Environmental Health Perspectives
Vol. 98, pp. 69–74, 1992

Polymorphisms for Aromatic Amine Metabolism in Humans: Relevance for Human Carcinogenesis

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The metabolic pathways associated with carcinogenic aromatic amines in humans provide an excellent example of polymorphisms that appear to be relevant to human carcinogenesis. In this regard, the *N*-acetylation of arylamines and the *O*-acetylation of their *N*-hydroxy metabolites are catalyzed preferentially by a genetically polymorphic acetyltransferase, high activity of which has been correlated with decreased risk for urinary bladder cancer and increased susceptibility to colorectal cancer. Cytochrome P450IA2, the principal liver enzyme involved in aromatic amine *N*-oxidation, exhibits a wide interindividual variation that appears trimodal in several populations and is clearly inducible by cigarette smoking and probably other host factors as well. UDP-Glucuronosyltransferases, which catalyze the *N*-glucuronidation of *N*-hydroxyarylamines and are likely to be responsible for their transport to the colon, show widely varied but unimodal distributions in humans. In contrast, human liver sulfotransferase activity for *N*-hydroxyarylamines, which would be expected to decrease their transport through the circulation, is catalyzed by a polymorphic enzyme(s) that is expressed at higher levels in blacks, as compared to whites, and could contribute to their relatively lower incidence of urinary bladder cancer. Peroxidative activation of aromatic amines can also occur, especially from prostaglandin *H* synthase in the urinary bladder and myeloperoxidase in the lungs of cigarette smokers, and both show considerable individual variability, apparently due to the extent of tissue inflammation. In a pilot study, we have examined two of these polymorphisms, acetyltransferase and cytochrome P450IA2, in colorectal cancer/polyp cases (n = 38) and controls (n = 100) and found that individuals who are both rapid acetylators and rapid *N*-oxidizers are indeed more prevalent ($p < 0.008$) among cases (37%) than among controls (16%).

Introduction

The wide variation in carcinogen metabolism in humans has long been regarded as an important determinant of individual susceptibility to chemical carcinogenesis (*1,2*). In the case of aromatic amine carcinogens, it has become apparent that the biochemical basis for these differences may be the polymorphic distribution of specific carcinogen-metabolizing enzymes involved in their activation and/or detoxification (*3,4*). These polymorphisms can arise from both heritable and environmental factors, which can be assessed in epidemiological studies. With the recent development of methods for metabolic phenotyping and genotyping, together with current techniques to detect carcinogen-protein and carcinogen-DNA adducts in human tissues, it should now be possible to assess much more accurately both susceptibility and exposure that may be truly predictive of individual cancer risk.

The role of aromatic amines has been well established in cancer of the human urinary bladder. In the early part of this century, industrial exposures to 4-aminobiphenyl (ABP), 2-naphthylamine, and benzidine were clearly associated with a high incidence of transitional urothelial-cell carcinomas (*5*); recently, occupational exposures to 4,4′-methylenebis(2-chloroaniline) (MOCA) and *o*-toluidine have also been correlated with increased bladder cancer risk (*6,7*). Likewise, cigarette smoking has often been implicated as a causative factor in urinary bladder carcinogenesis (*8*); and this association has been supported by findings that aromatic amines such as ABP are present in nanogram quantities in cigarette smoke (*9*) and that smokers have much higher level of aromatic amine-hemoglobin adducts than nonsmokers (*10*). Recently, we showed that several smoking-related DNA adducts present in human urothelium are characteristic of aromatic amine-C8-deoxyguanosine adducts and identified *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct (*11*).

An increasing body of evidence indicates that aromatic amines may play a significant role in the etiology of human

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colorectal cancer. Greater susceptibility to this disease has long been associated with dietary factors, including consumption of well-done red meats (*12*) and of cured and smoked meats (*13*). During the cooking process, pyrolysis of meat liquids can result in the formation of mutagenic and carcinogenic heterocyclic amines (*14*), such as 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1). Several of these aromatic amines are known to induce tumors in experimental animals in a variety of tissues, notably the liver and intestine (*15*); and they have also been detected in urine of individuals who consume a normal, meat-containing diet (*16*). Their metabolic activation pathways have been extensively studied in rodents, and we have recently shown that human liver and colon tissues possess comparable enzyme systems (*17*).

The involvement of aromatic amines in other human cancers is unclear, although limited epidemiological evidence suggests a role in cancers of the lung, liver, colon, and pancreas (*18-20*). In this regard, ABP–DNA adducts have been detected at high levels in the lungs of both smokers and nonsmokers (*20*).

In this report, we examine the biochemical properties of the major aromatic amine metabolizing enzymes in human tissues, their polymorphic distribution in human populations, and the implications of these findings for individual susceptibility to aromatic amine-induced cancers. The discussion will include the acetyltransferases, cytochromes P450, UDP-glucuronosyltransferases, sulfotransferases, and peroxidases. Finally, the application of current phenotyping methods in an on-going epidemiological study will be discussed.

Acetyltransferases

Acetyltransferase activity in humans is coded by two distinct genes, designated *NAT1* and *NAT2* (*21–23*). The former activity appears to be monomorphically distributed in humans, while the latter exhibits a polymorphism that allows the detection of phenotypically slow and rapid metabolizers. The polymorphism of *NAT2* arises from point mutations in coding and noncoding regions that can result in decreased expression, low activity, or enzyme instability. In human colon mucosa, high activities of both *NAT1* and *NAT2* have been found; however, only *NAT2* is expressed in the liver and at high levels in rapid acetylator individuals (*17*). At low substrate concentrations, *NAT1* selectively catalyzes the *N*-acetylation of *p*-aminobenzoic acid, while *NAT2* selectively *N*-acetylates sulfamethazine and several arylamine drugs and carcinogens (*20*). *NAT2* can also catalyze the *O*-acetylation of the *N*-hydroxyarylamine metabolites, which results in their conversion to an ultimate carcinogen that forms arylamine–DNA adducts (*24*). Overlapping substrate specificity between *NAT1* and *NAT2* is also apparent in the case of 2-aminofluorene *N*-acetylation and the *O*-acetylation of certain *N*-hydroxyarylamines (*17,21,24*).

The *NAT2* polymorphism can have a significant effect on individual susceptibility to aromatic amine-induced cancers. For urinary bladder carcinogenesis, *N*-acetylation of arylamines such as ABP and 2-naphthylamine represents a competing pathway for *N*-oxidation, a necessary metabolic activation step occurring in the liver. The unconjugated *N*-hydroxy metabolite can then enter the circulation, undergo renal filtration, and be transported to the urinary bladder lumen, where reabsorption and covalent binding to urothelial DNA can occur (*25*). Consequently, a number of studies have demonstrated increased cancer risk in phenotypically slow acetylators with documented occupational exposure to arylamines (*26*). Moreover, among cigarette smokers, the slow acetylator individual forms significantly higher levels of ABP–hemoglobin adducts (*27*).

Two of three reports on human colorectal cancer, (*13,28,29*) have found that rapid acetylators are overrepresented among cases as compared to controls. In this context, we have shown that the heterocyclic amines, which have been implicated as a risk factor in this disease, are poor substrates for *N*-acetylation in human liver; however, they readily undergo hepatic *N*-oxidation and subsequent *N*-glucuronidation (*vide infra*), resulting in conjugated *N*-hydroxy metabolites that can be transported to the colonic lumen, hydrolyzed by β-glucuronidases, and reabsorbed. In colonic mucosa, the *N*-hydroxy derivatives are good substrates for *O*-acetylation, which results in reactive *N*-acetoxyarylamines that form covalent DNA adducts (*17*). Thus, the association of higher risk for colorectal cancer with rapid acetylator phenotype is consistent with both an etiologic role for heterocyclic amines and their metabolic activation pathway in humans.

Cytochromes P450

The cytochromes P450 (CYPs) in humans consist of at least 20 different proteins that are coded by a multi-gene superfamily (*30*). Studies conducted thus far suggest, however, that only about four isoforms of these CYPs play a major role in carcinogen metabolism (*31*). These are CYP1A1, CYP1A2, CYP2E1, and CYP3A4. In this regard, polycyclic aromatic hydrocarbons appear to be activated principally by CYP1A1 and CYP3A4; low-molecular-weight nitrosamines, hydrazines, halogenated hydrocarbons, and vinyl compounds by CYP2E1; mycotoxins by CYP3A4; and most carcinogenic aromatic amines by CYP1A2 (*31*). CYP1A2 catalyzes the *N*-oxidation of ABP, 2-naphthylamine, IQ, MeIQx, PhIP, Glu-P-1, and a variety of other heterocyclic and bicyclic aromatic amines. 6-Aminochrysene and MOCA, however, are *N*-oxidized primarily by CYP3A4. Arylamine *N*-oxidation by human CYP1A1 may also occur to a limited extent; however, comparison with data in experimental animals (*32*) indicates that a role for CYP1A1 in detoxification through ring-hydroxylation is more likely.

CYP1A2, CYP2E1, and CYP3A4 are expressed at high levels in human liver; while CYP1A1 appears to be present only at low levels in human lung, placenta, and lymphocytes; and CYP3A4 is found at low levels in human intesti-

nal mucosa (*31*). Each of these CYPs appears to exhibit wide interindividual variation, and evidence for their inducibility by drugs and by environmental and dietary factors has been reported. Whether or not genetic polymorphisms exist is as yet unknown, although an apparently bimodal distribution for CYP1A2 in liver microsomes has been described (*33,34*). Since the identification of caffeine 3-demethylation (CYP1A2), chlorzoxazone 6-hydroxylation (CYP2E1), and cortisol 6β-hydroxylation (CYP3A4) as selective assays for these CYPs (31), studies are currently in progress in several laboratories on the application of phenotyping (and genotyping) methods to human populations.

Caffeine has been used for the determination of aromatic amine acetylation phenotype (*35*) on the basis of the urinary ratio of two metabolic end-products, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X). Since caffeine 3-demethylation was found to be selectively catalyzed by CYP1A2 and also represents the major pathway for caffeine disposition in humans (*36*), we decided to examine its use for phenotyping human populations to assess their proficiency for both aromatic amine acetylation and *N*-oxidation *in vivo*. The analytical procedure that we have developed is a modification of the acetylator phenotyping method (*35*) and involves high-performance liquid chromatography with quantification by diode array detection. In order to select a urinary ratio that accurately reflects caffeine 3-demethylation (and aromatic amine *N*-oxidation), a pharmacokinetic study was carried out in 12 individuals in whom the levels of caffeine and 15 of its metabolites in blood and urine were measured over a 48-hr period after caffeine dosing (3 mg/kg). The results indicated that the urinary ratio of [1,7-dimethylxanthine (17X) + 1,7-dimethyluric acid (17U)]/caffeine (137X), obtained 4-5 hr after caffeine ingestion, is better correlated ($r = 0.73$; $p = 0.007$) with the proficiency of caffeine 3-demethylation ($K_{137X \rightarrow 17X}/K_{137X \rightarrow urine}$) than are the ratios of 17X/137X ($r = 0.54$; $p = 0.07$) or [AFMU + 1X + 1U]/17U ($r = 0.33$; $p = 0.30$), which were suggested previously for this purpose (*37,38*).

Metabolic phenotyping of cytochrome CYP1A2, using the urinary ratio of (17X + 17U)/137X, has now been carried out on several groups of normal subjects from Arkansas (n = 100), Italy (n = 95), and China (n = 39). Pearson's chi-squared test of normality indicates that this urinary ratio is not normally distributed ($p < 0.01$) in any of these populations. Moreover, probit analysis and frequency plots show apparent trimodal distributions with different cut-off points in smokers and nonsmokers. These data provide strong evidence that CYP1A2 is induced in humans by cigarette smoking and are consistent with results from previous studies that show that smoking increases caffeine clearance (*39*), phenacetin *O*-deethylation (*40*) and levels of CYP1A2 in human liver microsomes (33,34). More importantly, the apparent trimodal distribution allows for the designation of slow, intermediate, and rapid metabolizers within these populations. These differences may arise from a genetic polymorphism in the *CYP1A2* gene or in a regulatory gene, from individual differences in exposure to inducing agents, as well as from other epigenetic factors. Nevertheless, although it is not yet known if this polymorphism represents a genotypic or phenotypic determinant, the caffeine metabolic phenotyping procedure is suitable for use in epidemiological studies and may provide a means for assessing the role of aromatic amines in human carcinogenesis.

UDP-Glucuronosyltransferases

The human UDP-glucuronosyltransferases (UGTs) appear to consist of two multigene superfamilies, designated *UGT1A* and *UGT2A/UGT2B* (*41*). These transferases exhibit distinct but overlapping substrate specificity and are known to catalyze the glucuronidation of a variety of phenols, arylamines, steroids, and bilirubin. Enzymatic activity is highest in the liver, followed by kidney and intestinal mucosa (*42*). We have shown that human liver microsomes can catalyze the *N*-glucuronidation of several *N*-hydroxyarylamines, including those derived from ABP and 2-naphthylamine (*43*), and from IQ, MeIQx, PhIP, and Glu-P-1 (*44*). By comparison with the findings from animal studies (*43,45*), these results suggest that the *N*-hydroxyarylamine *N*-glucuronides may also serve as proximate carcinogens in humans as a consequence of their transport via the urine or bile to the carcinogen-target tissue—the urinary bladder or colon, respectively. Hydrolysis of the *N*-glucuronide in the urinary bladder lumen under slightly acidic conditions can regenerate the *N*-hydroxyarylamine and the corresponding arylnitrenium ion, which can react with DNA in the urothelium (*25,43*). In the colonic lumen, β-glucuronidase can cleave the glucuronide to yield the *N*-hydroxyarylamine, which can then undergo further activation by *O*-acetylation in the colonic mucosa [(*17*) *vide supra*].

In human liver, at least five different UGT gene products are expressed (*46*). At present, it is not known which UGT isozyme is responsible for *N*-glucuronidation of *N*-hydroxyarylamines. Studies (K. Kaderlik, B. Burchell, and F. F. Kadlubar, unpublished data) using cultured cells that express cloned human UGT1A1 failed to show any activity for the *N*-hydroxy derivatives of Glu-P-1 or PhIP. With a variety of substrates, a wide variation (4- to 19-fold) in liver UGT activity has been reported (*47–49*); however, the distribution appears to be unimodal (*49*). Except in the case of UGT1A2, which is associated with defective bilirubin conjugation diagnostic of the Crigler–Najar syndrome (*50*), there is as yet no evidence for a polymorphism in human populations. Since UGT activities are known to be inducible in humans by cigarette smoking and by barbiturates (*50*), however, characterization of this activity may allow an assessment of host factors that could affect susceptibility to aromatic amine induced carcinogenesis in humans.

Sulfotransferases

Phenol sulfotransferases, which catalyze the sulfate conjugation of catechol and phenolic drugs and other xenobiotics, have been studied in several human tissues, including the liver, small intestine, cerebral cortex, kidney,

and blood platelets (*42,51*). Two forms of sulfotransferase have been characterized: a thermolabile (TL) form that catalyzes the sulfation of dopamine and other phenolic monoamines and is relatively resistant to inhibition by 2,6-dichloro-4-nitrophenol; and a thermostable (TS) form that catalyzes the sulfation of a variety of phenols, such as *p*-nitrophenol, and is highly sensitive to 2,6-dichloro-4-nitrophenol inhibition. The TL and TS enzymes are independently regulated (*52*) and appear to be expressed at highest levels in the liver, but the levels can vary widely in tissues from different individuals. For example, there is no correlation between levels of TL activity in platelets and in liver and small intestine from different individuals; while levels of platelet, liver, and intestinal TS activity are well correlated in this regard (*53,54*). Furthermore, several studies now indicate that the TS sulfotransferase exhibits a genetic polymorphism in humans that is polygenic and is responsible for regulation of both the level of activity and the thermal stability of the enzyme (*55-57*). A racial difference in TS sulfotransferase activity has also been reported, the mean level of platelet activity in blacks being about 2-fold higher than that in whites (*58*).

We recently examined the role of the liver TS and TL sulfotransferases in the conjugation of the *N*-hydroxy metabolites of several carcinogenic aromatic and heterocyclic amines (*59*). *N*-Hydroxy-2-aminofluorene, *N*-hydroxy-ABP, *N*-hydroxy-MOCA, and, to lesser extent, *N*-hydroxy-PhIP and *N*-hydroxy-Glu-P-1 were substrates for metabolic activation; but no activity was detected for the *N*-hydroxy derivatives of IQ and MeIQx. Studies of inhibition and thermal stability further indicated that it was the human liver TS sulfotransferase that was primarily responsible for these activities, while the TL enzyme may also play a significant role in the metabolic activation of *N*-hydroxy-MOCA, *N*-hydroxy-PhIP, and *N*-hydroxy-Glu-P-1.

Although the hepatic sulfate conjugation of *N*-hydroxyarylamines results in formation of a reactive electrophile and could increase the risk for initiation of liver neoplasia, the facile solvolysis of these esters to phenolic sulfates also represents a major detoxification pathway and usually accounts for the major excretion product of aromatic amine bladder carcinogens in experimental animals (*60*). Thus, higher levels of hepatic sulfotransferase in humans would be expected to decrease the amount of free *N*-hydroxy metabolite available to enter the circulation and be transported to the urinary bladder (*25*). Accordingly, the TS sulfotransferase polymorphism might be expected to result in individual differences in susceptibility to aromatic amine-induced urinary bladder carcinogenesis. In particular, the higher levels of TS sulfotransferase in American blacks could account for their observed lower relative risk to cigarette-smoking related urinary bladder cancer (61).

Hepatic sulfotransferase activity could also serve as a competing pathway for the *N*-glucuronidation of *N*-hydroxyarylamines. The relatively poor substrate specificity of the phenol sulfotransferases for the *N*-hydroxy heterocyclic amines suggests, however, that TS sulfotransferase polymorphism is unlikely to be a risk factor for aromatic amine-induced colorectal cancer.

Peroxidases

Another pathway for the activation of aromatic amines may occur in human extrahepatic tissues and involves peroxidative metabolism to reactive intermediates that form covalent adducts with DNA and proteins. At present, two separate enzyme systems have been implicated: prostaglandin *H* synthase and myeloperoxidase. Prostaglandin *H* synthase has been measured by both enzymatic and immunochemical criteria in human urinary bladder, prostate, and lung; and it has been shown to mediate the co-oxidation of benzidine, ABP, 2-naphthylamine, Glu-P-1, and IQ (*62*). The levels of this peroxidase in most tissue samples examined were comparable to that observed in experimental animals, where the contribution of this activation pathway to DNA adduct formation appears to be minor (*25*). However, because levels of prostaglandin *H* synthase are increased by physiological responses, including inflammation, the tissue peroxidase levels in some individuals are 20- to 100-fold higher, and they could play a major role in aromatic amine activation. This may be particularly relevant in the case of urinary tract infections, including schistosomiasis, which have long been established as high risk factors for bladder cancer (*63*).

Human myeloperoxidase, which is present in neutrophils and monocytes, is also stimulated by the inflammatory response. In addition, this enzyme has been shown to carry out the metabolic activation of several arylamine drugs and carcinogens (*64,65*) and has been suggested to be involved in several kinds of bone marrow toxicity induced by drugs such as procainamide, dapsone, and sulfamethoxazole (*66,67*). Recently, we characterized a peroxidase in human lung microsomal preparations that activates benzidine, ABP, 2-naphthylamine, 2-aminofluorene, and 4,4′-methylenebis(2-chloroaniline) and appears to be identical with myeloperoxidase, on the basis of cofactor requirements, inhibition, and solubilization with high salt (S. J. Culp and F. F. Kadlubar, unpublished studies). This activity varied by 10-fold in lung samples from smokers and ex-smokers and appears to be due to massive infiltration of neutrophils into peripheral lung tissue (*68*) around the particulate matter arising from deposition of cigarette tar. While this peroxidase activity is apparently a consequence of cosedimentation with the microsomal fraction of lung homogenates, it does represent the highest peroxidase activity ever observed in a mammalian tissue and it may have important toxicological significance in the extracellular matrix.

Acetyltransferase and CYP1A2 Polymorphism in Patients with a History of Colorectal Cancer and Polyps

The recent development of a noninvasive caffeine phenotyping method for both acetyltransferase and CYP1A2 now allows for epidemiological studies on two of the polymorphic enzymes involved in aromatic amine metabolism.

In view of the possible role of heterocyclic aromatic amines in the etiology of human colorectal cancer (*vide supra*), we decided to examine the prevalence of rapid and slow metabolizers of acetyltransferase and CYP1A2 in a case–control study. The control group was recruited from central Arkansas by telephone using random-digit dialing, and was matched for age, sex, ethnicity, and smoking status. Because colon polyps are regarded as preneoplastic lesions, we elected to include patients with polyps with the colorectal cancer cases and then stratify our analysis by diagnosis. Once patient consent had been obtained, the subject was provided with written instructions, a schedule, a modified "Health Habits and History Questionnaire," and a quantity of coffee (two packets of Maxwell House Instant). A follow-up phone call was used to answer questions, reinforce instructions, and improve participation. When the subject arrived to provide a 4- to 5-hr urine sample, the questionnaire was reviewed by the study nurse for completeness of response.

At present, 100 control subjects, 20 colorectal cancer patients, and 18 polyp patients have been entered into the study. Urine samples from these individuals were analyzed by high-performance liquid chromatography to determine AFMU/1X for acetyltransferase phenotyping and (17X + 17U)/137X for CYP1A2 phenotyping. Probit analysis of values obtained from the control group provided cut-off points of 0.6 for AFMU/1X and of 10.0 (nonsmokers) and 18.7 (smokers) for (17X + 17U)/137X. Control subjects consisted of 42% rapid acetylators and 43% rapid *N*-oxidizers; while cancer and polyp patients combined consisted of 61% rapid acetylators and 55% rapid *N*-oxidizers. Using a chi-square test with 2 × 2 contingency tables, a trend toward an increased proportion of the rapid acetylator phenotype ($p = 0.05$) and rapid CYP1A2 phenotype ($p = 0.19$) was apparent for colorectal cancer and polyp patients. A comparison of the prevalence of individuals who are both rapid acetylators and rapid *N*-oxidizers, however, showed that 16% of the controls, 33% of the polyp patients, and 40% of the colorectal cancer cases possess this rapid-rapid phenotype (37% of the polyp and cancer cases combined). This difference is highly significant ($p = 0.014$ for cancer cases; $p = 0.008$ for combined) and provides evidence that metabolic polymorphisms for foodborne carcinogenic heterocyclic amines can be used to predict individual susceptibility to colorectal carcinogenesis.

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

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