Indian J Med Res 143, May 2016, pp 542-544
DOI:10.4103/0971-5916.187101

Commentary

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[1-3].

The slow and rapid acetylated phenotypes of INH were described about 60 years ago in tuberculosis patients[4]. 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[5].

NAT2 is polymorphic and about 108 *NAT2* alleles have been assigned by Arylamine N-acetyl transferase Gene Nomenclature Committee[6]. An Indian study reported the presence of 35 different alleles in Indian populations[7]. *NAT2*4* has historically been designated as "wild type" since it is the most commonly occurring allele in some but not all ethnic groups[3]. 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)[8].

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[9]. 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[5]. 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[10]. A Japanese study also reported good concordance between *NAT2* genotype and metabolism of INH in patients with tuberculosis[11]. However, in patients with AIDS there was discordance between acetylator genotype and phenotype of NAT2 as measured by caffeine as a probe drug[12].

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[13]. The risk of phenytoin toxicity is higher if INH is given along with it which is supported by several reports[14,15]. However, Kay *et al*[16] 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[16].

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 upto 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[17-19]. INH induced phenytoin toxicity has been widely reported[14,15,20]. 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[13]. 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*[21] 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[22]. 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[21]. 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, anti-epileptics, antivirals, antiplatelets, chemotherapeutic agents, proton pump inhibitors as well as several antidepressants[23]. 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[24]. Similar clinical studies need to be conducted with other drugs which are metabolized by polymorphic drug metabolizing enzymes.

C. Adithan[1,*] & A. Subathra[2]
[1]Central Interdisciplinary Research Facility &
Department of Pharmacology
Mahatma Gandhi Medical College &
Research Institute, Pillaiyarkuppam,
Puducherry 607 403 &
[2]Department of Radiodiagnosis,
Jawaharlal Institute of Postgraduate
Medical Education & Research (JIPMER),
Puducherry 605 006, India
[*]*For correspondence*:
adithan50@gmail.com

References

1. Butcher NJ, Boukouvala S, Sim E, Minchin RF. Pharmacogenetics of the arylamine N-acetyltransferases. *Pharmacogenomics J* 2002; *2* : 30-42.
2. Harmer D, Evans DA, Eze LC, Jolly M, Whibley EJ. The relationship between the acetylator and the sparteine hydroxylation polymorphisms. *J Med Genet* 1986; *23* : 155-6.

3. Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, *et al*. Molecular genetics and epidemiology of the *NAT1* and *NAT2* acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev* 2000; *9* : 29-42.

4. Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am Rev Tuberc* 1954; *70* : 266-73.

5. Zabost A, Brzezińska S, Kozińska M, Błachnio M, Jagodziński J, Zwolska Z, *et al*. Correlation of N-acetyltransferase 2 genotype with isoniazid acetylation in Polish tuberculosis patients. *Biomed Res Int* 2013 : 853602.

6. Arylamine N-acetyl transferase Gene Nomenclature Committee. Available from: *http://nat.mbg.duth.gr/Human%20NAT2%20alleles_2013.htm*, accessed on May 1, 2016.

7. Khan N, Pande V, Das A. *NAT2* sequence polymorphisms and acetylation profiles in Indians. *Pharmacogenomics* 2013; *14* : 289-303.

8. Parkin DP, Vandenplas S, Botha FJ, Vandenplas ML, Seifart HI, van Helden PD, *et al*. Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. *Am J Respir Crit Care Med* 1997; *155* : 1717-22.

9. Ng CS, Hasnat A, A1 Maruf A, Ahmad MU, Pirmohamed M, Day CP, *et al*. N-acetyltransferase 2 (NAT2) genotype as a risk factor for development of drug-induced liver injury relating to antituberculosis drug treatment in a mixed-ethnicity patient group. *Eur J Clin Pharmacol* 2014; *70* : 1079-86.

10. Singh N, Dubey S, Chinnaraj S, Golani A, Maitra A. Study of *NAT2* gene polymorphisms in an Indian population: association with plasma isoniazid concentration in a cohort of tuberculosis patients. *Mol Diagn Ther* 2009; *13* : 49-58.

11. Kita T, Tanigawara Y, Chikazawa S, Hatanaka H, Sakaeda T, Komada F, *et al*. N-acetyltransferase2 genotype correlated with isoniazid acetylation in Japanese tuberculous patients. *Biol Pharm Bull* 2001; *24* : 544-9.

12. Wolkenstein P, Loriot MA, Aractingi S, Cabelguenne A, Beaune P, Chosidow O. Prospective evaluation of detoxification pathways as markers of cutaneous adverse reactions to sulphonamides in AIDS. *Pharmacogenetics* 2000; *10* : 821-8.

13. Desta Z, Soukhova NV, Flockhart DA. Inhibition of cytochrome P450 (CYP450) isoforms by isoniazid: potent inhibition of CYP2C19 and CYP3A. *Antimicrob Agents Chemother* 2001; *45* : 382-92.

14. Adole PS, Singh A, Kharbanda PS, Sharma S. Phenotypic interaction of simultaneously administered isoniazid and phenytoin in patients with tuberculous meningitis or tuberculoma having seizures. *Eur J Pharmacol* 2013; *714* : 157-62.

15. Miller RR, Porter J, Greenblatt DJ. Clinical importance of the interaction of phenytoin and isoniazid: a report from the Boston Collaborative Drug Surveillance Program. *Chest* 1979; *75* : 356-8.

16. Kay L, Kampmann JP, Svendsen TL, Vergman B, Hansen JE, Skovsted L, *et al*. Influence of rifampicin and isoniazid on the kinetics of phenytoin. *Br J Clin Pharmacol* 1985; *20* : 323-6.

17. Giancarlo GM, Venkatakrishnan K, Granda BW, von Moltke LL, Greenblatt DJ. Relative contributions of CYP2C9 and 2C19 to phenytoin 4-hydroxylation *in vitro*: inhibition by sulfaphenazole, omeprazole, and ticlopidine. *Eur J Clin Pharmacol* 2001; *57* : 31-6.

18. McNamara JO, Pharmacotherapy of the epilepsies. In: Brunton LL, Chabner BA, Knollman BC, editors. *Goodman & Gilman's the pharmacological basis of therapeutics*. 12th ed. New York: McGraw-Hill Companies; 2011. p. 583-607.

19. Hvidberg EF, Dam M. Clinical pharmacokinetics of anticonvulsants. *Clin Pharmacokinet* 1976; *1* : 161-88.

20. Walubo A, Aboo A. Phenytoin toxicity due to concomitant antituberculosis therapy. *S Afr Med J* 1995; *85* : 1175-6.

21. Adole PS, Kharbanda PS, Sharma S. N-acetyltransferase 2 (*NAT2*) gene polymorphism as a predisposing factor for phenytoin intoxication in tuberculous meningitis or tuberculoma patients having seizures - A pilot study. *Indian J Med Res* 2016; *143* : 581-90.

22. Murali M, Manjari T, Madhuri B, Raghavan S, Jain DC, Vivekanandhan S. Genetic polymorphism of *NAT2* metabolizing enzymes on phenytoin pharmacokinetics in Indian epileptic patients developing toxicity. *CNS Neurosci Ther* 2012; *18* : 350-8.

23. Umamaheswaran G, Kumar DK, Adithan C. Distribution of genetic polymorphisms of genes encoding drug metabolizing enzymes & drug transporters - a review with Indian perspective. *Indian J Med Res* 2014; *139* : 27-65.

24. Azuma J, Ohno M, Kubota R, Yokota S, Nagai T, Tsuyuguchi K, *et al*. Pharmacogenetics-based tuberculosis therapy research group. *NAT2* genotype guided regimen reduces isoniazid-induced liver injury and early treatment failure in the 6-month four-drug standard treatment of tuberculosis: a randomized controlled trial for pharmacogenetics-based therapy. *Eur J Clin Pharmacol* 2013; *69* : 1091-101.