Oral Administration of 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) Yields PhIP-DNA Adducts but Not Tumors in Male Syrian Hamsters Congenic at the N-acetyltransferase 2 (NAT2) Locus

Adrian J. Fretland,*† 1 Uday S. Devanaboyina,*2 Yi Feng,*†, Matthew A. Leff,*† Gong H. Xiao,* Stephanie J. Webb,* and David W. Hein*† 3

*Department of Pharmacology and Toxicology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota 58202; and †Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky 40292; and 2 Present address: Hoffmann La-Roche Pharmaceuticals, 340 Kingsland Street, Nutley, NJ 07110—1199.

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2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is a heterocyclic amine carcinogen present in well-done meat. PhIP must undergo host-mediated bioactivation to exert its mutagenic and carcinogenic effects. Following N-hydroxylation, N-acetyltransferases catalyze the O-acetylation (activation) of N-hydroxy-PhIP to an electrophile causing DNA damage. A well-defined genetic polymorphism in N-acetyltransferase 2 (NAT2) activity exists in humans and the Syrian hamster. Since some human epidemiological studies suggest an association between acetylator genotype and cancer susceptibility in individuals who consume well done meats, this study was designed to investigate the specific role of acetylator genotype in PhIP-induced tumors using a Syrian hamster model congenic at the NAT2 locus. Following oral administration of PhIP to male rapid and slow acetylator Syrian hamsters, DNA adducts were identified in each tissue examined with levels in the relative order: pancreas > heart and urinary bladder > prostate, small intestine and transverse colon > ascending colon, liver, cecum, descending colon, and rectum. However, no tumors were observed in male rapid and slow acetylator congenic hamsters administered 11 oral doses of PhIP (75 mg/kg) and maintained on a high fat diet for one year.

Key Words: N-acetyltransferase 2 (NAT2); 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, PhIP; acetylator genotype; DNA adducts; Syrian hamster.

2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is considered the most prevalent heterocyclic amine in the human environment, with primary exposure through the consumption of well-done meats (Layton et al., 1995). Consumption of well-done meat in the diet has been associated with higher risk of colorectal (Sinha et al., 1999), breast (Zheng et al., 1998) and possibly prostate (Norrish et al., 1999) cancers in humans. Recently, PhIP was specifically associated with human breast cancer (Sinha et al., 2000). Rats administered PhIP develop tumors of the prostate, mammary gland, and colon (Hasegawa et al., 1993; Ito et al., 1991; Shirai et al., 1997, 1999), and rats administered N-hydroxy-PhIP develop tumors of the urinary bladder (Archer et al., 2000). In contrast, a recent Swedish case-control study reported that normal dietary exposure to heterocyclic amines was not associated with colon, rectal, kidney, or urinary bladder cancer in humans (Augustsson et al., 1999). Thus, the carcinogenicity of heterocyclic amines in humans remains unclear, but the question is of major significance to public health, due to ubiquitous and potentially preventable exposure to heterocyclic amines in the human diet.

PhIP must undergo host-mediated biotransformation to genotoxic species in order to exert mutagenic and carcinogenic effects (Alexander et al., 1994; Buonarati et al., 1990; Kaderlik et al., 1994a). Following N-hydroxylation, N-acetyltransferases catalyze the O-acetylation of N-hydroxy-PhIP to N-acetoxy-PhIP (Buonarati et al., 1990; Hein et al., 1994b; Minchin et al., 1992). N-acetoxy-PhIP is unstable and spontaneously hydrolyzes to an arylnitrenium ion that binds DNA. PhIP does not undergo N-acetylation (deactivation) in vitro, unlike many aromatic amine carcinogens (Hein et al., 1993), but phenotypic differences in O-acetylation of N-hydroxy-PhIP may lead to differences in DNA adduct formation. PhIP binds to the C-8 position of guanine nucleotides forming N-(deoxyguanosin-8-yl)-PhIP (Lin et al., 1992). The formation of DNA adducts may induce mutations in proto-oncogenes and tumor suppressor genes leading to the formation of neoplastic lesions. Thus, genetic differences in O-acetyltransferase phenotype may result in differential cancer susceptibility following exposure to heterocyclic amine carcinogens such as PhIP.

A genetic acetylation polymorphism exists in humans (Hein et al., 2000) and Syrian hamster (Hein et al., 1997). Slow acetylator congenic Syrian hamsters are homozygous for the NAT2*16A allele containing a premature stop codon (Ferguson et al., 1994a) which encodes a deficient NAT2 enzyme (Fer-
Rapid and slow acetylator Syrian hamsters differ in N- and O-acetyltransferase activity (Hein et al., 1994), and in levels of hemoglobin-adduct (Feng et al., 1994), DNA-adduct (Feng et al., 1996b) and aberrant crypt foci (Feng et al., 1996c; Paulsen et al., 1996) induced by aromatic amine carcinogens. Phenotypic differences in O-acetyltransferase catalyzed by NAT2 may lead to differences in bioactivation of heterocyclic amines, DNA adduct formation, and tumor incidence.

As recently reviewed, numerous human epidemiological studies have investigated the role of acetylator genotype in cancer susceptibility (Hein et al., 2000). Some studies suggest a role for the NAT2 rapid acetylator genotype and cancer incidence, but not all investigations support this relationship. Epidemiological studies often are limited in their power to study the role of the metabolizing enzymes in cancer susceptibility because of numerous factors including lack of a homogeneous population, insufficient exposure data, and other confounding factors.

The congenic hamster model provides a useful model to investigate the relationship between acetylator genotype and heterocyclic amine carcinogenesis. The construction and characterization of the rapid- and slow-acetylator congenic hamster model has been described (Hein, 1991; Hein et al., 1994a). Rapid and slow acetylator congenic hamsters are theoretically 99.975% genetically identical differing only at the NAT2 locus (Ferguson et al., 1994a, 1996) or other tightly linked loci. This virtually eliminates genetic polymorphism in other metabolism, DNA repair, and oncogenesis pathways facilitating investigation into the specific role of the acetylation polymorphism in tumorigenesis. Urinary bladder and colon tumors are induced in the Syrian hamster by 3,2-dimethyl-4-aminobiphenyl (Feng et al., 1999; So and Winder, 1972; Williams et al., 1981), an aromatic amine carcinogen related to PhIP. Thus, we utilized Syrian hamsters, congenic at the NAT2 locus, to investigate the specific role of the acetylation polymorphism in PhIP-induced carcinogenesis.

MATERIALS AND METHODS

Animals and chemicals. Hamsters were bred and maintained first at the University of North Dakota and subsequently at the University of Louisville School of Medicine. Hamsters were housed singly in polycarbonate cages and fed Rodent Laboratory Chow 5001 (Purina Mills, Inc., St. Louis, MO) and tap water ad libitum. PhIP was purchased from Toronto Research Chemicals, Inc. (Toronto, ON Canada). Synthetic PhIP-DNA adduct standard, N-(deoxyguanosin-8-yl)-PhIP, was kindly provided by Fred F. Kadlubar (National Center for Toxicological Research, Jefferson, AR).

PhIP-DNA adducts. PhIP-DNA adduct levels were measured by the 32P-postlabeling method, as previously described (Left et al., 1999). Briefly, genomic DNA was isolated by digestion with proteinase K, followed by phenol:chloroform extraction. PhIP-DNA adducts were resolved by thin-layer chromatography, quantified using the Instant Imager electronic autoradiography system (Packard Instruments, Chicago, IL), and compared with synthetic PhIP-DNA-adduct standard, N-(deoxyguanosin-8-yl)-PhIP.

PhIP bioassay. Twelve-week-old rapid- (n = 23) and slow- (n = 23) acetylator congenic male hamsters were administered 11 doses of PhIP (75 mg/kg) by oral gavage. The dosing regimen consisted of 2 doses per week for 4 weeks, followed by a single dose per week for 1 week and finally, 1 dose every other week for the final 2 doses. Three rapid- and 3 slow-acetylator congenic hamsters received vehicle alone as control. At the conclusion of dosing, all hamsters were placed on a polyunsaturated high-fat diet consisting of 23.5% corn oil. The PhIP dosing regimen followed by the high-fat diet is a slight modification from a PhIP dosing regimen (10 oral doses of 75 mg/kg PhIP over a 2-week period) that yields tumors in the female rat (Ghoshal et al., 1994; Snyderwine et al., 1998). All hamsters were monitored daily for morbidity and mortality. Hamsters were sacrificed fifty-six weeks after the final dose, and all tissues were examined for gross abnormalities. In addition, each prostate and pancreas was fixed in 10% buffered formalin for 24 h, followed by storage in 70% ethanol. Tissues were embedded in paraffin and sectioned serially. The tissue sections were stained with hematoxylin-eosin-saffron and scored blind by a veterinary pathologist.

RESULTS

A single DNA adduct spot was observed in all hamsters treated with PhIP that co-migrated with synthetic N-(deoxyguanosin-8-yl)-PhIP after resolution by thin layer chromatography (Fig. 1). No adducts were observed in hamsters treated with vehicle. PhIP-DNA adducts were observed in each tissue examined of rapid and slow acetylator congenic hamsters, with levels in the relative order: pancreas > heart and urinary bladder > prostate, small intestine, and transverse colon > ascending colon, liver, cecum, descending colon, and rectum (Fig. 2). PhIP-DNA adduct levels did not differ significantly between rapid and slow acetylator congenic hamsters in any tissue.

All treated and control hamsters survived the entire study
Period. No significant differences were observed in body weight or general health and grossly apparent tumors were not found in any tissue of either the treated or control groups. After histopathological analysis, prostate from both treated and control groups were normal. The pancreas from both treated and control groups exhibited lipomatosis, minimal lymphocytic steatitis, increased mast cells, and islet enlargement. However, they are considered age-related lesions and were observed to an equivalent extent in both treated and control animals.

**DISCUSSION**

PhIP-DNA adducts were examined in rapid and slow acetylator congenic Syrian hamsters as a biomarker for DNA damage. The high level of PhIP-DNA adducts in the pancreas and low levels in the liver are consistent with previous findings in the rat (Fretland et al., 1997; Friessen et al., 1996; Kaderlik et al., 1994a; Pfau et al., 1997; Takayama et al., 1989). The basis for this differential tissue level of DNA adducts is not known, but may be due, at least in part, to high levels of hepatic conjugation of N-acetoxy-PhIP with glutathione and/or glucuronidation of N-hydroxy-PhIP (Kaderlik et al., 1994b; Lin et al., 1994). Despite the high level of DNA adducts, tumors were not observed in the pancreas of PhIP-treated male Syrian hamster, a result consistent with previous findings in the rat (Ito et al., 1991) and a recent report in the Syrian hamster (Yoshimoto et al., 1999).

No statistically significant differences in PhIP-DNA adduct levels were observed between rapid and slow acetylator congenic hamsters administered PhIP. This result is similar to the finding that rapid and slow acetylator congenic hamsters showed no differences in DNA adduct formation when administered 3,2’-dimethyl-4-aminobiphenyl (Feng et al., 1996a). Recent studies show that N-hydroxy-PhIP is activated to a much greater extent by recombinant Syrian hamster N-acetyltransferase I than recombinant Syrian hamster NAT2 (manuscript in preparation). Since rapid- and slow-acetylator congenic hamsters do not have a functional difference in N-acetyltransferase 1 phenotype (Ferguson et al., 1994b), this could account for the lack of difference in DNA adduct levels between rapid- and slow-acetylator hamsters that might have been observed following administration of other doses of PhIP. However, in preliminary studies with lower doses, PhIP-DNA adduct levels were very low to non-detectable in most major organs of the hamster. Recent studies show that human sulfotransferase is very important in the metabolic activation of N-hydroxy-PhIP (Chou et al., 1995; Wu et al., 2000). Rapid and slow acetylator congenic hamsters do not differ in N-hydroxy-PhIP O-sulfotransferase activity (Fretland et al., in press), which may explain the lack of a difference in PhIP DNA adduct formation in rapid- and slow-acetylator congenic hamsters.

No tumors were observed in male rapid- and slow-acetylator congenic Syrian hamsters following oral administration of PhIP and maintenance on a high-fat diet for a year. The absence of prostate tumors following administration of PhIP to Syrian hamsters differs from the rat, in which PhIP induces prostate tumors (Shirai et al., 1997, 1999). However, the PhIP-dosing regimen used in the rat studies (400 ppm in the diet for 52 weeks) differs substantially from the present hamster study (11 oral doses of 75 mg/kg). It is possible that alternative PhIP dosing regimens would induce prostate tumors in the hamster. The regimen used in the current study is a slight modification of the one that induces tumors in the female rat (Ghoshal et al., 1994; Snyderwine et al., 1998). We recently reported lack of aberrant crypt foci and tumors in the gastrointestinal tract of male and female congenic hamsters administered PhIP (Steffensen et al., 2000). These results suggest that the male Syrian hamster is relatively resistant to PhIP tumorigenesis despite the widespread formation of PhIP-DNA adducts in target tissues. Absence of PhIP-induced tumors also was observed recently in the female hamster (Fretland et al., in press). In contrast, levels of DNA adducts (Purewal et al., 2000a) and aberrant crypt foci (Purewal et al., 2000b) were higher in rapid than slow acetylator inbred rats administered PhIP. Since human exposures to PhIP are much lower but for a much longer period of time than carried out in the present study, further studies are necessary to understand the role of the NAT2 acetylation polymorphism on
DNA-adduct formation and susceptibility to PhIP-induced cancers in humans.

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