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Effect of Rapid Human N-acetyltransferase 2 Haplotype on DNA Damage and Mutagenesis Induced by 2-Amino-3-methylimidazo [4,5-f] quinoline (IQ) and 2-Amino-3,8-dimethylimidazo-[4,5-f] quinoxaline (MeIQx)

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Abstract

Heterocyclic amines such as 2-amino-3-methylimidazo [4,5-f] quinoline (IQ) and 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx) are dietary carcinogens generated when meats are cooked well-done. Bioactivation includes N-hydroxylation catalyzed by cytochrome P4501A2 (CYP1A2) followed by O-acetylation catalyzed by N-acetyltransferase 2 (NAT2). Nucleotide excision repair-deficient Chinese hamster ovary (CHO) cells stably transfected with human *CYP1A2* and either *NAT2*4* (rapid acetylator) or *NAT2*5B* (slow acetylator) alleles were treated with IQ or MeIQx to examine the effect of NAT2 genetic polymorphism on IQ- or MeIQx-induced DNA adducts and mutagenesis. MeIQx and IQ both induced decreases in cell survival and significantly ($p < 0.001$) greater number of endogenous *hypoxanthine phosphoribosyl transferase* (*hprt*) mutants in the CYP1A2/NAT2*4 than the CYP1A2/NAT2*5B cell line. IQ- and MeIQx-induced *hprt* mutant cDNAs were sequenced and over 85% of the mutations were single base substitutions with the remainder exon deletions likely caused by splice-site mutations. For the single-base substitutions, over 85% were at G:C base pairs. Deoxyguanosine (dG) -C8-IQ and dG-C8-MeIQx adducts were significantly ($p < 0.001$) greater in the CYP1A2/NAT2*4 than the CYP1A2/NAT2*5B cell line. DNA adduct levels correlated very highly with *hprt* mutants for both IQ and MeIQx. These results suggest substantially increased risk for IQ- and MeIQx-induced DNA damage and mutagenesis in rapid NAT2 acetylators.

1. Introduction

2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo-[4,5-f] quinoxaline (MeIQx) are potent and abundant mutagens in the human diet, formed during high temperature cooking of meats [1]. IQ and MeIQx are two of the four heterocyclic amine carcinogens designated as “reasonably anticipated to be a human carcinogen” [2]. IQ- and

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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MeIQx-induced DNA adduct formation and mutagenesis requires N-hydroxylation catalyzed by P450s such as cytochrome P450 1A2 (CYP1A2) which occurs at relatively high rates in humans [3]. Following N-hydroxylation, N-hydroxy-IQ or MeIQx may be further O-acetylated by N-acetyltransferase 2 (NAT2) to acetoxy-derivatives that are highly unstable, leading to electrophilic intermediates that form DNA adducts potentially leading to mutagenesis [4]. Major IQ- and MeIQx-induced DNA adducts form at the C8 position of deoxyguanosine and have been identified in human tissues [4]. Bulky DNA adducts such as these are recognized by the nucleotide excision repair (NER) pathway [5]. Recent studies report that IQ and MeIQx also can be metabolized to nitroso derivatives subject to NAT2 catalysis ultimately leading to the same C8 deoxyguanosine adducts [6].

Both IQ and MeIQx are reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity in experimental animals and supporting genotoxicity data. When administered orally, they induce tumors in both mice and rats at multiple tissue sites [2]. Case control studies suggest that MeIQx may increase the risk of colon adenoma [7] and lung cancer [8]. DNA adducts are an informative biomarker for investigations of genetic variation in carcinogen metabolism. Mutagenesis induced by heterocyclic amines and their N-hydroxy metabolites have been investigated at both the *hprt* [9–12] and *aprt* [13,14] gene loci of Chinese hamster cell lines.

Since heterocyclic amines require metabolic activation to exert their carcinogenic effects, genetic polymorphisms in carcinogen metabolizing enzymes such as NAT2 may modify cancer risk following exposure. Humans exhibit genetic polymorphism in NAT2 resulting in rapid and slow acetylator phenotypes. The *NAT2*4* allele is associated with a rapid acetylator phenotype whereas the *NAT2*5B* allele is associated with a slow acetylator phenotype [15, 16]. Epidemiological studies suggest a role for NAT2 genetic polymorphism in susceptibility to various cancers [17,18]. However, the findings are often inconsistent [19], suggesting the need for laboratory-based experiments to support the biological plausibility and the conclusions inferred from these epidemiological studies.

Previous studies showed that transfection of CYP1A2 and NAT2 increases mutagenesis by IQ or MeIQx [20–22]. The authors speculate that the human NAT2 acetylation polymorphism would modify this effect, but this has yet to be tested. In order to test this hypothesis directly, we used NER-deficient Chinese hamster ovary (CHO) cells transfected with human *CYP1A2*, human *NAT2*4* (rapid acetylator) or human *NAT2*5B* (slow acetylator) alleles.

2. Materials and methods

2.1 Sources for chemicals and cell lines

IQ and MeIQx were purchased from Toronto Research Chemicals, Ontario, Canada. N-hydroxy-IQ and N-hydroxy-MeIQx were purchased from Midwest Research Institute, Kansas City, MO. dG-C8-IQ was a kind gift of Dr. Paul Vouros, Northeastern University, Boston, MA. dG-C8-MeIQx and dG-C8-MeIQx-d3 were generously provided by Dr. Robert Turesky of the Wadsworth Center, Albany, NY. The UV5-CHO cell line, a nucleotide excision repair-deficient derivative of the AA8 line [23], was obtained from the ATCC. The UV5-CHO cell line lacks nucleotide excision repair due to a mutation in the XPD (ERCC2) gene [24].

2.2 Cell culture

Cells were grown in alpha-modified minimal essential medium (Cambrex) without L-glutamine, ribosides, or deoxyribosides, supplemented with 10% fetal bovine serum (Hyclone), 100 units/mL penicillin, 100 µg/mL streptomycin (Cambrex), and 2 mM L-glutamine

(Cambrex) at 37°C in 5% CO₂. Media were supplemented with selection agents as previously described [10] appropriate for maintenance of stable transfectants.

2.3 Construction of UV5-CHO Cells expressing CYP1A2 and NAT2*4 or NAT2*5B

The construction of UV5-CHO cells expressing human *CYP1A2* and *NAT2*4* or *NAT2*5B* has been reported and characterized [10]. Briefly, a pFRT/*lacZeo* plasmid was transfected into nucleotide excision repair-deficient UV5 cell lines to generate a UV5 cell line containing a single integrated FRT site (UV5FRT). Purified human NADPH cytochrome P450 reductase (POR) and CYP1A2 polymerase chain reaction (PCR) products were digested and ligated into similarly treated pIRES vector and transformed into DH5 α competent cells. The pIRES plasmid containing cDNAs of human *CYP1A2* and *POR* was transfected into the newly established UV5FRT cell line. The colonies of these cells were expanded, and intact geneticin-resistant cells were assayed for 7-ethoxyresorufin O-deethylase (EROD) activity as described previously (Metry et al., 2007). EROD catalytic activity is undetectable (<0.2 pmoles/min/10⁶ cells) in untransfected UV5 cells, whereas *CYP1A2*-transfected cells (with and without further transfection with *NAT2*) have EROD catalytic activities about 3 pmol/min/10⁶ cells [10].

The open reading frames of *NAT2*4* and *NAT2*5B* were amplified by PCR and inserted into the pcDNA5/FRT vector. The pcDNA5/FRT plasmid containing human *NAT2*4* or *NAT2*5B* was co-transfected with pOG44, a Flp recombinase expression plasmid, into UV5FRT/CYP1A2 cells. Integration of the pcDNA5/FRT construct into the FRT site was confirmed by PCR. The *NAT2*4*- and *NAT2*5B*-transfected cells were characterized for *N*-acetylation of sulfamethazine, a NAT2-selective substrate. NAT2 catalytic activities are undetectable (<20 pmol/min/mg total protein) in untransfected UV5 and UV5/CYP1A2 cells, about 1.5 nmol/min/mg total protein in CYP1A2/*NAT2*4* cells, and about 0.1 nmol/min/mg total protein in CYP1A2/*NAT2*5B* cells [10].

2.4 O-acetyltransferase assays

The metabolic activation of N-hydroxy-MeIQx and N-hydroxy-IQ via O-acetylation were measured by high performance liquid chromatography (HPLC) with modifications of an assay previously described [9,25]. Briefly, reaction mixtures (10 mM sodium phosphate buffer, 0.5 mM EDTA, 0.5 mM DTT, pH 7.4) containing cell lysate, 1 mg/mL deoxyguanosine, substrate (100 μ M N-hydroxy-MeIQx or 15 μ M N-hydroxy IQ), and 1 mM acetyl coenzyme A were incubated 10 min at 37 °C and stopped by the addition of water saturated ethyl acetate. The reactions were centrifuged for 10 min and the organic phase was transferred, evaporated to dryness, and resuspended in 100 μ l of 10% acetonitrile. HPLC separation of dG-C8-IQ or dG-C8-MeIQx adducts was achieved using a gradient of 85:15 sodium perchlorate pH 2.5:acetonitrile to 0:100 sodium perchlorate pH 2.5:acetonitrile over 10 min. DNA adducts were quantitated against a dG-C8-IQ or dG-C8-MeIQx standard curve by absorbance at 305 nm. The limit of detection was 10 pmoles. Protein levels were determined with the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA). O-acetyltransferase activities were normalized to reaction minutes and mg protein in the assay. Baseline measurements using lysates of UV5 and *CYP1A2*-transfected cells were subtracted from measurements using lysates from the *CYP1A2/NAT2*4*- and *CYP1A2/NAT2*5B*-transfected CHO cell lines.

2.5 Assays for survival and mutagenesis at the hprt locus

Assays for cell survival and mutagenesis were modified slightly from methods previously described [10]. Briefly, cells were grown for 12 doublings, with selective agents in complete HAT medium (30 μ M hypoxanthine, 0.1 μ M amethopterin, and 30 μ M thymidine). Cells were plated at a density of 5 $\times$ 10⁵ cells/T-25 flask and incubated for 24 hr, after which media were changed and the cells treated for 48 hr with various concentrations of IQ or MeIQx dissolved

in DMSO or vehicle control (0.5% DMSO). Survival was determined by colony forming assay and expressed as percent of vehicle control. The remaining cells were replated and subcultured for 7 days of growth. Then, cells were plated for cloning efficiency in complete media and for *hprt* mutants in complete medium containing 40 μ M 6-thioguanine (Sigma). Dishes were seeded with 1×10^5 cells/100 mm dish (10 replicates) and incubated for 7 days in 6-thioguanine media; cloning efficiency dishes were seeded with 100 cells/well/6-well plate in triplicate and incubated for 6 days.

2.6 Identification and quantitation of IQ- and MeIQx-DNA adducts

Cells grown in 15-cm plates were treated separately with IQ or MeIQx as described above for the survival and mutagenesis assays. Cells were harvested after 48 h treatment. DNA was extracted and quantified as previously described [9]. DNA quality was monitored by UV spectroscopy using $A_{260/280 \text{ nm}}$ and this ratio was consistently above 1.9. DNA samples (200 μ g) added to 1 ng (3.3 adducts per 10^6 DNA bases) deuterated internal standard (dG-C8-MeIQx-d3) were digested at 37°C with 10 units DNase I (US Biological) for 1 h followed by 5 units micrococcal nuclease (Sigma), 5 units nuclease P1 (US Biological), 0.01 units spleen phosphodiesterase (Sigma), and 0.01 units snake venom phosphodiesterase (Sigma) for 6 h followed by 5 units alkaline phosphatase (Sigma) overnight. Two volumes of acetonitrile were added to the digest, which was then filtered and concentrated to 100 μ L in a speed vacuum.

Samples were subjected to binary gradient HPLC and introduced into a Micromass Quattro LC triple quadrupole mass spectrometer using a custom-built nanospray as described previously [9,26]. Multiple reaction monitoring (dwell time, 0.5 s; span, 0.4 Da) was used to measure the $[M+H]^+$ to $[(M-116)+H]^+$ (loss of deoxyribose) mass transition. Multiple reaction monitoring in the electrospray ionization-positive ion mode was carried out using argon as the collision gas. Capillary and cone voltages and collision energies were optimized for cleavage of the glycosidic bond. Samples used for quantitative analysis of dG-C8-IQ and dG-C8-MeIQx were spiked with 1 ng dG-C8-MeIQx-d3 internal standard before sample treatment. dG-C8-IQ was monitored using the transition from m/z 464 to m/z 348 (Figure 1). dG-C8-MeIQx was monitored using the transition from m/z 479 to m/z 363 and dG-C8-MeIQx-d3 internal standard was monitored using the transition from m/z 482 to m/z 366 as previously described [9].

2.7 Sequencing of *hprt* mutants

To obtain independent *hprt* mutants for sequence analysis, one viable colony was picked from each 6-thioguanine containing culture dish of either 1 μ M IQ- or MeIQx- treated *CYP1A2/NAT2*4*-transfected cells. They were propagated until one confluent 10-cm dish was obtained. The cells were harvested and pelleted. RNA was extracted immediately using the RNeasy Mini Kit (Qiagen), or pellets were snap-frozen in liquid nitrogen and stored at -80°C for later use. RNA (1 μ g) was reverse transcribed with oligo (dT) primer from the SuperScript III First-Strand Synthesis System (Invitrogen) as described by the manufacturer. The *hprt* coding region was amplified by a PCR reaction consisting of P1, $_{68}\text{ctcggcgccctcctcgcgg}_{49}$, P2, $_{721}\text{cctaattttactgggaacat}_{702}$, RNase-free water, PCR buffer, 3.0 mM MgCl_2 , 0.2 mM dNTPs, 5 μ L of the cDNA synthesized above, and Taq polymerase in a total volume of 20 μ L. Amplification was performed for 35 cycles consisting of 95°C for 30 sec, 62°C for 30 sec, and 72°C for 1 min and then an extension step at 72°C for 5 min. The 780 base pair products were purified using the Qiaquick PCR purification kit (Qiagen). Purified PCR products were sequenced using both S1, ctcctcgcgggcttct and S2, $\text{agatccattcccatgactgtagatt}$ with the Big Dye Terminator kit (Beckman, Fullerton, CA) as described by the manufacturer.

3. Results

3.1 O-acetylation of N-hydroxy-IQ and N-hydroxy-MeIQx

Cell lysates from UV5 and each of the transfected CHO cell lines were tested for their capacity to activate (via O-acetylation) N-hydroxy-IQ and N-hydroxy-MeIQx to form dG-C8-IQ, and dG-C8-MeIQx adducts respectively. dG-C8-IQ adducts were below the limit of detection (10 pmoles) in each cell line. The N-hydroxy-MeIQx O-acetyltransferase activity in CYP1A2/NAT2*4 cells was about 4-fold higher ($p=0.0101$) than in the CYP1A2/NAT2*5B cells (Figure 2).

3.2 Cell survival and mutagenesis

No detectable effect on cell survival was observed in the UV5 cell line at any of the IQ or MeIQx concentrations tested (up to 2 μ M). Only the CYP1A2/NAT2*4 cell line showed concentration-dependent cell death and *hprt* mutagenesis following treatment with IQ or MeIQx (data not shown). IQ-induced *hprt* mutants (Figure 3) and MeIQx-induced mutants (Figure 4) were significantly higher in the CYP1A2/NAT2*4-transfected cell line as compared to all other cell lines at all concentrations tested.

3.3 Mutation characterization

We characterized 22 independent IQ-induced *hprt* mutants (Table 1). Mutations were primarily (89%) single base substitutions while the remaining 11% were exon deletions likely caused by splice-site mutations. For the single-base substitutions, 89% were at GC base pairs, with 75% G:C→T:A, 6% G:C→A:T, and 19% G:C→C:G. We characterized 21 independent MeIQx-induced *hprt* mutants (Table 2). MeIQx-induced *hprt* mutations also were primarily (88%) single base substitutions, whereas the remaining 12% were splice-site mutations resulting in an exon deletion. Further, 88% of the single base substitutions were at G:C base pairs, with 62% G:C→T:A, 7% G:C→A:T, and 31% G:C→C:G. The most common mutation induced by IQ (18%) and MeIQx (9.5%) was a G:C>T:A transition (Gly>Val) at position 47 in exon 2. Bioactivation of both IQ and MeIQx caused two single-base substitutions at A:T base pairs but in different locations. The number of mutants in vehicle-treated cells was less than 10% of those from IQ- or MeIQx- treated cells and were not sequenced. The locations of the single base substitutions in the *hprt* cDNA sequence are illustrated in Figure 5.

3.4 Identification and quantitation of DNA adducts

With low energy multiple reaction monitoring mass spectrometry scanning conditions, protonated dG-C8-IQ and dG-C8-MeIQx ions were detected at m/z 464 and m/z 479, respectively, by positive ion electrospray mass spectrometry. No other adducts were detected. dG-C8-IQ (Figure 6) and dG-C8-MeIQx (Figure 7) adduct levels were significantly greater in the CYP1A2/NAT2*4 cell line than all other cell lines tested. dG-C8-IQ and dG-C8-MeIQx adduct levels correlated very highly with IQ ($r^2 = 0.96$) and MeIQx ($r^2 = 0.91$) -induced *hprt* mutants, respectively.

4. Discussion

We investigated IQ- and MeIQx-induced DNA adducts and mutagenicity in NER-deficient CHO cells transfected with CYP1A2 only or CYP1A2 and NAT2*4 or NAT2*5B. Our study showed that dose-dependent DNA adducts and mutagenesis following treatment with IQ or MeIQx occurred only in the CYP1A2/NAT2*4-transfected cell line. DNA adduct levels and mutants in the CYP1A2/NAT2*5B-transfected cell line were not significantly different from CYP1A2- or untransfected UV5 cells. These results are consistent with previous studies reporting the importance of rapid acetyltransferase NAT2 in mutagenicity following incubation of

CHO cells with IQ [13], MeIQx [9], 4-aminobiphenyl [27] and 2-amino-9H-pyrido[2,3-b]indole (AαC) [12] but differ from findings reported for 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [10,11]. dG-C8-IQ and dG-C8-MeIQx were the only adducts identified following treatment with IQ and MeIQx. High correlations between *hprt* mutagenesis and dG-C8 adduct levels were observed for both IQ and MeIQx.

We also determined the mutation spectrum of IQ-, and MeIQx- induced *hprt* mutants. Sequence analysis was performed on mutants that were derived from populations that had at least a 10-fold induction. The majority of *hprt* mutations were single base substitutions, in which 89% and 88% were at G:C base pairs for IQ and MeIQx respectively. These results strongly implicate adduct formation on deoxyguanosine as the premutagenic lesion and are consistent with previous studies with other heterocyclic amines such as PhIP [10]. In order to compare single-base substitutions induced by IQ, MeIQx, and PhIP (the latter reported previously in Metry et al., [10]), we mapped these mutations in the *hprt* cDNA sequence (Figure 5). It is of interest that for each of these heterocyclic amines, the majority of targeted G:C substitutions, 93% for IQ, and 88% for both MeIQx and PhIP arose from putatively adducted dG residues on the nontranscribed strand. This strand distribution is well-known and has been noted with aromatic amine carcinogens, such as N-acetyl-N-acetoxyaminofluorene [28]. In each of these cases, the strand distribution of premutagenic adducts is overwhelmingly toward the nontranscribed strand and cannot be ascribed to preferential repair of the transcribed strand since in each of these cases the cells are nucleotide excision repair-deficient. Data now indicate that mutations induced by chemical carcinogens, and those by ultraviolet radiation, differ in ways that may shed light on the mechanisms involved. The Y-family DNA polymerase ι acts preferentially on the leading strand template and its homolog DNA polymerase η on the lagging strand [29]. These data are consistent with error-prone bypass of lagging-strand chemical adducts by pol ι .

Similarities in the location of single-base substitutions at G:C base pairs were noted among the three heterocyclic amine carcinogens. For PhIP, most mutations occurred in exons 3 and 6, with several mutations at positions 418 and 419 of exon 6 within a GpG dinucleotide [10]. There were also MeIQx and IQ mutations at this site. However, position 47 was more common for MeIQx- and IQ-induced mutations. These results are similar to previous studies with PhIP and IQ [13]. Further, these arylamine carcinogens predominantly induced G to T transversions, which have been detected in proto-oncogenes and tumor-suppressor genes, including *Ki-ras*, *Ha-ras*, *Apc*, *p53*, and *beta-catenin*, implicating these adducts in cancer etiology [2].

N-hydroxy-IQ O-acetyltransferase activity was undetectable in all cell lines tested. This may have been due to the small amount of N-hydroxy-IQ substrate available and tested (15 μ M) or to DNA adduct assay sensitivity (10 pmoles). N-hydroxy-MeIQx O-acetyltransferase activity was about 4-fold higher in *CYP1A2/NAT2*4*-transfected cells than in *CYP1A2/NAT2*5B*-transfected cells which is similar to previous studies in yeast [30] and in CHO cells [9]. Our results confirm previous studies suggesting that NAT2 is an important activation enzyme for IQ and MeIQx genotoxicity [9,13,20–22,31]. Furthermore, the effect of NAT2 on both MeIQx and IQ-induced DNA adduct formation and mutagenesis was restricted to the rapid acetylator *CYP1A2/NAT2*4*-transfected cell line at each concentration tested. This effect is consistent with studies in which higher levels of N-hydroxy-IQ and –MeIQx O-acetylation have been reported in human [32] and Syrian hamster [25] tissues and in primary cultures of human [31] and rat [33] mammary epithelial cells derived from rapid acetylators.

A number of human epidemiologic studies have reported associations or lack of associations between surrogate measures of heterocyclic amine exposure (i.e., well done meat intake) and cancer incidence. Furthermore, some of these studies have explored a possible modifying effect of NAT2 acetylator polymorphism. For example, NAT2 rapid acetylator phenotype is

associated with increases in colorectal [34–36], breast [37,38], and lung [39] cancer risk in individuals exposed to heterocyclic amine carcinogens. In contrast, other studies [40–42] did not find this association. Due to the need to clarify epidemiological studies and provide laboratory based experiments with a more controlled environment, we developed CHO cells stably transfected with human *CYP1A2* and either human rapid or slow acetylator NAT2, and investigated the genotoxicity of IQ and MeIQx, common heterocyclic amines found in well-done meat. By measuring DNA adduct formation and mutagenicity, we provide further evidence for the role of NAT2 acetylation capacity in increased cancer risk with exposure to these environmental carcinogens. Furthermore, these studies demonstrate a clear link between adduct formation and mutagenesis, illustrated by the strong linear correlation between dG-C8-adduct formation and induced *hprt* mutants, as well as the majority of *hprt* mutations occurring at deoxyguanosine. Hepatic dG-C8-MeIQx adducts are higher in rapid than slow NAT2 acetylator congenic rat lines administered MeIQx [43]. These results and the current study strongly suggest that rapid NAT2 acetylators are at greater risk than slow NAT2 acetylators to the genotoxic and hence carcinogenic effects of IQ and MeIQx.

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Fig. 1. Structure and ion spectra of dG-C8-IQ. Collision induced dissociation fragmentation of dG-C8-IQ at 50 V collision energy. The major fragment produced is the aglycone ion of m/z 348.3.

Fig. 2. N-hydroxy-MeIQx O-acetyltransferase activity in CHO cells transfected with CYP1A2/NAT2*5B or CYP1A2/NAT2*4. Each bar represents mean $\pm$ SEM for three determinations. The difference between the CYP1A2/NAT2*5B and the CYP1A2/NAT2*4 CHO cell line was significant ($p = 0.0101$).

Fig. 3.

IQ-induced mutations at the *hprt* locus for UV5 (solid squares), UV5/CYP1A2 (open squares), UV5/CYP1A2/NAT2*4 (solid triangles), and UV5/CYP1A2/NAT*5B (open triangles) CHO cell lines treated with IQ. The background mutant frequency was very low due to negative preselection in HAT medium, typically $< 5 \times 10^{-6}$. This value has been subtracted from the observed mutant frequency to obtain the induced mutant frequency. Data presented as mean $\pm$ SEM for 4 independent experiments. Some error bars fall within the symbol. The UV5/CYP1A2/NAT2*4 cell line had significantly greater ($p < 0.001$) *hprt* mutants as compared to all other cell lines at each concentration tested.

Fig. 4.

MeIQx-induced mutations at the *hprt* locus for UV5 (solid squares), UV5/CYP1A2 (open squares), UV5/CYP1A2/NAT2*4 (solid triangles) and UV5/CYP1A2/NAT*5B (open triangles) CHO cell lines treated with MeIQx. The background mutant frequency was very low due to negative preselection in HAT medium, typically $< 5 \times 10^{-6}$. This value has been subtracted from the observed mutant frequency to obtain the induced mutant frequency. Data presented as mean $\pm$ SEM for 4 independent experiments. Some error bars fall within the symbol. The UV5/CYP1A2/NAT2*4 cell line had significantly greater ($p < 0.001$) *hprt* mutants as compared to all other cell lines at each concentration tested.

Fig. 5. Locations of heterocyclic amine-induced base substitutions in the *hprt* cDNA sequence. Odd numbered exons are highlighted in gray and beginning and ending nucleotides between exons are bolded. I: IQ; M: MeIQx; P: PhIP. PhIP-induced mutations from [10].

Fig. 6. dG-C8-IQ adduct levels. dG-C8-IQ adduct levels were quantitated relative to a dG-MeIQx-d3 internal standard and thus reflect relative rather than absolute adduct levels. Cells were treated with 0.5 (open bars), 1.0 (hatched bars), or 1.5 (solid bars) μ M IQ. Data represent mean $\pm$ SEM for three independent experiments in each of the transfected CHO cell lines. dG-C8-IQ adduct levels were set as 1 in the UV5 cells treated with 0.5 μ M IQ and all other dG-C8-IQ adduct levels are relative to this value. dG-C8-IQ adduct levels were significantly greater in the UV5/CYP1A2/NAT2*4 cell line as compared to all other cell lines at each concentration tested.

Fig. 7. dG-C8-MeIQx DNA adduct levels. Cells were treated with 0.5 (open bars), 1.0 (hatched bars), or 1.5 (solid bars) μM MeIQx. Data represent mean ± SEM for three independent experiments in each of the transfected CHO cell lines. dG-C8-MeIQx DNA adduct levels were significantly greater in the UV5/CYP1A2/NAT2*4 cell line as compared to all other cell lines at each concentration tested ($p < 0.001$).

Table 1

IQ-induced *hprt*^{*} mutations in UV5/CYPIA2/NAT2^{*} 4 Cells

Single-base substitutions					Mutation	Surrounding Sequence [†]	Amino Acid Change
Mutant	Position	Exon					
1	47	2			G:C > T:A	ccaggctat	Gly > Val
2	47	2			G:C > T:A	ccaggctat	Gly > Val
3	47	2			G:C > T:A	ccaggctat	Gly > Val
4	47	2			G:C > T:A	ccaggctat	Gly > Val
5	129	2			G:C > T:A	attatggac	Met > Ile
6	148	3			G:C > C:G	cttgccega	Ala > Pro
7	149	3			C:G > A:T	cttgccega	Ala > Asp
8	173	3			G:C > C:G	atggaggac	Gly > Ala
9	296	3			T:A > C:G	gattttatc	Phe > Ser
10	355	4			G:C > C:G	gggtgggat	Gly > Arg
11	358	4			G:C > T:A	ggggatgat	Asp > Tyr
12	370	4			A:T > C:G	tcaacttta	Thr > Pro
13	419	6			G:C > A:T	aciggtaaa	Gly > Asp
14	425	6			C:G > A:T	aaaacuatg	Thr > Lys
15	448	6			G:C > T:A	ctgggtcaag	Val > Phe
16	509	7			G:C > T:A	tctggaagt	Arg > Leu
17	565	8			G:C > T:A	gtgtgttga	Val > Phe
18	634	9			G:C > T:A	actgggaaa	Gly > Trp
Deletions(Δ)^{††}							
19	385–402	Δ5			Deletion	gaaagAgacat	Frameshift
20	486–532	Δ7			Deletion	ctgctAtgct	Frameshift
21	533–609	Δ8			Deletion	agactAcatat	Frameshift
22	533–609	Δ8			Deletion	agactAcatat	Frameshift

^{*} GenBank sequence accession # J00060.

[†] Mutated or inserted nucleotides are bolded.

^{7/7} The actual mutation in the splice-site region which led to the deletions cannot be determined with certainty because sequencing was performed on mutant cDNAs.

Table 2

MeIQx-induced *hprt** mutations in UV5/CYP1A2/NAT2* 4 CHO cells

Single-base substitutions					
Mutant	Position	Exon	Mutation	Surrounding Sequence [†]	Amino Acid Change
1	47	1	G:C > T:A	ccaggctat	Gly > Val
2	47	1	G:C > T:A	ccaggctat	Gly > Val
3	58	2	G:C > C:G	ctagattta	Asp > His
4	118	2	G:C > T:A	catggagtg	Gly > Stop
5	145	3	C:G > T:A	agacttgcc	Leu > Phe
6	172	3	G:C > C:G	atgggagggc	Gly > Arg
7	209	3	G:C > T:A	aagggggggc	Gly > Val
8	403	5	G:C > T:A	gagacata	Asp > Tyr
9	419	6	G:C > C:G	actggtaaa	Gly > Ala
10	437	6	T:A > A:T	actctgctt	Leu > Gln
11	464	6	T:A > C:G	aacctcaaa	Leu > Pro
12	500	7	G:A > T:A	aaaaggacc	Arg > Met
13	539	8	G:C > T:A	gttggatt	Gly > Val
14	569	8	G:C > T:A	gttgatgat	Gly > Val
15	589	8	G:C > T:A	aalgagtag	Glu > Stop
16	601	8	G:C > C:G	aggagtttg	Asp > His
Deletions(Δ)^{††}					
17	385–402	Δ 5	Deletion	gaaagAgacat	Frameshift
18	403–485	Δ 6	Deletion	ttgagActtgc	Frameshift
19	403–485	Δ 6	Deletion	ttgagActtgc	Frameshift
20	486–532	Δ 7	Deletion	ctgctAttgct	Frameshift
21	610–654	Δ 9	Deletion	atcatΔStop	Frameshift

* GenBank sequence accession # J00060.

[†] Mutated or inserted nucleotides are bolded.

^{††} The actual mutation in the splice-site region which led to the deletions cannot be determined with certainty because sequencing was performed on mutant cDNAs.