What Do Diet-Induced Changes in Phase I and II Enzymes Tell Us about Prevention from Exposure to Heterocyclic Amines?\textsuperscript{1–3}

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Expanded Abstract

Well-done cooked protein containing foods derived from muscle can contain 1–200 parts per billion of mutagenic/carcinogenic heterocyclic amines. The most abundant of these compounds, 1-methyl-6-phenyl-1Himidazo[4,5-b]pyridin-2-amine (PhIP),\textsuperscript{4} has recently been classified by the National Toxicology Program (NTP) to be a “reasonably anticipated human carcinogen.” This is in part because of the extensive human epidemiology data associated with these heterocyclic amines (1). In >30 human epidemiology studies done to correlate cancer incidence and well-done meat consumption, 80% of them show a positive correlation. This includes cancer sites in breast, colon, rectum, esophagus, larynx, lung, pancreas, and prostate. The relative risks range from 2 to 3 in the breast to 8 in the colon and prostate.

Human exposure to these carcinogens predominantly occurs by consumption of well-done cooked meats. Once ingested, these compounds are rapidly metabolized and distributed throughout the body, where they can cause mutations in multiple tissues. Studies have shown that the internal dose of these compounds can be reduced by binding up the heterocyclic amines by other foods in the diet (2). Artificial digestion of muscle foods and subsequent human studies, where total urinary metabolites were examined after consumption of heterocyclic amines from grilled chicken, show that foods traditionally high in fiber such as pasta, prunes, and beans may be able to bind to the carcinogens, thus reducing the internal dose of these carcinogens when these foods are consumed in the diet at the same time.

Numerous laboratories have shown that heterocyclic amines are first metabolized by cytochrome P4501A2 to bioactive N-hydroxy intermediates that can then be either detoxified by Phase II conjugation enzymes such as UDP-glucuronosyltransferase (UGT) or further activated by other Phase II enzymes (i.e., acetyltransferase, sulfotransferase). These reactive enzymatic products presumably act as leaving groups. The reactive free radicals then bind almost exclusively to the C-8 of guanine, causing DNA adducts, mutations, chromosomal abnormalities, and cancer in multiple tissues.

Human differences in response to the internal exposure to these compounds most likely depends on 2 major factors: 1) individual genotypic differences in the activities of the Phase I and II enzymes and 2) the dietary modulation of the enzyme levels by natural inducers or inhibitors found in our food.

An example of individual differences that might promote DNA damage can best be illustrated by those related to UGTs. Polymorphic expression of several UGT isoforms has been reported. These polymorphisms have been implicated as risk factors for several diseases, including cancer (3). The most notable polymorphisms are variants in the UGT1A1 gene that result in significant downregulation of UGT1A1 activity. The most common polymorphism is characterized by an allelic variant in the UGT1A1 gene that contains an additional (TA)\textsubscript{n} dinucleotide repeat in the “A(TA)\textsubscript{n}TAA” box region of the promoter (4). Wild-type UGT1A1 activity is associated with 6 repeats, whereas the variant allele contains 7 TA repeats (UGT1A1*28). This variant results in significant downregulation of UGT1A1 activity and occurs in 7–10\% of the general population. It is also the polymorphism associated with Gilbert’s syndrome, which is a benign form of hyperbilirubinemia that results from a reduced capacity to glucuronidate bilirubin.

There is evidence to suggest that individuals with Gilbert’s syndrome may be at greater risk from exposure to heterocyclic amines that are conjugated by UGT1A1 because their ability to detoxify these compounds would be diminished (5). For example, a recent study has reported a correlation between the UGT1A1*28 polymorphism and a decreased ability to glucuronidate and detoxify N-hydroxy-PhIP in human liver microsomes (6). Subjects possessing the UGT1A1*28 allelic variant (which contains (TA)\textsubscript{n} repeats) showed significant decreases in UGT1A1 protein expression and N-hydroxy-PhIP glucuronidation activity in liver microsomes when compared with subjects having the wild-type UGT1A1*1 genotype.

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\textsuperscript{4} Abbreviations used: NTP, National Toxicology Program; PAH, polycyclic aromatic hydrocarbon PhIP, 1-Methyl-6-phenyl-1H-imidazo[4,5-b]pyridin-2-amine; UGT, UDP-glucuronosyltransferase.

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Cytochrome P450 1A2, which is the major enzyme in the oxidation step for these heterocyclic amines (7), is inducible by signaling from the Ah receptor. Flavonoids (β-naphthoflavone) and polycyclic aromatic hydrocarbons (PAHs; e.g., benzo[a]pyrene) have been known for 30 y to be inducers of this Phase I pathway. PAHs such as benzo[a]pyrene are produced from the fat dripping on hot coals during barbecuing. Therefore, an individual consuming heterocyclic amines from charred barbecued meat will have much faster production of N-OH intermediates (because of induction of CYP1A2 by PAHs) compared with eating pan-fried meat. The risk from the change in kinetics for the formation of these intermediates in humans is not known and should be investigated.

The induction of UGT glucuronosyltransferases by several flavonoids (quercetin, luteolin, apigenin, baicalein chrysin, flavone) is of interest because many flavonoids that induce UGTs are also inducers of the P450 oxidation step. The kinetics of the induction may very well determine whether more or less adducts are formed in the DNA. Other naturally occurring compounds that induce these Phase II enzymes are benzo[a]pyrene, coumarin, α-angelicalactone, ellagic acid, and fericulic acid. The structural characteristics of these compounds and the differences in binding affinity for the enzyme active site may very well set the stage for which compounds can actually lead to prevention.

The most mass abundant heterocyclic amine, PhIP, causes mammary tumors in rats and has also been shown to induce an estrogenic response by binding to the estrogen receptor. Other heterocyclic amines inhibit this response, possibly by binding to another site on the receptor protein. The ratio of the heterocyclic amines in the cooked meat coupled with the binding kinetics may be important for understanding their true contribution to cell proliferation and/or tumor promotion in humans who are exposed to a mixture of these compounds. Additionally, assays in MCF-7 cells have shown that coinubation of heterocyclic amines (with the exception of PhIP) with estradiol inhibits estradiol-induced estrogen activation. This suggests that exposure to heterocyclic amines may also modulate normal estradiol-related functions (8).

In conclusion, steps can be taken to reduce the exposure to these dietary mutagenic and carcinogenic heterocyclic amines. These include paying close attention to food preparation methods and including foods in your diet that either adsorb the heterocyclic amines or change their metabolic pathways to favor detoxification. We recommend not cooking food to “well-done” stage, cooking at a lower temperature (below 200°C), and cooking more slowly for a longer period of time. Premicrowaving meat is also an efficient way to reduce exposure. This technique releases the heterocyclic amine reaction precursors from the meat; thus, when it is cooked “well-done”, lower levels of carcinogens are generated. Marinating and flipping meat serve to reduce the surface temperature of the meat, which helps lower heterocyclic amine formation. Eating fiber-containing foods such as beans and pasta with your meat may lower the internal dose by absorbing some of the heterocyclic amines before they can become bioactivated. Vegetables high in flavonoids have been shown to induce both Phase I and II enzymes. Which pathways (detoxification or bioactivation) pre-dominate will dictate what enzymes are induced. It has been shown that induction of UGT activity greatly increases the detoxification of these compounds. This is seen clearly by a reduction in PhIP-induced mutation and cell killing in CHO cells with and without induced UGT activity (9). These simple dietary changes can significantly reduce one’s exposure to food-derived heterocyclic amines and, in turn, reduce the potential carcinogenic effects from these compounds.

Literature Cited