vitro, 4 (1990) 415.
[18] F. Morel, P. Beaune, D. Ratanasavanh, J.P. Flinois, C.S. Yang, F.P. Guengerich and A. Guillouzo, Eur. J. Biochem., 191 (1990) 437.
[19] D. Ratanasavanh, P. Beaune, G. Baffet, M. Rissel, P. Kremers, F.P. Guengerich and A. Guillouzo, J. Histochem. Cytochem., 34 (1986) 527.
[20] Z. Abdel-Razzak, P. Loyer, A. Fautrel, J.C. Gautier, L. Corcos, B. Turlin, P. Beaune and A. Guillouzo, Mol. Pharmacol., 44 (1993) 707.
[21] M. Maurice, L. Pichard, M. Daujat, I. Fabre, H. Joyeux, J. Domergue and P. Maurel, FASEB. J., 6 (1992) 752.
[22] M. Eichelbaum, S. Mineshita, E.E. Ohnhaus and C. Zekorn, Br. J. Clin. Pharmacol., 22 (1986) 49.
[23] P. Dayer, T. Kronbach, M. Eichelbaum and U.A. Meyer, Biochem. Pharmacol., 36 (1987) 4145.
[24] P. Dayer, J. Desmeules, T. Leemann and R. Striberni, Biochem. Biophys. Res. Commun., 152 (1988) 411.
[25] S.G. Al Dabbagh, J.R. Idle and R.L. Smith, J. Pharm. Pharmacol., 33 (1981) 161.
[26] G.C. Kahn, M. Rubenfield, D.S. Davies, S. Murray and A.R. Boobis, Drug. Metab. Dispos., 13 (1985) 510.
[27] A.R. Boobis, C.E. Seddon and D.S. Davies, Biochem. Pharmacol., 35 (1986) 2961.
[28] P.F. Churchill, S.A. Churchill, M.V. Martin and F.P. Guengerich, Mol. Pharmacol., 31 (1987) 152.
[29] A. Kupfer, S.G. Al Dabbagh, J.C. Ritchie, J.R. Idle and R.L. Smith, Biochem. Pharmacol., 31 (1982) 3193.
[30] T. Zysset, T. Zeugin and A. Kupfer, Biochem. Pharmacol., 37 (1988) 3155.
[31] N.L. Kerry, A.A. Somogyi, G. Mikus and F. Bochner, Biochem. Pharmacol., 45 (1993) 833.
[32] G. Mikus, A.A. Somogyi, F. Bochner and M. Eichelbaum, Biochem. Pharmacol., 41 (1991) 757.
[33] C.L. Crespi, B.W. Penman, H.V. Gelboin and F.J. Gonzalez, Carcinogenesis, 12 (1991) 1197.
[34] B.W. Penman, J. Reece, T. Smith, C.S. Yang, H.V. Gelboin, F.J. Gonzalez and C.L. Crespi, Pharmacogenetics, 3 (1993) 28.
[35] V. Fischer, B. Vogels, G. Maurer and R.E. Tynes, J. Pharmacol. Exp. Ther., 260 (1992) 1355.
[36] V. Fischer, A.E. Vickers, F. Heitz, S. Mahadevan, J.P. Baldeck, P. Minery and R. Tynes, Drug. Metab. Dispos., 22 (1994) 269.
[37] S.W. Ellis, M.S. Ching, P.F. Watson, C.J. Henderson, A.P. Simula, M.S. Lennard, G.T. Tucker and H.F. Woods, Biochem. Pharmacol., 44 (1992) 617.
[38] G.T. Tucker, M.S. Lennard, S.W. Ellis, H.F. Woods, A.K. Cho, L.Y. Lin, A. Hiratsuka, D.A. Schmitz and T.Y. Chu, Biochem. Pharmacol., 47 (1994) 1151.
[39] E. Matsunaga, U.M. Zanger, J.P. Hardwick, H.V. Gelboin, U.A. Meyer and F.J. Gonzalez, Biochemistry, 28 (1989) 7349.

[40] T. Kronbach, D. Mathys, J. Gut, T. Catin and U.A. Meyer, Anal. Biochem., 162 (1987) 24.
[41] A.R. Boobis, S. Murray, C.E. Hampden and D.S. Davies, Biochem. Pharmacol., 34 (1985) 65.
[42] E.I. Minder, P.J. Meier, H.K. Muller, C. Minder and U.A. Meyer, Eur. J. Clin. Invest., 14 (1984) 184.
[43] P. Dayer, T. Leemann and R. Striberni, Clin. Pharmacol. Ther., 45 (1989) 34.
[44] P. Dayer, T. Leemann, R. Striberni and P. Meyer, Schweiz. Med. Wochenschr., 117 (1987) 1971.
[45] B.A. Osikowska-Evers and M. Eichelbaum, Life Sci., 38 (1986) 1775.
[46] J.W. Ho and D.E. Moody, Anal. Biochem., 203 (1992) 348.
[47] Y.W. Lam and S.Y. Rodriguez, Ther. Drug Monit., 15 (1993) 300.
[48] J. Gut, T. Catin, P. Dayer, T. Kronbach, U. Zanger and U.A. Meyer, J. Biol. Chem., 261 (1986) 11734.
[49] F.P. Guengerich, D.R. Umbenhauer, P.F. Churchill, P.H. Beaune, R. Bocker, R.G. Knodell, M.V. Martin and R.S. Lloyd, Xenobiotica, 17 (1987) 311.
[50] S.V. Otton, R.U. Brinn and L.F. Gram, Drug. Metab. Dispos., 16 (1988) 15–7.
[51] R. Fonne Pfister and U.A. Meyer, Biochem. Pharmacol., 37 (1988) 3829.
[52] M.V. Relling, W.E. Evans, R. Fonne Pfister and U.A. Meyer, Cancer Res., 49 (1989) 68.
[53] T. Inaba, M. Jurima, W.A. Mahon and W. Kalow, Drug. Metab. Dispos., 13 (1985) 443.
[54] S.V. Otton, T. Inaba and W. Kalow, Life Sci., 34 (1984) 73.
[55] T. Inaba, R.E. Tyndale and W.A. Mahon, Br. J. Clin. Pharmacol., 22 (1986) 199.
[56] S.V. Otton, H.K. Crewe, M.S. Lennard, G.T. Tucker and H.F. Woods, J. Pharmacol. Exp. Ther., 247 (1988) 242.
[57] E. Skjelbo and K. Brosen, Br. J. Clin. Pharmacol., 34 (1992) 256.
[58] S. Kobayashi, S. Murray, D. Watson, D. Sesardic, D.S. Davies and A.R. Boobis, Biochem. Pharmacol., 38 (1989) 2795.
[59] M.P. Manns, E.F. Johnson, K.J. Griffin, E.M. Tan and K.F. Sullivan, J. Clin. Invest., 83 (1989) 1066.
[60] M. Manns, U. Zanger, G. Gerken, K.F. Sullivan, K.H. Meyer zum Buschenfelde, U.A. Meyer and M. Eichelbaum, Hepatology, 12 (1990) 127.
[61] U.M. Zanger, H.P. Hauri, J. Loeper, J.C. Homberg and U.A. Meyer, Proc. Natl. Acad. Sci. USA, 85 (1988) 8256.
[62] H.K. Kroemer, G. Mikus, T. Kronbach, U.A. Meyer and M. Eichelbaum, Clin. Pharmacol. Ther., 45 (1989) 28–33.
[63] F. Broly, C. Libersa, M. Lhermitte and B. Dupuis, Biochem. Pharmacol., 39 (1990) 1045.
[64] K. Brosen, T. Zeugin and U.A. Meyer, Clin. Pharmacol. Ther., 49 (1991) 609.
[65] N.L. Kerry, A.A. Somogyi, F. Bochner and G. Mikus, Br. J. Clin. Pharmacol., 38 (1994) 243.
[66] L.M. Distlerath and F.P. Guengerich, Proc. Natl. Acad. Sci. USA, 81 (1984) 7348.
[67] R.F. Tyndale, F.J. Gonzalez, J.P. Hardwick, W. Kalow and T. Inaba, Pharmacol. Toxicol., 67 (1990) 14.
[68] T. Wolff, L.M. Distlerath, M.T. Worthington, J.D. Groopman, G.J. Hammons, F.F. Kadlubar, R.A. Prough, M.V. Martin and F.P. Guengerich, Cancer. Res., 45 (1985) 2116.
[69] B. Osikowska-Evers, P. Dayer, U.A. Meyer, G.M. Robertz and M. Eichelbaum, Clin. Pharmacol. Ther., 41 (1987) 320.
[70] U.A. Meyer, J. Gut, T. Kronbach, C. Skoda, U.T. Meier, T. Catin and P. Dayer, Xenobiotica, 16 (1986) 449.
[71] A.R. Boobis, C.E. Hampden, S. Murray, P. Beaune and D.S. Davies, Br. J. Clin. Pharmacol., 19 (1985) 721.
[72] F. Broly, N. Vandamme, C. Libersa and M. Lhermitte, Br. J. Clin. Pharmacol., 32 (1991) 459–66.
[73] L. Firkusny, H.K. Kroemer and M. Eichelbaum, Biochem. Pharmacol., 49 (1995) 1777–1784.