NAT2 gene polymorphism: covert drug interaction causing phenytoin toxicity

Important drug metabolizing phase I enzymes such as cytochrome P450 enzymes (CYP2C9, CYP2C19, CYP2D6 and CYP3A4) and phase II enzymes n-acetyltransferase 2 (NAT2), UDP-glucuronosyltransferase (UGT), thiopurine S-methyltransferase (TPMT) are located in the liver. These enzymes are encoded by specific genes and polymorphism of such genes may inhibit or increase enzyme activity. Besides genetic polymorphism, environmental factors and concomitant drug intake can modulate the activity of drug metabolizing enzymes. In this context, drug interactions can occur indirectly mediated through genes. This covert gene-drug interaction is an area of great clinical importance and needs to be investigated in detail.

NAT2 is located in the liver and catalyzes the acetylation of isoniazid (INH), hydralazine, sulphadoxine, procainamide, dapsone and other clinically important drugs. It also catalyzes the acetylation of aromatic and heterocyclic carcinogens. It is implicated in the modification of risk factors in the development of malignancies involving the urinary bladder, colorectal region, breast, prostate, lungs and the head and neck region. It is also shown to be involved in the development of Alzheimer’s disease, schizophrenia, diabetes, cataract and parkinsonism.

The slow and rapid acetylated phenotypes of INH were described about 60 years ago in tuberculosis patients. This difference was shown to be due to genetic variability of NAT2 enzyme which mediates the biotransformation of INH to its metabolite acetyl INH. This is hydrolyzed to acetyl hydrazine and further acetylated by NAT2 to non-toxic diacetyl hydrazine. When there is low NAT activity, acetyl hydrazine is predominantly oxidized by CYP2E1 leading to increased hepatotoxicity.

NAT2 is polymorphic and about 108 NAT2 alleles have been assigned by Arylamine N-acetyl transferase Gene Nomenclature Committee. An Indian study reported the presence of 35 different alleles in Indian populations. NAT2*4 has historically been designated as “wild type” since it is the most commonly occurring allele in some but not all ethnic groups. Based on NAT2 genotypes, there can be three enzymatic phenotypes namely fast (rapid) acetylators (having two fast alleles), intermediate acetylators (one fast and one slow allele) and slow acetylators (two slow alleles).

Slow acetylator status of a patient is clinically more important than the other two phenotypes. People with slow acetylator phenotype are more susceptible to drug interactions with INH and other INH induced toxicity. The clinical significance of NAT2 slow acetylator status has been investigated worldwide. In a Polish study, the average plasma concentration of INH was 2 to 7 fold higher among slow acetylators compared to other types. A study done in Maharashtra, India, reported higher plasma concentration of INH in slow acetylators which correlated with the variant NAT2 genotypes in tuberculosis patients. A Japanese study also reported good concordance between NAT2 genotype and metabolism of INH in patients with tuberculosis. However, in patients with AIDS there was discordance between acetylator genotype and phenotype of NAT2 as measured by caffeine as a probe drug.

Tuberculous meningitis patients are treated with both INH and phenytoin. INH is reported to decrease the clearance of many drugs including phenytoin, carbamazepine, diazepam, vincristine, primidone and acetaminophen. The risk of phenytoin toxicity is higher if INH is given along with it which is supported by several reports. However, Kay et al showed that concomitant administration of INH and rifampicin...
increased the clearance of phenytoin. This has been attributed to rifampicin induced enzyme induction which is not adequately counteracted by INH.

Phenytoin has a saturable pharmacokinetic property. It is mainly metabolized by CYP2C9 (90%) and to a small extent by CYP2C19. The rate of elimination of phenytoin varies as a function of its concentration. Its elimination follows first order kinetics up to 10 µg/ml of plasma concentration and beyond this level it follows saturation kinetics. As a result, any small change in the dose leads to disproportionately higher plasma concentration of phenytoin. INH induced phenytoin toxicity has been widely reported. An in vitro study done in human liver microsomes found that INH was a potent and concentration dependent inhibitor of CYP2C19 and CYP3A enzymes, but it did not produce significant inhibition of CYP2C9 enzyme. The therapeutic concentration of INH causes minimum inhibition of CYP2C9 enzyme, the primary metabolizing enzyme of phenytoin. It appears that INH induced phenytoin toxicity is not due to involvement of CYP2C9 enzyme but due to inhibition of CYP2C19 enzymes which is the alternative pathway when plasma phenytoin level exceeds 10 µg/ml. This is supported by a study published in this issue by Adole et al. demonstrating NAT2 gene polymorphism as a predisposing factor for phenytoin toxicity in patients receiving INH. In this study, the plasma phenytoin level was more than 15 µg/ml in all patients with phenytoin toxicity suggesting saturation kinetics of phenytoin in them. This could be due to indirect effect of NAT2 polymorphic gene increasing the INH level which in turn caused inhibition of phenytoin metabolism. In this pilot study, the plasma INH level was not measured. Therefore, there was no direct evidence that INH levels were elevated by NAT2 polymorphic genes. Further, the frequency of variant alleles of CYP2C9 and C19 were not estimated. CYP2C9 and C19 variant alleles could have caused phenytoin toxicity per se. In the absence of these data, authors could only speculate the contribution of NAT2 mutant alleles that decreased the clearance of phenytoin leading to its toxicity. A previous Indian study reported that even in the absence of concomitant INH administration, genetic polymorphism of NAT2 per se was associated with phenytoin toxicity. It is difficult to explain this finding since NAT2 is an acetylating phase II enzyme which has no direct role in the oxidation of phenytoin. However, in this study also the frequency of variant alleles of CYP2C9 and C19 was not determined which are more important for phenytoin metabolism.

The suggestion that NAT2 mutant alleles inhibited INH metabolism thereby increasing their plasma concentration, and that increased INH levels inhibited CYP2C19 resulting in phenytoin toxicity is interesting. It may suggest a covert gene (NAT2)-drug (INH)-enzyme (CYP2C19) interaction - a new area of clinical pharmacogenomics research. The variant allele of CYP2C19 is present in more than 30 per cent of Indian population which metabolizes several important drugs including antimalarial, oral anticoagulants, antiepileptics, antivirals, antiplatelets, chemotherapeutic agents, proton pump inhibitors as well as several antidepressants. In any patient who may receive INH and happens to be NAT2 slow acetylator type, NAT2 genotype by covert action may influence the clinical response of above drugs. Further studies with other substrates of CYP2C19 may be required to confirm their clinical importance.

In order to translate pharmacogenomics findings into clinical practice, there is a need to develop higher level of evidence such as randomized controlled clinical trials to demonstrate that genotype guided drug therapy will be beneficial and cost-effective. In a Japanese study, NAT2 genotype guided regimen of anti-tuberculosis drugs reduced isoniazid induced liver injury or early treatment failure when compared to patients who received conventional standard treatment. Similar clinical studies need to be conducted with other drugs which are metabolized by polymorphic drug metabolizing enzymes.

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References


