Genetic polymorphism in drug metabolism and toxicity: Linking animal research and risk assessment in man

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Abstract
One of the major challenges facing toxicology is to bridge the gap between animal research and risk assessment in man. In this meeting, the genetic polymorphism of drug metabolizing enzymes in relation to drug toxicity will be described.

Keywords: genetic polymorphism, drug metabolism, drug toxicity, species differences, isozymes

Introduction
Advances in molecular toxicology have shown that diversity of drug metabolizing enzymes becomes extremely important to consider the drug toxicity. For an increasing number of such enzymes, allelic variants with different catalytic activities from those of the wild-type form have been identified. It is possible to phenotype or genotype a person with respect to a particular genetic variant, and it is likely that such characterization will be increasingly useful in individualizing drug therapy. As the toxicological aspects of genetic polymorphism, idiosyncratic toxicity of drugs and chemicals has been reported. In this meeting, troglitazone-induced hepatotoxicity will be described.

General implication of genetic variation in drug metabolism
The Pharmacologic and toxic effects of certain drugs are exaggerated in a significant percentage of the population due to a heritable deficiency in a P450 enzyme(Tucker, 1994; Meyer, 1994; Smith et al., 1998). The two major polymorphically expressed P450 enzymes are CYP2D6 and CYP2C19 (Fig. 1). About 70 single nucleotide polymorphisms (SNPs) have been identified in the CYP2D6 gene. Based on the ability of the drug metabolizing enzymes, four phenotypic subpopulations of metabolizers exist: poor(PM), intermediate(IM), extensive(EM), and ultrarapid(UM). Importantly, their frequency varies according to racial background.

Individuals lacking CYP2D6 and 2C19 were initially identified as poor metabolizers of debrisoquine (an antihypertensive drug metabolized by CYP2D6), whereas less than 1 percent of Japanese subjects are defective in CYP2D6 activity. In contrast, 20 percent of Japanese subjects are poor metabolizers of S-mephenytoin (an anticonvulsant metabolized by CYP2C19), whereas less than 5 percent of Caucasians are so affected(Kaneko et al, 1999).

When several P450 enzymes catalyze the same reaction, their relative contribution to xenobiotic biotransformation is determined by the kinetic parameter, Vmax/Km, which is a measure of in vitro intrinsic clearance at low substrate concentrations(<10 percent of Km)(Houston, 1994).

Genetic polymorphism of conjugating enzymes
The N-acetylation of xenobiotics is catalyzed by N-acetyltransferases(NATs) and requires the cofactor acetyl-coenzyme A(acetyl-CoA). These enzymes are located in the cytosolic fraction of liver and many other tissues of most mammalian species, with the exception of the dog and fox, which are unable to acetylate xenobiotics. In contrast to other drug metabolizing enzymes, the...
number of N-acetyltransferases is limited (Vatsis et al., 2000). Humans, rabbits, and hamsters express only two N-acetyltransferases, known as NAT1 and NAT2, whereas mice express three distinct forms of the enzymes, namely NAT1, NAT2, and NAT3. NAT is the official gene symbol for arylamine N-acetyltransferase (EC 2.3.1.5).

Among the conjugating enzymes in drug metabolism, NAT2 was one of the first to be found to have a genetic basis some 50 years ago. This isoform is involved in the metabolism of about 16 common drugs including isoniazid (Fig. 2), procainamide, and caffeine. About 15 allelic variants have been identified, and some of which are without functional effect, but others are associated with either reduced or absent catalytic activity. Considerable heterogeneity is present in the worldwide population frequency of these alleles, so that the slow-acetylator phenotype frequency is about 60% in American whites and 60% in Blacks, but only 10% to 20% in Southeast Asians (Table 1).

Similarly, genetic variability in the catalytic activity of glutathione S-transferases (GSTs) may be linked to individual susceptibility to drug toxicity. GSTs are well recognized to be involved in the conjugation and detoxification of drugs and chemicals. Acetaminophen is metabolized by cytochrome P450 to form the active metabolite having severe hepatotoxicity. The active metabolite is then conjugated by GST to form the inactive metabolite, and excreted in urine.

Toxicologic aspects of genetic polymorphism: Idiosyncratic toxicity of troglitazone

The idiosyncratic toxicity of drugs due to the genetic polymorphism is one of the serious problem in pharmaceutical development and clinical use. Troglitazone has been introduced into the market in 1997 as a new drug for the treatment of insulin-resistant diabetes mellitus. However, it was withdrawn from the market in 2000 due to an incidence of rare but severe hepatotoxicity including death in some patients.

The hepatotoxicity of troglitazone was later diagnosed as an idiosyncratic, hepatocellular injury. A tremendous amount of safety data collected from the experimental animals before regulatory approval failed to predict the adverse reaction of this drug. Toyoda et al. (2001) reported that troglitazone causes apoptosis when added to cultured rat hepatocytes. Funk et al. (2001) reported that the sulfate of troglitazone inhibits the canalicular bile export pump (Bsep), possibly causing the accumulation of bile acids in the hepatocytes and cholestasis. This was also considered an unlikely mechanism since the hepatotoxic patients exhibited signs of hepatocellular injury but not particularly those of cholestasis.

As shown in Fig. 3, Kassahun et al. (2001) demonstrated that CYP3A4 catalyzes the production of chemically reactive forms of troglitazone metabolites which were all detected in vitro as glutathione conjugates. Yamamoto et al. (2002) reported the production of an epoxide metabolite of troglitazone using the CYP3A4 expression system. Gene analysis showed that 40% of the case patients possessed the null genotype of both GSTT1 and GSTM1 (Table 2). The results indicated that a patient-specific deficiency in the scavenger enzymes but not the amount of reactive metabolites produced could be the underlying cause of the hepatotoxicity (Watanabe, 1982).

Table 1. Ethnic differences in the distribution of acetylation phenotype

<table>
<thead>
<tr>
<th>Population</th>
<th>% Slow</th>
<th>% Hetero Rapid</th>
<th>% Homo Rapid</th>
</tr>
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<tbody>
<tr>
<td>South Indians</td>
<td>59</td>
<td>35.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Caucasians</td>
<td>58.6</td>
<td>35.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Blacks</td>
<td>54.6</td>
<td>38.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Eskimos</td>
<td>10.5</td>
<td>43.8</td>
<td>45.7</td>
</tr>
<tr>
<td>Japanese</td>
<td>12</td>
<td>45.3</td>
<td>42.7</td>
</tr>
<tr>
<td>Chinese</td>
<td>22</td>
<td>49.8</td>
<td>28.2</td>
</tr>
</tbody>
</table>


Table 2. Genotyping analysis of troglitazone-induced hepatotoxicity

<table>
<thead>
<tr>
<th>GST gene</th>
<th>Control Patients</th>
<th>Patients with Hepatotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (% )</td>
<td>n (%)</td>
</tr>
<tr>
<td>GSTT1</td>
<td>GSTM1</td>
<td></td>
</tr>
<tr>
<td>wild wild</td>
<td>25 (29%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>wild null</td>
<td>27 (32%)</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>null wild</td>
<td>20 (24%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>null null</td>
<td>13 (15%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td>Total</td>
<td>85 (100%)</td>
<td>25 (100%)</td>
</tr>
</tbody>
</table>

Conclusion: Double null mutation of GSTT1 and GSTM1 might cause troglitazone-induced hepatotoxicity (odds ratio 3.692) \( p = 0.043 \)


Fig. 2 Genetic polymorphism of N-Acetyltransferase
et al., 2003). However, another mechanism is quite likely involved since 15% of the control patients also had the same genotype. It is known that most cases of the idiosyncratic hepatotoxicity are accompanied with some form of immune reactions.

**Conclusions**

The Term "Pharmacogenetics" is the study of genetically controlled variations in drug response. The key concepts and terms of genetic polymorphism include monogenic, polygenic and polymorphic. Monogenic variation is due to allelic variation at a single gene, and polygenic is due to variation at two or more genes. Polymorphic variation is frequently occurring monogenic variation in more than 1%. Thus, characterization of the variants of the drug metabolizing enzymes will become increasingly useful in individualizing drug therapy, especially for drugs with a narrow therapeutic index.

**References**


