Variability in Human Drug Response
G.T. Tucker, Editor

Pharmacokinetic / pharmacodynamic impact of genetic polymorphism of drug metabolizing enzymes

Heyo K. Kroemer and Werner Siegmund

Department of Pharmacology, Ernst Moritz Arndt-University, Greifswald, Germany.

ABSTRACT

Discovery of genetic polymorphisms in the expression of drug metabolizing enzymes has contributed to our understanding of the variable dose vs. effect relationship of drugs. Unexpected side effects were initially noticed by physicians and simple tests were developed to assign the extensive metabolizer (EM) or poor metabolizer (PM) phenotype (ie the visual expression of the genotype) to an individual patient. Later, molecular biology techniques were employed to characterize the individual gene locus and identify mutations responsible for aberrant enzyme function (assignment of genotype).

The present article highlights the pharmacokinetic and pharmacodynamic impact of genetic polymorphisms of drug metabolizing enzymes using the example of cytochrome P4502D6 (CYP2D6). This polymorphism is suitable to discuss the problem for various reasons: first it affects a substantial fraction of our population (about 7 to 10 percent of Caucasians), second the enzyme CYP2D6 oxidizes numerous frequently used drugs and third, the molecular biology underlying polymorphic CYP2D6 expression has been described in detail. The pharmacodynamic impact of the genetic polymorphism of CYP2D6 depends on the pharmacological features of the individual compound and cannot be generalized.

In summary, while the pharmacokinetic consequences are readily understood, the pharmacodynamic impact is complex and has to be evaluated for each compound.

Key words: genetic polymorphism, drug metabolizing enzymes, CYP2D6.

Correspondence: Prof. Heyo K. Kroemer, Department of Pharmacology, Ernst Moritz Arndt-University, Friderich-Löfflerstrasse 23d, 17489 Greifswald, Germany, Tel: +49-3834-86-5630, Fax: +49-3834-86-5631

INTRODUCTION

Aside from identifying new targets for yet untreatable diseases variability in response to a given dose of a drug has proven to be a major challenge for further improvement of pharmacotherapy. For example, the effects of a cancer chemotherapy treatment regimen are well established in large patient populations, while response in the individual person remains

largely unpredictable.

Part of this variable response is due to inherited eg genetic factors and it was AE Garrod who postulated inborn errors of metabolism to contribute to this phenomenon [1]. His assumption was based on the observation that patients with alkaptonuria either excreted homogentisic acid in normal amounts or not at all: "its appearance in traces or in gradually increasing or diminishing quantities has never been observed". Multimodal distribution of activity of drug metabolizing enzymes has ever since been the hallmark of the new discipline of pharmacogenetics. The term itself has been coined by Vogel [2] and was intertwined with the assumption of Motulski that exaggerated response to drugs may be a consequence of genetically determined deficiencies in enzyme activities [3].

An early observation of unexpected drug effects followed the introduction of the depolarizing muscle relaxant succinylcholine. This compound was characterized by rapid onset and short duration of action. Administration of 1 mg/kg leads to complete paralysis within 2 minutes. The half life of 2 to 6 minutes guarantees complete recovery within a short period of time. Due to this favourable pharmacokinetics the drug gained widespread application. The use in large populations, however, revealed an unexpected feature of succinylcholine. Some patients remained completely paralyzed for several hours requiring mechanical ventilation. The pioneering work of Kalow [4] could identify the reason for the prolonged action of succinylcholine in these patients. Normally the drug is rapidly cleaved by butyrylcholine esterase. The patients with exaggerated effects showed reduced enzyme activity based on a genetic deficiency which was inherited in an autosomal recessive fashion. Later on the molecular bases of this enzyme deficiency has been unravelled [5]. The gene encoding for butyrylcholine esterase is located on chromosome 3. Nucleotides 208 to 210 have the sequence GAT and therefore encode aspartic acid at position 70 of the protein. Patients with decreased activity have a mutation in nucleotide 209 leading to GGT. The latter triplet encodes for the monocarbonic glycine instead of the dicarbonic aspartic acid in the wild type. The second carboxylic group is required for binding of the quarternary amine of succinylcholine and its loss leads to reduced affinity and therefore reduced clearance of the drug. As a consequence plasma concentrations remain elevated for a prolonged period of time. Later on several other mutant forms of succinylcholine esterase have been identified. Simple measures for phenotyping patients have been developed (dibucaine challenge). The current knowledge has been summarized by Lockridge [6].

Butyrylcholine esterase is an impressive example for a close chain of evidence from the molecular level to the clinical consequences of a genetically determined enzyme deficiency. A variety of such deficiencies has been described in the meantime (Table 1). While the above described defect in butyrylcholinesterase affects only a limited number of patients other enzymes match the definition of genetic polymorphisms. A genetic polymorphism is a monogenic trait which occurs in the population in at least two geno- or phenotypes, neither of which has a frequency of less than one percent [7].

Similar to the discovery of the genetic defect in succinylcholine esterase the initial observations of unexpected response to drugs came from physicians. Later on, the biochemistry and the molecular biology of each deficiency has been explored. Methods for assignment of phenotype and/or genotype to an individual patient were developed which allow to identify patients at risk and to adjust the dose in an individual manner. Although the tools for integrating pharmacogenetics into clinical practice are now available this discipline has not yet found its way into day to day pharmacotherapy. One reason may be that both the

pharmacokinetic and the pharmacodynamic impact of such polymorphic drug metabolism is very complex thereby precluding any generalizations. In this article I will focus on the cytochrome P4502D6 polymorphism for several reasons: first the molecular bases for this genetic deficiency has been explored in great detail. Second, a substantial fraction of the population is affected by polymorphic expression of this enzyme, which in turn catalyzes the oxidation of more than 50 frequently used drugs. Third, although all prerequisites for a clinically relevant source of interindividual variability in drug disposition and action are present the individualization of dose based on pheno- or genotype has not been widely applied in clinics which may be due to the complex nature of the therapeutic consequences. Therefore, the CYP2D6 polymorphism appears to be a good example for potentials and pitfalls of pharmacogenetics.

Table 1.
Drug metabolizing enzymes, the activity of which is genetically determined

Type of enzyme	Enzyme affected	Clinical consequences of enzyme deficiency
Esterase	Butyrylcholinesterase	Prolonged action of succinylcholine
Transferase	N-Acetyltransferase	Enhanced side effects of isoniazide
	Methyltransferase	Enhanced toxicity of mercaptopurine
	UDP-Glucuronosyltransferase	Crigler-Najjar or Gilbert's Syndrome
Dehydrogenase	Aldehyde dehydrogenase	Flush after ethanol intake
Oxidoreductase	Flavin-containing monoxygenase	Fish odor syndrome
Monoxygenase	CYP2D6, CYP2C19	Depends on pharmacology of the compound

THE POLYMORPHISM OF CYP2D6

In 1972 the department of Internal Medicine at the University of Bonn performed a routine pharmacokinetic study to characterize the pharmacokinetics of the antiarrhythmic sparteine. The investigators identified one volunteer, who experienced serious side effects such as nausea, diplopia and blurred vision, which are hallmarks of intoxications with this type of antiarrhythmics. Analysis of urine samples identified a distinct metabolic pattern in this volunteer: he excreted almost the entire dose as unchanged sparteine in urine, while all other volunteers excreted the major portion of the dose as hydroxy metabolites. In accordance with these data was the observation of a dramatically decreased apparent oral clearance of sparteine in this volunteer which was reduced by 80 percent as compared to the other volunteers. Subsequent family studies revealed the deficient sparteine metabolism to be inherited as an autosomal recessive trait in about seven to ten percent of the Caucasian population [8]. A similar observation was reported at the same time for the antihypertensive debrisoquine [9].

Both sparteine and debrisoquine were used to assign phenotypes (ie the visual expression of genotypes) to individual patients. Patients affected are referred to as Poor Metabolizers (PM) while the remainder of the population is called Extensive Metabolizers (EM). Later on two further phenotypes have been denoted IM (intermediate metabolizer; patients whose metabolic capacity is between EM and PM) and finally the phenotype of ultra rapid metabolizer. The latter phenotype has been previously reported by Bertilsson and coworkers [10], again based on a patient with an unusually high dose requirements for the antidepressant nortriptyline.

The polymorphism of CYP2D6 would have been of rather limited clinical interest if the enzyme affected would catalyze only the oxidation of sparteine and debrisoquine. Further studies, however, identified numerous drugs from different therapeutic areas and a wide array of chemical structures to be substrates of CYP2D6 (a list is provided in Table 2).

Table 2.
Compounds whose metabolism cosegregates with that of sparteine

Cardiovascular drugs

Alprenolol, Flecainide, Metoprolol, N-Propylajmaline, Perhexiline, Propafenone, Propranolol, Timolol.

CNS drugs

Amitryptyline, Clomipramine, Clozapine, Desipramine, Imipramine, Nortriptyline, Thioridazine.

Others

Codeine, Dihydrocodeine, Dextromethorphan, Ethylmorphine, Hydrocodone, Norcodeine, Oxycodone, Tropisetrone.

The polymorphism of cytochrome P450 2D6 has been thoroughly characterized on the molecular level [11]. In brief, the wild type CYP2D6 gene is located on the long arm of chromosome 22 and is part of a gene cluster which besides CYP2D6 is thought to carry two pseudogenes (CYP2D7 and CYP2D8). The CYP2D6 gene locus has proven to be highly polymorphic eg both the EM and the PM phenotype are encoded by numerous allelic variants (a list of variants is shown in TABLE 3). These variants range from complete deletion of the entire CYP2D6 gene to gene amplification leading to up to 12 copies of the CYP2D6 gene in one individual. The latter phenomenon has been identified as the molecular base for the ultrarapid metabolizer phenotype [12].

The availability of probe drugs with exactly defined pharmacokinetics in combination with detailed knowledge about the molecular mechanisms underlying the CYP2D6 polymorphism should allow for an exact match of phenotype and genotype (predictions made based on the genotype of a patient should translate into enzyme expression and hence pharmacokinetics of a drug). A large scale study trying to combine genotype and phenotype of CYP2D6 has been recently reported in a detailed study by Griese and coworkers [13]. Most interestingly they found that the same genotype can be associated with a wide range of enzyme activity (Figure 1). Therefore "postgenomic factors" must contribute to the interindividual variability in

CYP2D6 activity. These data clearly point to the possibility of substantial gene regulation of CYP2D6. Interestingly, several recent reports indicate regulation of CYP2D6 activity and its analogue in rats CYP2D1 by xenobiotics such as clozapine or denaverine [14,15]. The aspect of environmental vs genetic factors in expression of CYP2D6 deserves further attention.

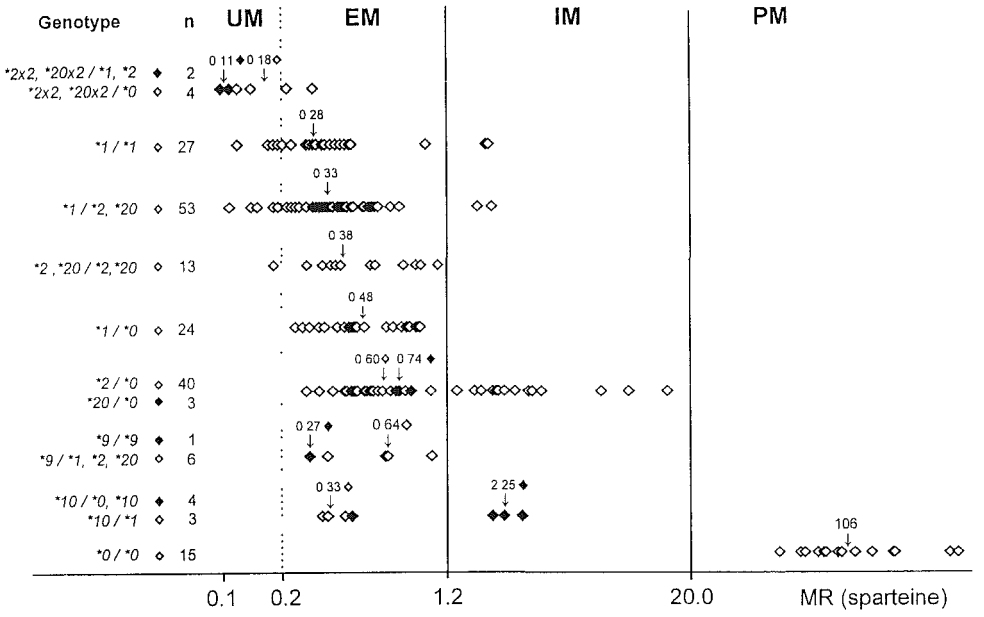

Figure 1. Distribution of sparteine metabolic ratio (MR; a measure of CYP2D6 activity) in 195 Caucasian individuals in relation to genotypes. *0 indicates the presence of any of the seven tested nonfunctional alleles. Number above the lines give the median for each group (Reproduced with permission from Griese EU *et al.* 1988 [13])

Asides from scientific interest the possibility of gene regulation supports the importance of phenotype assignment. Administration of a probe drug to an individual patient with subsequent evaluation of the excretion of parent compound and or metabolite is the only way to experimentally assess the individual enzyme function. The probe drug approach, however, carries several disadvantages. First, it is required to administer a pharmacologically active compound which always carries the potential of side effects. Second, the probe drugs used are either not widely available (eg sparteine or debrisoquine) or not specific for CYP2D6 (eg dextromethorphane or debrisoquine). Third, metabolism of the probe may be hampered by other CYP2D6 substrates concomitantly administered to the same patient thereby mimicking a reduced oxidative activity even in patients homozygous for wild type CYP2D6.

The pharmacokinetic impact of genetic polymorphism in CYP2D6 expression is readily explained. Depending on individual enzyme activity, clearance of compounds oxidized by CYP2D6 varies over a wide range. Consequently, concentrations achieved during chronic administration will show pronounced variability following administration of a fixed dose to a population of patients. In some cases, however, prediction of the pharmacokinetic consequences carried some problems, for example in the case of the antiarrhythmic flecainide.The drug has been identified as CYP2D6 substrate in panel studies (ie comparing pharmacokinetics in EM and PM volunteers) following administration of single doses [16]. Based on these data it was predicted that chronic administration of flecainide should result in accumulation of the drug in the PM phenotype. In contrast to this assumption the actual clinical experiment resulted in steady state plasma concentrations that showed no significant relationship to phenotype [17].

The pharmacodynamic impact of genetic polymorphism is even more difficult to predict. The initial observation which led to the first description of the CYP2D6 polymorphism was that of exaggerated response to regular doses. Based on this first experience it was generally assumed that patients with reduced metabolizing capacity have a high risk to experience side effects. There have been in fact numerous examples of drugs with a higher incidence of side effects in the poor metabolizer population. One example is the calcium channel antagonist perhexiline, which was associated with a high incidence of peripheral neuropathy and liver damage. Retrospective evaluation revealed about 50 percent of patients with side effects to have the PM phenotype [18]. Based on individual enzyme activity extreme dose adjustments may be necessary. One impressive example has been reported by Bertilsson *et al.* for the antidepressant nortriptyline [10]. Whereas PMs require about 10 mg per day of nortriptyline to develop therapeutic plasma concentrations ultra rapid metabolizers should be treated with 500 mg per day. The upper limit of dose recommended by the manufacturer is 150 mg and 225 mg per day for outpatients and inpatients, respectively. Thus, it is evident that this phenotype of patients has a high risk to be treated with inefficiently low doses.

A different problem occurs, when a drug which is metabolized via CYP2D6 requires metabolic activation to gain therapeutic efficacy. One example is the analgesic codeine. CYP2D6 catalyzed O-demethylation to morphine represents the analgesic mode of action [19]. Poor metabolizers were shown to form smaller amounts of morphine after administration of codeine and consequently had a reduced analgesic activity. Recent interaction studies with quinidine, a potent blocker of CYP2D6 activity, however, led to a more complex picture [20]. Although coadministration of quinidine blocked formation of morphine from codeine completely, analgesic activity of codeine was still significantly higher than in PM. It has been speculated that CYP2D6 in the CNS may locally bioactivate codeine and that this process is not blocked by quinidine.

The examples given highlight the problems of drug therapy induced by genetic polymorphism in drug oxidation. Metabolic capacity and hence concentration after administration of a given drug is an individual feature of the patient and not a characteristic disadvantage of this particular compound. Side effects due to genetically determined polymorphic oxidation (both lack of efficacy and exaggerated response) can therefore be defined as a consequence of inadequate dosing in relation to the individual metabolic capacity.

In view of the data available it has to be expected that knowledge about genetic polymorphisms in drug oxidation will be implemented into clinical practice in the near future. A recent article in the JAM states that adverse drug reactions are the fourth leading cause of

death in the United States [21]. Polymorphic expression of drug metabolizing enzymes can result in adverse drug reactions and may therefore contribute to this drug-related mortality. Prospective trials are underway to proof this hypothesis.

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Discussion: Pharmacokinetic / pharmacodynamic impact of genetic polymorphism of drug metabolizing enzymes

M.M. Reidenberg:
You pointed out how this genetic information is obtained, but not how it is utilised. What do you see as the primary barrier to the next step?

H.K. Kroemer:
I think the primary barrier to the next step is the lack of easy assays functionally measuring enzyme capacity. Easy assays for phenotyping patients should be available before administration of drugs. I think there is really a lack of good, reliable, and fast assays in terms of assessing enzyme function. If you look to the probes we are using and the ways the analytical methods are handled, there is certainly nothing a doctor in private practice can do easily.

P. Rolan:
You mentioned that, even for the same genetic expression for CYP2D6, there is quite a lot of variability in the actual phenotype, and you made a very brief comment that you think that xenobiotics may be modulating the activity there. However, I thought that CYP2D6 was relatively non-inducible and not very much affected by xenobiotics. Could you comment on this?

H.K. Kroemer:
You summarised well the current thinking on this issue. I would interpret as an induction the data of these papers where they try to induce sparteine metabolism with rifampizine. Using a statistical analysis the desired result, i.e. no induction effect, was obtained but if you look at the data there is induction. Ivar Roots group in Berlin (Sachse C *et al.*, Eur J Clin Pharmacol (Abstract), 1997) have shown that in a cell culture using clozapine, you can induce CYP2D6. Other people have used certain compounds to down-regulate CYP2D6 in cell lines. These data at least suggest, in combination with the available *in vitro* evidence, that there is some regulation of CYP2D6. Therefore we should put much more emphasis on the interaction of environment and genetics when addressing these issues.

A.J.J. Wood:
People claim that there is no effect, citing studies in which the possibility of changing poor metabolisers (PMs) into extensive metabolisers (EMs) by induction was being investigated, and this obviously was not possible. A shift to the left in EMs was achieved by induction and we have obtained similar results. I find it hard to believe that well recognised inhibitors for CYP2D6 do not also exist in the environment and that we can phenocopy an EM into a PM by giving quinidine. With respect to the effects of codeine, there is a fairly good relationship between the measurement of CYP2D6 activity and the measurement of respiratory depression, which is a much more easily measurable continuous variable. I think there is also an effect on induction in the CNS and this is particularly seen in the effect of quinidine. But certainly within the EMs, there is a fairly good relationship between CYP2D6 activity and respiratory depression produced by codeine.

H.K. Kroemer:
Aside from these global pharmacokinetic parameters like genotype or phenotype, it may be necessary to examine in greater detail the local bioactivation, for example in the CNS, or the local inactivation, for example in the lung. More studies should be done on the local area around the receptors.

M.M. Reidenberg:
I have had a patient with inflammatory bowel disease needing 600 to 900 mg of codeine a day to control the diarrhoea because other treatments did not work. This would cause CNS effects. When I added a small daily dose of quinidine to it, he was able to tolerate these doses of codeine without disabling CNS effects.

J. Benítez:
Your previous comment about statistics and sparteine induction was very interesting. Statistics can be misleading. For example, there does not necessarily exist an intermediate between an ultra-fast metaboliser and a poor metaboliser. When a patient is concerned the population data is of limited value. You have also said we lack easy essays, but I think we lack general knowledge too. For example, you have presented a graph showing a poor correlation between geno and phenotype. We have also observed this but we have not been able to interpret it. Thirdly, we have shown that maybe psychiatric patients control their treatment themselves, just by caffeine intake. Caffeine is not only a stimulating drug, but it also could block metabolism by CYP1A2, and could lead to dangerous intoxication with clozapine and with other drugs, possibly. And finally, it is not only important to take into account genetic and environmental interactions, but also interactions between different isotypes of the cytochrome P450.

G.T. Tucker:
Pregnancy is one clear example of a factor associated with up-regulation of CYP2D6. There is a very Swedish study showing that blood levels of metoprolol, a major 2D6 metabolite, are almost non-existent during pregnancy, but they return to normal levels after delivery.

J. Urquhart:
There is an incredible pricing problem that arises when you have a hundred-fold range of dose requirement. And most of the European pricing authorities still want to do milligram-based pricing, which is not possible under these circumstances.

H.K. Kroemer:
Also there can eventually be problems with the cost of treatments. But in reply to your comment, it depends on how companies should act. Tropisetron is a good example of these problems because it is under-dosed due to safety reasons.

J. Urquhart:
Treatment pricing is difficult because dosage forms are frequently cut to save money. Nevertheless, pharmaceutical companies are not alone in this. The pricing policies being set by governments also has a role.