1998 INTERNATIONAL MEETING ON THE ARYLAMINE N-ACETYLTRANSFERASES:
SYNOPSIS OF THE WORKSHOP ON NOMENCLATURE, BIOCHEMISTRY, MOLECULAR
BIOLOGY, INTERSPECIES COMPARISONS, AND ROLE IN HUMAN DISEASE RISK

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ABSTRACT:

On October 22–24, 1998, a workshop was held at Kuranda, Queensland, Australia. The purpose of the meeting was to provide a forum for discussion of a number of diverse research areas of the biochemistry and molecular biology of arylamine N-acetyltransferases and to foster collaboration among several major groups of investigators around the world. In addition, participants were asked to consider how the nomenclature system for arylamine N-acetyltransferases could be strengthened to cope with the burgeoning number of new alleles discovered in the last 3 years. The full text of all meeting abstracts can be viewed at http://www.pharm.uwa.edu.au/workshop/prog.html.

Synopsis

Molecular Aspects of Arylamine N-Acetyltransferases (NATs)

Delgoda and coworkers undertook structural studies of NATs using the homologous NAT from Salmonella typhimurium. This NAT has about 25% identity to the human amino acid sequences. NMR studies were used to investigate the Ping Pong Bi Bi reaction mechanism while the enzyme itself was crystallized from sodium potassium tartrate. Preliminary X-ray diffraction data showed an orthorhombic unit cell with an unusual 6-fold symmetry. Computational analysis suggested a highly conserved α helical N-terminal region that packs against a more variable predominantly β sheet C terminus. Hanna and coworkers also had made considerable progress toward growing crystals of hamster NAT1, after having developed an efficient novel bacterial expression system for this isoform.

Fakis and colleagues provided preliminary information on the physical location of NATs in the human and mouse genomes. The genomic restriction fragment length polymorphism marker D8S21 (used by the Human Genome Project) was found to be located within the NAT2 gene on chromosome 8. NAT2 and NATP were much closer to each other, whereas NAT1 and NAT2 are somewhat farther apart. Family studies showed that there was a significant linkage disequilibrium between the NAT1*10 allele and the NAT2*4 allele such that the haplotype occurs 3 times more frequently than would be expected. However, the significance of this observation is unclear. In the mouse, Nat1 and Nat2 were much closer to each other and also on chromosome 8. Nat3 appeared to be within 1 Mb of the other two loci.

NAT and Development

Work from Edith Sim’s group using selective NAT1 [p-aminobenzoic acid (PABA) and p-aminobenzoylglutamate (PABG)] and NAT2 [sulfamethazine (SMZ)] substrates showed that NAT1 activity was predominant in placenta and that it was present in all three trimesters (1 > 2 > 3 < at birth). PABA and PABG metabolism were highly correlated and the metabolite N-acetyl-PABG was identified by gas chromatography-mass spectrometry. In other experiments (Payton and colleagues), mouse Nat2 (the functional equivalent of human NAT1) was found to metabolize PABG. Activity was greater in the C57Bl/6 than in the A/J mouse strain. PABG metabolism was detected in a wide range of tissues, including brain, and thus murine NAT2 might have a role in the endogenous catabolism of folate. Antibodies against murine NAT2 were used to demonstrate that there was intense staining of the mouse embryo neural tube at 9.5 days of gestation, which is just before closure of the tube. These studies should provide the tools for further investigation of the hypothesis that NATs may play a pivotal role in neural tube defects in humans.

Other studies in mice (Estrada-Rogers and coworkers) showed that over the first 80 days of life, PABA metabolism is increased 2.5- and 5.8-fold in females and males, respectively. Nat2 mRNA was selectively increased in the male kidney but not in the liver. Studies of the promoter region of murine Nat2 showed a palindromic hormone responsive element site that mediated androgenic regulation of the gene in the kidney. In the 5′-UTR of the NAT1 gene, Minchin and colleagues characterized a minimum promoter sequence. An AP-1-like motif, flanked by ATCATTT repeats, was shown to be functional in several human cell lines.

The mouse also was used as a model system to assess pre- and postnatal acetylation of the carcinogen 4-ABP (McQueen and coworkers). Both Nat1 and Nat2 were present in fetal tissues at days 10 and 15 of development. Only Nat2 was seen in fetal liver on day 18 of development and day 3 postnatal. Adducts of 4-ABP were demonstrated in fetal tissues after its administration to the mother. These
data indicate that in situ formation of reactive metabolites of aromatic amines could play a role in their developmental toxicology.

**NAT and Disease.** Contributions in this area included studies on the association of the NAT’s with lung, colon, and urinary bladder cancer, and asthma. Overall, an important point arising from the discussions was that single gene effects are often not easy to demonstrate but gene interactions are increasingly important in such associations and will require larger study populations.

Despite the long history of a strong association between bladder cancer and the slow NAT2 phenotype, important new studies are still being undertaken. Improvements in genotyping/phenotyping methods offer a more detailed analysis than was possible in the past. Grant and coworkers confirmed the lack of CYPIA2 in bladder epithelium and the significant presence of prostaglandin H synthase, suggesting that the latter could play a role in local carcinoma bioactivation. NAT1 activity in bladder was some 400 times higher than NAT2 activity, but in a case-control study \((n = 96)\), there were no significant differences between patients and controls in terms of either phenotype or genotype frequencies. This negative result was in marked contrast to a number of previous studies showing a positive association between bladder cancer and NAT2 phenotype. It was suggested that previous studies with negative findings may not have been published because of “negative publication bias”. Furthermore, in discussion it transpired that if genotype did have a significant role to play, it would be for those subjects with an intermediate level of exposure to occupational carcinogens. High levels of exposure would most likely lead to tumors irrespective of genotype whereas for low levels of exposure, genotype would be unlikely to alter the incidence of cancer significantly.

Links between occupational exposure to asbestos and lung cancer were investigated by Saarikoski and coworkers who found that the prevalence of NAT2 slow acetylator genotype and glutathione S-transferase M1 null genotype was similar for both patients and controls. However, Hirvonen and colleagues identified NAT1 genotype as an important modifier of individual lung cancer risk in French Caucasian smokers. In particular, the slow NAT1 genotype was associated with significantly greater risk. By contrast, NAT2 genotype showed no association with lung cancer.

Wikman and colleagues found a significant association between occupational exposure to isocyanates and asthma prevalence. These chemicals are widely used in the manufacture of plastic foams and are first hydrolyzed to amines before being acetylated. Individuals with combined NAT1 and NAT2 slow genotypes had a 2.6-fold greater risk of developing asthma.

Hein and coworkers studied human NAT1 and NAT2 genotype and phenotype determinations in cytosolic preparations from surgical human colon specimens and confirmed that colon tissue from patients with NAT2 intermediate and slow genotypes showed high and low SMZ activity in vitro, respectively. NAT1-dependent PABA activity in colon was higher, but was not related to NAT2 or NAT1 genotypes. Moreover, there was no association between NAT1*10 and PABA activity, suggesting that the NAT1*10 allele did not affect enzyme activity. The authors suggested that much larger numbers of subjects would be needed to evaluate the validity of the previously demonstrated epidemiological relationship between NAT1*10 and colon cancer.

**NAT in Bacteria and Other Species.** The idea that the study of NAT in intestinal bacteria might provide insight into structure-function relationships, contribute to bioactivation of arylamine carcinogens in humans, or lead to new mutagenicity tests was explored by Dupret and colleagues. NAT activities in a wide variety of eubacteria were probed with a *Salmonella typhimurium* DNA fragment and signals were detected in seven Gram-negative species. NAT activity \((K_m, V_{max})\) was also determined using 2-aminofluorene and SMZ as substrates, and significant activity was found in seven species. *Citrobacter freundii*, *Citrobacter koseri*, and *Pseudomonas aeruginosa* had higher catalytic activity than *Salmonella typhimurium*. No species metabolized p-aminosalicylic acid or PABA and in discussion it transpired that, contrary to some published reports, *Helicobacter pylori* did not exhibit NAT activity. The suggestion was made that the function of NAT in bacteria could be a protective mechanism for folate conservation, given that bacteria have to derive their folate requirements from their environment.

**Characterization and Localization of NAT.** Site-directed mutagenesis was used by Goodfellow and coworkers to show that the size of the amino acid side chain at position 125 in the NAT gene produces selectivity for NAT2, whereas charge of the amino acid at position 127 was a key determinant of NAT-type behavior. Using fluorescence in situ hybridization, Johnson and colleagues investigated the loss of heterozygosity in exfoliated cells from bladder cancer patients. In a significant proportion of cells, both NAT1 and NAT2 were deleted from chromosome 8 at the 8p22 region. Because this region may also contain important tumor suppressor genes, further mapping in this region of chromosome 8 is warranted. NAT1 and NAT2 themselves were considered unlikely to have tumor suppressor functionality but could be used as markers of investigated loss of heterozygosity.

Data on the location of NAT activity in human gut were presented by Sim and coworkers. NAT2 activity (SMZ) was generally higher in the upper than in the lower gut, whereas NAT1 activity (PABA) generally was similar in all areas of the gut. Moreover, NAT1 activity was only present in the gut mucosa and not in the underlying muscularis. Histochemical studies showed that NAT1 was present in the tips of the colonic villi but not in the crypts.

**Down-Regulation of NAT.** This was a novel topic that engendered considerable interest at the meeting. Significant environmental control in the expression of NAT1 (PABA) activity in human peripheral blood mononuclear cells was demonstrated by Butcher and coworkers. Although the underlying cause of this variability was not identified, regulation of NAT1 activity by PABA in vitro was demonstrated. Preliminary findings suggested a negative regulatory effect of PABA on NAT1 translation. Kundub and coworkers showed that addition of the coffee lipids kahweol and cafestol to the diet was able to decrease 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-DNA adducts in rat colon. Moreover, in rat liver and cultured rat hepatocytes, both NAT1 activity (orthologous to human NAT2) and mRNA were also decreased.

**Nomenclature of NAT.** Weber led the discussion on this topic and started with an update on the number of new alleles that have been discovered since the 1995 review paper by Vatsis et al. (1995). As of September 1998, a total of 31 NAT1* (22 human and 9 other species) and 40 NAT2* (26 human and 14 other species) alleles had been described. There was general agreement that, irrespective of species and as per the original rules, alleles should continue to be classified on the basis of nucleotide sequence homology. New alleles should be given a name irrespective of whether they were published in a journal. It was pointed out that sequences could and should be published in Genebank. The complete sequence of the gene for any new allele must be submitted so that confusion with previously named alleles can be avoided. A committee comprising D. Hein, D. Grant, and E. Sim volunteered to continue the ongoing task of assigning names to new alleles. A web site (http://www.louisville.edu/medschool/pharmacology/NAT.html) where information on new NAT’s will be readily accessible to all researchers has been established. However, some
journals and the Human Genome Project may require an alternative enzyme-naming system to be used and it was agreed therefore that all relevant journal editors and other appropriate persons would be approached with a view to achieving a uniform nomenclature system.

Conclusions and Future Directions

These can be summarized as follows:

- To avoid confusion, the nomenclature of new NATs needs to be clearly defined.
- Structural characterization of NATs using techniques such as X-ray crystallography and NMR spectroscopy should provide a basis for understanding enzyme-substrate interactions at the molecular level.
- The relationship between NATs and disease requires clinical studies with large n values so that gene effects can be dissected with appropriate statistical power.
- Regulation of NATs at the gene level will be an important area for future study and may assist our understanding of genotoxicity and its association with human disease.
- The role of NATs in embryonic development deserves further study as it has the potential to assist in identifying a putative endogenous substrate(s) for NAT1.

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Reference