The impact of genetic polymorphisms in risk assessment of drugs

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Genetic variation is a fundamental characteristic of all living organisms. In humans marked individual variability has been demonstrated at every level of investigation. With regard to drug metabolism variability in responsiveness to a given dose originates from many factors, e.g. environmental influences, alteration of absorption, distribution, excretion and metabolism.

The most important drug metabolizing enzymes are by tradition categorized into two groups, phase I and phase II, according to the chemical reactions they catalyze. Phase I biotransformations include oxidation, reductions and hydrolyses, while phase II reactions (sometimes designated synthetic reactions) are conjugation to glucuronic acid, sulfate, glutathione, etc. Originally, phase I metabolism followed by phase II metabolism was considered to be the rule. It is now realized that many compounds undergo only one of the phases. From a toxicological point of view there are only very few examples of drugs that are activated into toxic metabolites by phase II metabolism whereas phase I metabolism can frequently yield toxic metabolites. This example of paracetamol (acetaminophen) to be discussed later in this paper.

In man the CYP (cytochrome P450) isozymes are the major catalysts of drug oxidation, and within the last decade considerable progress has been made in identifying the genetic basis for the intra-individual variation in drug metabolism. Although genetic polymorphisms are recognized as major determinants of individual variation in the activities of drug metabolizing enzymes, it must be stressed that also with regard to non-polymorphic enzymes large interindividual variability as well as equally important implications can be found.

In the context of metabolizing chemicals a genetic polymorphism is the presence within a population of at least two groups with distinctly different ability of metabolizing xenobiotics. Presently, a polymorphism is defined by a frequency in the population of at least 1% of at least two phenotypes (Vogel and Motulsky 1982). In this review special focus is on the relevance of genetic polymorphisms in drug metabolism for the risk assessment of drugs.

Genetic polymorphisms in man:

CYTP2D6:

CYTP2D6 is found in human liver (Gonzalez et al. 1988) and in the brain (Tsuda et al. 1991). A polymorphism in oxidation of the antihypertensive drug debrisoquine was observed (Dahloob et al. 1977) because of a particular slow elimination and pronounced pharmacodynamic effect in about 8% of the Caucasian population. Many drugs are now realized to be oxidized by CYTP2D6 (Brezen and Grann 1989).

The genetic defects in the CYTP2D gene are well characterized (Kagimoto et al. 1990, Gandig et al. 1991). Furthermore, the phenotypes for debrisoquine hydroxylation can be predicted by DNA- testing (Graf et al. 1992).

Phenotyping: Sparteine, debrisoquine or dextromethorphan (Schofl et al. 1985) can be used as probes for phenotyping individuals. Some polymorphs (PM) are defined from a metabolic ratio (parent compound/metabolite) in urine of >20 for sparteine (Eichelbaum et al. 1982), >12.6 for debrisoquine (Evans et al. 1980) and <0.3 for dextromethorphan (Schofl et al. 1983).

Genotyping: DNA obtained from leukocytes after preparation of a conventional venesection can be used for PCR based screening of the CYTP2D6 mutations D6-A, D6-B and D6-C (Henn and Meyer 1992).

Example: Codeine is an old analgesic used in many composite analgesic formulations. It is activated to morphine by CYTP2D6 (Dyer et al. 1988) and its analgesic action is dependent on biotransformation. It is intriguing that while metabolizers have no analgesic effect of codeine fast metabolizers with quinidine induced poor metabolizer phenotypes still have the analgesic effect (Weinstein and Slade 1980, Sindrup et al. 1990). Presumably, local bioactivation of codeine in the brain plays a role (Wahlström et al. 1988) and this mechanism may not be inhibited by quinidine.

CYPA1-L2:

CYPA1 has been detected in human placenta, skin, lymphocytes and peripheral lung, whereas it is notably absent in human liver (Kadiashvili and Gogeburg 1992). This enzyme is among the best conserved among xenobiotic-metabolizing enzymes (Nebert 1989). The only available clinically example of a drug that is metabolized by CYPA1 is coal tar used by dermatologists, the major focus on this enzyme is its role in activation of carcinogens and teratogens (Nebert 1989). In this respect it is noteworthy that the enzyme constitutively has a low expression and that many substances can induce its activity many fold, e.g. cigarette smoke, polyaromatic hydrocarbons etc. In relation to drug metabolism the most interesting feature is whether drugs have CYPA1 inducing properties that might change the susceptibility of the treated individual to carcinogens or mutagens. In such a case long term treatment might increase the teratogenic or carcinogenic risk.

CYPA2 has only been identified in the liver and there appears to be a 50-100 fold variability in its activity (Gorumirth and Shimada 1991). CYPA2 is important for the metabolism of important drugs, e.g. caffeine, phenacetin and pemecaine, and for a large number of xenobiotics, e.g. heterocyclic amines. A polymorphism has been suggested from a ratio of the xxCaffeine metabolites (Butler et al. 1992). However, in studies using the conventional caffeine metabolic ratio on more than 300 persons such a polymorphism could not be detected (Vogelius et al. 1992). The conventional CYPA2 index correlates well with the caffeine clearance is between the two step demethylated metabolites of caffeine, i.e. 1-methyluric acid (1U) and 4-acetyl-6-formylaminol-3-methyl uracil (AFMU), and the one step de-methyl-
lated metabolite 1,7-dimethylurea (17U) (Campbell, ME et al. 1987). As stated below genotyping does not suggest any polymorphisms in CYP1A2. Presently it must be concluded that a polymorphism for CYP1A2 still needs to be verified.

Phenotyping: There are no available non-hazardous probes for determination of CYP1A activity in vivo, in vitro ethoxysresorfin appears to be a specific probe (Jensen et al. 1993).

CYP1A2 activity can be estimated from dietary caffeine metabolite ratios in urine as described above.

Genotyping: DNA from various tissues, e.g. with or without the PCR amplification be used to determine restriction fragment length polymorphisms in CYP1A1 (Kawajiri et al. 1990, Tefre et al. 1991).

CYP1A2 protein analysis of human liver has demonstrated variations, but no liver has been found that lacks this enzyme suggesting that mutant alleles of CYP1A2 are not present in humans (Wrighton et al. 1986, McM anus et al. 1988, Shimada et al. 1987).

Example: So far the only example of a drug with CYP1A1 inducing properties is coar
tar for topical application whereas the environmentally dominating source of such xenobiotics are tobacco smoke. An association of lung cancer with CYP1A1 poly-

morphism (EFIP) has been found in a Japanese population (Kawajiri et al. 1990) whereas this association could not be found in a Norwegian study (Tefre et al. 1991).

Regarding CYP1A2 a distinct polymorphism remains to be demonstrated, however, the 30-50 fold variability for individual variability and xenobiotic related effects. A recent example presumably of clinical importance is the inhibitory effect of the antidepressant fluoxetamine as demonstrated by a low K for inhibition of CYP1A2 in human microsomes (Brezen et al. 1993b).

CYP2E1.

In human CYP2E1 is found in the liver and in peripheral blood lymphocytes. An approximate 10-fold variation in hepatic enzyme activity has been reported (Peter et al. 1999). CYP2E1 was originally called MEOS (microsomal ethanol oxidizing system) and it metabolizes ethanol and other structurally related compounds as for example some of the volatile anesthetics (halothane). The muscle relaxant chlorothiazide (Peter et al. 1996) is metabolized almost exclusively by CYP2E1 and excreted into the urine, and the analgetic paracetamol (acetaminophen) is activated by CYP2E1 to its arylating metabolite. Presently, there are no systematic investigations available for comparison with the restriction fragment length polymorphisms identified for CYP2E1.

Phenotyping: The only suggested probe for CYP2E1 is chlorothiazone (Peter et al. 1999). Preliminary investigations in our laboratory show that recovery of the 6-OH metabolite is almost complete in urine and that plasma clearance is about 300 ml/min.

It would be of interest to see the clearance as a measure of CYP2E1 activity and to develop a simplified one sample method in analogy with the one developed for anilpyrine (Poulton and Loft 1988).

CYP2C.

Recently, two reports have described restriction fragment length polymorphisms of CYP2C1. One is based on PeT or Real restriction and localized to the transcription regulation region of CYP2C1 (Hayashi et al. 1991). The other polymorphism is localized to exons 6 and revealed by the Dual restriction enzyme (Ichimori et al. 1991).

Example: Paracetamol is activated to an arylating metabolite by CYP2E1, CYP1A2 and CYP1A4 (Thunell et al. 1992, Poulsen et al. 1993) following an overdose that depletes hepatic glutathione. Preliminary investigations in our laboratory points at CYP2C as the qualitatively important activating route in mice (unpublished observations), in man, however, another enzyme could be the most important. It is still debated if e.g. alcoholism is a risk factor for hepatic injury from paracetamol overdose, since CYP2E1 is thought to be induced. However, systematic investigations on this important clinical problem has yet to be done.

CYP2C2.

This cytochrome often called cytochrome P450m1 is controlled by an autosomal recessive gene resulting in its absence in approximately 2-3% of the caucasian population as opposed about 13% in orientals (Bertilsson et al. 1992). An increasing number of drugs are recognized to be metabolized by CYP2C, e.g. diazepam (Bertil-


Phenotyping: The simplest approach to determine the phenotype appears to be the ratio between the (R)- and the (S)-enantiomers in urine collected for 8 hours after oral admin-

istration of the racemate (Wigstrom et al. 1989).

Genotyping: Presently there are no available methods.

Example: The antimalarial drug quinapril is metabolized by CYP2C to cyclo-

quezenil (Ward et al. 1991), which is responsible for the antimalarial action. Poor metabolizers have only about 25% of the cycloquezenil plasma level found in fast metabolizers (Brennen et al. 1993a), whether this is reflected by a higher risk of therapeutic failure remains to be established.

N-acetyl transferase:

This enzyme is found in many tissues but is most abundant in the liver (Hem et al. 1987). It has been recognized for more than 30 years as genetically determined by two co-dominant alleles (Evans et al. 1960). The acetylation phenotype, i.e. fast and slow acetylators, predict the rate of elimination of many drugs metabolized by this enzyme, e.g. isoniazid, procainamide, sulphonamethazine, nitrazepam, caffeine (Ito 1990). Both O- and N-acetylation of hydroxylamines are under the same genetic control and the two reactions are important for the metabolic activation and deactivation of en-

zymatically carcinogenic, e.g. arylamines (Hem 1988).

The genetic defects are now well characterized. The dominant, autosomal allele for fast acetylators and the multiple alleles for slow acetylators have been located to chromo-

some 8 (Blum et al. 1990, Obukh and Deutsch 1990).
Phenotype determination. Acetylcholinesterase (AChE) phenotype may be determined by probing with carbamoyloxytrimethyl carbamate (Clarks 1983). It should be noted, however, that some drugs are metabolized equally well by acetylation regardless of the phenotype, e.g., p-aminosalicylate (Weber and Hein 1985). By means of dietary caffeine intake, acetylstarch phenotype can be determined from the urinary ratio between the acetylated metabolite-2-acetylcarnitine (AAM) and the altemative metabolite-1-acetylcarnitine (1X), or 1X plus 1-methylciclic acid (1U) plus 17 demethyluric acid (17O). This index discriminates the two phenotypes in accordance with other reports (Grant et al. 1983). Whether this discrimination methodology can distinguish between homoygous and heterozygous fast acetylators is debated (Gray et al. 1983, 1984, Visiezen et al. 1992).

Genotype determination. Genetic DNA obtained from peripheral blood leukocytes can be used for a polymerase chain reaction based test for determination of the wild type (wt) and the mutated genes (M1, M2 and M3) (Graf et al. 1992). By this technique homozygous and heterozygous fast acetylators can be distinguished from slow acetylator genotypes.

Example: Weber et al. (Weber and Hein 1985) concluded that slow acetylators are more likely to develop peripheral neuropathy than fast acetylators from homoygous, and patients suffering from homoygous neuropathy have a higher frequency of hepatic disorders. The rapid acetylator genotype of homozygous-induced liver toxicity in osteosarcoma (fast acetylators constitute 40-50% of the population) than in caustics (fast acetylators constitute 40-50% of the population).

Nevertheless, the main importance of this polymorphism is probably related to environmental and occupational exposure to aromatic amines and related cancer risk (Hein 1988).

Sulfation. Sulfation is suggested to be genetically polymorphic regulated. However, solid evidence of the protein and gene level is not available. A number of sulfur-containing drugs are eliminated by sulfonation. The formation of a metabolite from the monothiolic agent carbamazepine as assessed by a urinary metabolic ratio has appeared to be under genetic control (Mitchell et al. 1984). Although a low rate of metabolic excretion has been associated with various inflammatory and neurological diseases (Stevenson et al. 1989, Gordon et al. 1992), it has been related to a few reactions in sulfur-containing drugs, i.e., phenytoin (Emery et al. 1984), and this could be due to an apparently wrong clinical course in patients with this trait (Emery et al. 1992). In fact, the claim that the protective carbamazepine metabolism is a product of sulfonation and its importance have been disputed by others (Brockbom et al. 1991, Meese et al. 1991, Gregory et al. 1992). No definite polymorphism with respect to sulfonation of drugs has yet been identified.

Methylation. This reaction lacks in the chemical investigations that are available for the CYPs. However, conjugation of methyl groups to thiol, oxygen and nitrogen appear to be under separate genetic control (Weinshilboum 1988, 1993). Thiopurine methyltransferase (TPMT), a cytosolic enzyme expressed in all tissues, catalyzes the S-methylation of aromatic and heteroaromatic sulfhydryl compounds, including 6-mercaptopurine and thiopeptine. The activity of TPMT can be assessed from red blood cells. From population and family studies the activity appears to be controlled by a single gene with two alleles and gene frequencies for high and low activity of 34% and 66%, respectively (Weinshilboum 1992). Thus, in 228 subjects 88.6%, 11.1% and 0.3% had high, intermediate, and no activity, respectively (Weinshilboum and Sladek 1980). Whereas patients with low TPMT activity may suffer toxicity to the thiopurine drugs (Lemar et al. 1987, 1989), those with particularly high activity may risk underdosing and relapse more frequently when treated for leukemia (Lemar et al. 1990).

Thiol-methytransferase (TMT), a membrane-bound enzyme expressed in most tissues, catalyzes the methylation of sulfhydryl drugs, such as captopril and D-phenylciclyclic (Weinshilboum 1988, 1992). The activity as assessed from red blood cells appear to be under genetic control. Although particular TMT activity has been associated with some neurological disorders (Waring et al. 1989), no relationship with any drug reaction has been reported.

Cysteic acid-cysteine S-methytransferase (COMT) metabolizes some neurotransmitter and catechol drugs (Weinshilboum 1989, 1992). The distribution of COMT activity assessed from red blood cells in a population suggests regulation by a single locus with two alleles and almost equal gene frequencies. Whereas COMT activity correlates with the rate of metabolism of L-dopa and methyldopa (Roth et al. 1980, Campbell, NRC 1984), the clinical significance has yet to be demonstrated.

From a large family study the activity of N-methyltransferase expressed in red blood cells appears to be under genetic control (Scott et al. 1988). Although homovanillic acid is N-methylated by this enzyme, no drug has yet been identified as an important substrate.

Sulfates. Sulfate transferase belong to a family of enzymes found mainly in the cytoplasm of hepatocytes but also in platelets. In contrast to the CYPs they are not investigated in the same details regarding the proteins and the genes.

Both thermolabile and thermostable phenyl sulfotransferase as assessed from platelets appear to be under genetic control in population and family studies (Weinshilboum 1990). The extent of paraaminosalicylic acid correlated with the platelet activity of both forms in one study (Reiter and Weinshilboum 1982) but only with the thermostable form in another study (Bohman-Carter et al. 1983). In subjects supplemented with sodium salicylate the extent of methylsulfate sulfation correlated with only the thermolabile sulfotransferase activity of platelets (Campbell, NRC 1984), although
this does not predict the activity of this form in other tissues (Weinshilboum 1990). The clinical significance of genetic variation in the sulfation capacity, which may also be dependent on (activated) sulfate availability has yet to be determined.

Miscellaneous reactions: A variety of other reactions are not reviewed in details here because they either are considered irrelevant for drug metabolism, because they represent particular problems that should be addressed separately in the risk assessment of drugs (e.g., the hepatic cytosolic alcohol dehydrogenase) or because only sporadic information is available (e.g., some of the CYPs, the paracorone/zyloisomerase polymorphisms on the proteins and genes.

Phenotyping versus genotyping: Several consideration on the choice between phenotyping and genotyping can be made although it should be realized that the two methods are supplementary rather than alternatives. Phenotyping has the advantage of obtaining continuous scale measurement and can therefore determine potential drug interactions. For example, quicktime administration turn fast CYP2D6 metabolizers phenotype to slow metabolizer phenotype (Sindrup et al., 1992), an effect that cannot be revealed by genotyping. Analogous arguments are valid for inducing effects. Most polymorphisms show a considerable variability even in one of the genotypes. Very fast-fmetabolizers for example have a enzyme activity that cannot be distinguished from slow fast-metabolizers by genotyping, whereas phenotyping offers the possibility of revealing reactions related to such a trait. Furthermore, it can be argued that the huge variability that can be found in non-polymorphic enzyme activities can be of equal magnitude and importance for administration of drugs. The drawback of phenotyping is that it requires a trial that is planned for this purpose and that it requires administration of a test compound and sampling of relevant biological material.

The advantage of analysis on DNA for genotyping is that it only requires minute amounts of genomic DNA from e.g., lymphocytes because of the PCR technique and that it can be done retrospectively if such material is stored. Genotyping has a high potential for screening and the molecular techniques have a high capacity and a price that get cheaper and cheaper. It should be noted that only in case of CYP2D6 and N-acetyltransferase there is solid evidence that the genotyping predict the phenotype with sufficient accuracy. Interestingly, multiple genes are said to be found in fast fast-metabolizers of debrisoquine.

Which polymorphisms are relevant for drug development and registration? The polymorphisms in CYP2D6, CYP2C and N-acetyltransferase are clearly demonstrating of importance for the metabolism of many drugs. For a safety evaluation it is mandatory to include investigations on whether a new drug is metabolized by these enzymes. If it can be established that the metabolism by either of these pathways only

constitute a minor pathway further investigations are not necessary unless in the case(s) where the metabolism leads to conversion of a prodrug to an active metabolite, confer the coenzyme and program examples mentioned above. The safety evaluation should also include whether a new drug has inhibiting effects on either of these enzymes, e.g., by estimating metabolism of probe drugs in human microsome systems or transfected cellular systems. For example we have recently shown that the antidepressant Duvoxamine is a potent inhibitor of CYP2A2 (Brandt et al. 1993b). A similar effect has led to withdrawal of the drug forstyril due to its inhibition of caffeine metabolism leading to caffeine intoxication from dietary intake. Transfected cell lines can be used for specific elucidation of analogous effects (Jensen et al. 1993).

Recommendations: In the safety evaluation of new drugs it should be examined whether or not they are metabolized by the enzymes that exhibit genetic polymorphism, i.e., CYP2D6, CYP2C and N-acetyltransferase. If this is the case further safety studies are required particularly if the drug has a steep dose response curve and/or the pharmacokinetic effects include influence on vital functions, i.e., a narrow therapeutic index. Particular emphasis should be put on drug that are activated by polymorphic enzymes, in which case even a minor metabolite may be of considerable importance.

Regardless of the drug metabolism route it should be documented that new drugs do not inhibit the enzymes of the major drug-metabolism polymorphisms. These recommendations can be extended to existing drugs as well as to other important routes of drug elimination.

If a new drug depends on bioactivation by CYP2D6 or CYP2C about 4-10% of the population would have no therapeutic effect while they still may suffer from the side-effects. If a new drug is metabolized by a genetic polymorphic enzyme it should be determined if drug action depends on this and/or if the partial clearance is of a sufficient magnitude to result in interindividual variation in drug elimination.

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