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Review

Heart damage associated with cooked meat mutagens

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Mutagenic heterocyclic amines are produced during the ordinary cooking of meats and fish. When metabolically activated, heterocyclic amines will form covalent adducts with DNA, which, if not repaired, may affect the flow of genetic information in a cell. It has been proposed previously that heterocyclic amine mutagens contribute to the incidence of dietary-related cancers because they cause somatic cell mutations and induce tumors in rodents and nonhuman primates. Recent work has shown that some cooked food mutagens preferentially produce DNA damage in heart cells, DNA adduct levels are directly related to dose and duration of mutagen exposure, the dietary damage persists for long intervals in cardiac tissue, and mitochondrial DNA is more vulnerable than nuclear DNA to these mutagens. Because cardiac myocytes are terminally differentiated cells that have lost their ability to divide, the capacity to repair DNA damage is a critical factor in the proper function of cardiomyocytes, and cardiac myocytes seem to have limited DNA repair capabilities. DNA damage formed by dietary components, such as heterocyclic amines, might accumulate with time because of inefficient repair and thereby affect heart cell function or viability. The possibility that dietary habits play a role in idiopathic cardiomyopathies and congestive heart disease should be explored in greater depth. (J. Nutr. Biochem. 8:490–496, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Epidemiological studies indicate that diet is an important factor in the causes of cardiovascular disease.[1,2] Diets rich in saturated fatty acids correlate generally with increased risks and incidences of coronary heart disease,[2] but there are other components in food that could have adverse effects on the cardiovascular system. For example, cooked meats and fish contain a number of mutagenic and carcinogenic heterocyclic amines.[3–6] Heterocyclic amines are formed from all major protein sources of the American diet that are cooked under normal conditions. Major protein sources are meat and fish, but other high-protein foods such as eggs, beans, milk, and cheese can have mutagenic activity associated with heating or cooking these foods.[7] In fact, natural substances that contain amino acids, creatin(in)e, and sugars will, when heated, yield mutagenic heterocyclic amines.[8,9] These mutagens also are enriched in pan gravies and come off in heating smoke and fumes.[4,10] Greater biologic activity is generally associated with overcooking and charring meats, whereas lower temperature and increased water, e.g., stewing and poaching, generate less mutagens.[11] Although the distribution and yields of heterocyclic amines can vary with the method of cooking, all heating of meats results in some mutagenic activity,[4,8,9] and food-borne heterocyclic amines have been found in urine samples of healthy human subjects eating cooked meat in their daily diets.[12,13] Therefore, individuals who consume cooked meats in their regular diet may be continually exposed to potentially harmful compounds.

Metabolic processing of heterocyclic amines

To exert their biologic effects of cytotoxicity, mutagenicity, and carcinogenicity, heterocyclic amines require metabolic

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activation.[8] These compounds are absorbed rapidly from the gastrointestinal tract and transformed metabolically into several products. Metabolic activation occurs via a two-step process. The amino moiety of heterocyclic amines is first *N*-oxidized by cytochrome P450-dependent mono-oxygenase systems[14,15] that are most highly concentrated in liver but have been detected in other tissues.[16] In rodents, the isozymes CYP1A1 and CYP1A2 are both involved in *N*-hydroxylation of cooked meat mutagens.[17] On the other hand, human liver microsomes almost exclusively catalyze mutagen oxidation by CYP1A2.[18] Comparative studies using rat, nonhuman primate, and human liver microsomal fractions showed that human liver microsomes are more active than the others in catalyzing heterocyclic amine activation.[19,20] It is well known that different cytochrome P450 enzymes involved in the metabolism of xenobiotic substrates differ markedly in their response to enzyme inducers and inhibitors. Clearly, the expression, regulation, and specificity of P450-activating enzymes are species- and tissue-specific.

N-hydroxylated intermediates are converted by phase II enzymes to highly reactive electrophilic species capable of covalently binding to nucleic acids and proteins.[8] Such downstream activation systems have been described that include acetylation, sulfation, phosphorylation, and amino acylation, mediated by acetyltransferases, sulfotransferases, kinases, and amino acyl-tRNA synthetases, respectively.[20] More than one activation system can operate in a tissue, and indeed pathways frequently compete with one another. *N*-hydroxylamines are unstable in oxidative environments but are relatively nonreactive with DNA under physiological conditions. However, it has been shown that the acetyltransferase *N*-acetoxy-derivatives are very reactive with DNA in vitro, forming the same deoxyguanosine-C8 adduct that is a major in vivo product.[21–23] This ability to form DNA adducts has been demonstrated repeatedly for every activated heterocyclic amine examined, most of which are extremely potent mutagens in bacterial assays.[3–6] In contrast to activation, detoxification is another mechanism that affects genotoxicity. Metabolites that result from cytochrome P450-mediated ring oxidation followed by conjugation to sulfate or β-glucuronic acid, are excreted in urine and feces.[24–27] These elimination pathways are quite prominent in rodents, but show little activity in human assays.[18] Although the parent compounds can be detoxified by ring hydroxylation, once converted to the *N*-hydroxylamine, the proximal mutagen can be inactivated through conjugation with glucuronide again catalyzed by cytochrome P450 enzymes. The overall intracellular levels of ultimate mutagen/carcinogen will be determined by the balance between phase I activation/detoxification and phase II activation/detoxification. Furthermore, the health risk to humans from heterocyclic amines in the diet will depend on polymorphic activating and detoxifying enzymes that can exhibit extensive interindividual variability.[28,29]

Cooked food mutagens and cardiac damage

Twenty-one heterocyclic amines from cooked-meats have been identified and their structures determined.[30–32] All heterocyclic amine mutagens form DNA-adducts, normally most abundantly in liver tissue—the proximal site of activation. For the early years after their discovery, the focus of cooked food mutagen research was on their carcinogenicity. It is thought that these agents contribute to the incidence of dietary-related cancers because they cause somatic cell mutations and induce tumors in animal models.[3–6,30–32] Every heterocyclic amine tested thus far is carcinogenic in rodents and nonhuman primates.[5,30] More recently, there has been a consideration of the damage these compounds might do to nonreplicating cells that are long-lived.[33] There might be toxic effects in which case, cells in a nonrenewing cellular compartment could die. Alternatively, there might be long-lived, persistent damages that could cause abnormal pathology and compromise function.

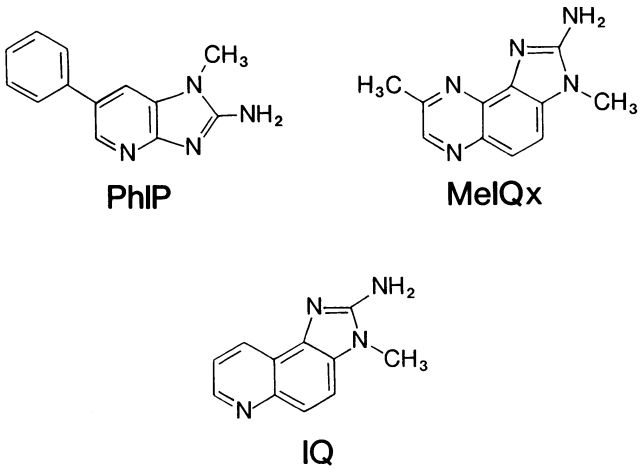

Figure 1 The chemical structures of the cooked meat mutagens 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ).

Recent interest has focused on the heterocyclic amines 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) which are aminoimidazoazaarenes, structures of which are shown in *Figure 1*. IQ, MeIQx, and PhIP, at parts per million levels in the diet, have been shown to induce tumors in multiple organs of rodents with different target organ specificities.[5,6,30,32] Furthermore, IQ, MeIQx, and PhIP have been implicated in causing heart damage.[33] PhIP and MeIQx are present in cooked beef at levels of 7.5–50 ng/g for PhIP and 0.1–5.9 ng/g for MeIQx.[3,34] The two heterocyclic amines account for roughly half of the total mutagenic potential of fried beef. Therefore by weight, PhIP is the most abundant heterocyclic amine in commonly fried meats and is estimated to be the most prevalent of these compounds in the American diet.[34] PhIP, however, is less mutagenic in routine bacterial mutagenicity tests than other abundant cooked meat mutagens, such as MeIQx, which initially caused this compound to be somewhat overlooked.[3] Interestingly, PhIP undergoes phase I (but not phase II) activation in the liver, thereby generating systemic N-OH-PhIP for subsequent metabolism in extrahepatic tissues.[24] Recently, several studies have indicated that PhIP may play a pathological role in tissues not normally associated with

chemically induced tumors. High levels of DNA adducts have been observed in heart tissue of monkeys and rats fed PhIP in their diet.[35–38] Treated animals displayed myocardial abnormalities, including foci of chronic inflammation and myocyte necrosis.[39,40] Tissue distribution studies demonstrated that of 12 tissues compared, the heart had the highest levels of PhIP-induced adducts.[35,37,38] For example, Takayama et al.[35] showed that after 4 weeks of dietary administration of PhIP to Fischer 344 rats, the highest level of DNA damage observed was in heart tissue, approximately 10-fold higher than liver tissue. Overvik et al.[41] subsequently showed that DNA adducts are formed in rat heart after dietary administration of MeIQx. The most remarkable result of these latter studies was that MeIQx adducts increased linearly in heart DNA throughout the 10-month period that MeIQx was administered and persisted for up to 5 months after the compound was withdrawn.[41] The data suggest that this class of DNA damage is relatively refractory to DNA repair processes in heart tissue and that these adducts can accumulate over a lifetime and exist at levels proportional to their dietary intake. Alternatively, DNA adduct levels in several other tissues of rats exposed to the same dietary concentration of MeIQx increased for only the first month or two, after which time a plateau was reached or the levels declined.[42] The development of MeIQx resistance in noncardiac tissues correlated with the expression of the multiple-drug-resistance gene, indicating that gene induction was involved in mutagen resistance.[42]

Comparative studies have shown that the metabolically activated forms of IQ, MeIQx, and PhIP are cytotoxic to cardiac myocytes in culture.[40,43] Cultured rat cardiac cells were exposed to varying concentrations of either the parent compounds or the *N*-hydroxylated mutagens. Unmodified IQ, MeIQx, and PhIP did not result in detectable levels of DNA adducts and were not cytotoxic, whereas their *N*-hydroxylamine derivatives were.[40,43] Cardiac myocytes were significantly more sensitive to the acute cytotoxic effects of activated MeIQx and PhIP than were nonmyocytes. Activated PhIP induced 10-fold more DNA damage in myocytes than did *N*-hydroxy-IQ,[40] showing different phase II substrate specificities in myocytes for the various heterocyclic amine mutagens. Cell-specific genotoxicity was also noted. For example, *N*-hydroxy-PhIP produced four times more adducts in myocytes than in nonmyocytes.[43] This latter observation may be related to differences in metabolic processing between the two cell types. Therefore, these results support the view that cardiac myocytes are capable of activating proximal food mutagens to DNA damaging species, and myocytes are therefore susceptible in vivo targets for dietary heterocyclic amines.

DNA damage and repair in cardiac cells

DNA damage is the primary lesion mediating many cytotoxic and mutagenic events, and there is increasing evidence that many forms of DNA damage occur naturally through exposure to dietary agents.[44,45] Because cardiac myocytes are postmitotic and exist in a state of terminal differentiation,[46] the capacity to repair DNA damage is a critical factor in a myocyte's proper function and longevity. The myocardium may be at risk for genotoxic agents because of limited DNA repair capabilities. Several in vitro studies have reported that as myoblasts differentiate into myocytes, their ability to repair DNA damage is reduced.[47,48] Rats fed MeIQx in the diet accumulated DNA damage in heart tissue in direct proportion to exposure dose, and once induced, adducts persisted for long periods, suggesting very limited DNA repair.[41] In addition, food-mutagen DNA adducts were removed from the genome of cultured cardiac myocytes much less efficiently than nonmyocytes.[43] In mammalian cells, DNA repair exhibits intragenomic heterogeneity—with some DNA sequences being repaired more efficiently than others.[50,51] In contrast to overall DNA repair levels that are attenuated in differentiated rat myoblasts, some transcriptionally active, tissue-specific genes are selectively repaired, although repair rates are low relative to proliferating cells.[49] Terminally differentiated myocytes in culture therefore seem to maintain DNA damage surveillance for selected genes or genomic domains, but the relevance of these in vitro results to gene-specific repair in myocardium in situ is not known because no investigations have been reported, although DNA lesions, such as aromatic adducts and alkyl adducts, have been shown to accumulate in mouse myocardium during aging.[52,53]

The consequences of persistent lesions in a cellular genome depend on where the lesions are located and whether or not the cell is replicating. It has been proposed that DNA lesions, not mutations, are likely to exert their most profound effects in nondividing cells.[54] DNA acts as a template to duplicate itself, and deleterious mutations would disrupt the flow of information through progeny cells—many of which might die or be selected against. Alternatively, the other major function of DNA is to guide the formation of RNA molecules, which continually turn over and need to be replaced. Lesions involving DNA that interfere with RNA synthesis might be lethal to postmitotic cells if new proteins cannot be made, or physiologic functions might be reduced if less protein is made or altered proteins are synthesized.[54–56] The genetic flow of information in myocytes could be more compatible with accumulated damage rather than lethal lesions. In postmitotic heart tissue, for example, myocytes are typically binucleate and depending on the species, can be polypoid as they are for humans.[57] Therefore, genetic complementation can occur. If one allele is inactivated via a bulky adduct or other damage that affects gene expression, the other allele can supply the missing gene product. However, the effective gene dosage would be cut in half, with the possibility of less protein being made. Thus, function would be diminished as opposed to lost. There is suggestive evidence that DNA damage can selectively alter cardiac gene transcription. For example, experiments showed a major depressive effect of adriamycin on rat myocardial α-actin mRNA.[58] No depression of troponin C or glyceraldehyde-3-phosphate dehydrogenase mRNAs was observed. This inhibitory effect of adriamycin might be attributable to blocking of RNA polymerase II by DNA damage.[59] Recent studies have, in fact, demonstrated PhIP-DNA adducts inhibit plasmid reporter gene expression in mammalian cells.[60] However, no

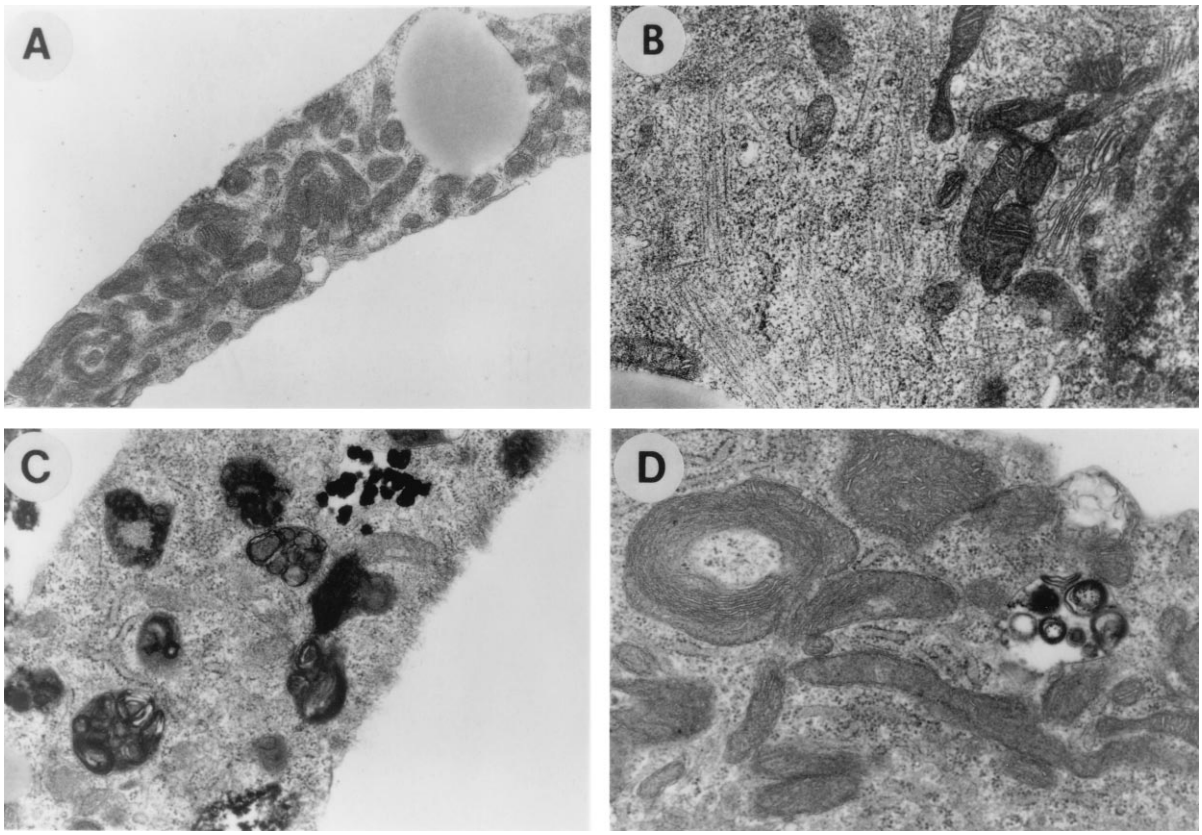

Figure 2 Transmission electron micrographs of neonatal rat cardiac myocytes in culture. Control myocytes are shown in *A* and *B*. Myocytes exposed to 50 μM N-OH-PhIP for 2 hr, then washed, placed in culture medium, and fixed for EM 24 hr later are shown in *C* and *D*.

in vivo studies have been performed examining the effects of cooked-food-mutagen damage on gene expression.

Damage to mitochondria

To gain a better understanding of the influence of dietary factors on a myocyte's ability to maintain differentiated function for many years, it is important to consider damage to the mitochondrial genome in addition to the nuclear genome, because damaged mitochondria might alter cellular energy stores and thereby affect the ability of myocytes to overcome injury caused by dietary components. The mitochondrial genome is an important cellular target for cytotoxic compounds.[61] Emerging evidence, moreover, indicates significant mitochondrial DNA instability associated with chronic diseases and aging of human myocardium.[62,63] One potential outcome of accumulated damage or mutations in the mitochondrial population is a reduction in oxidative phosphorylation and thus, ATP.[63,64] Indeed, accumulations of mutations and deletions in mitochondrial DNA with their associated defects in oxidative phosphorylation have been implicated in a diverse group of clinical problems such as ischemic heart disease, diabetes, Parkinson's disease, Alzheimer's disease, cancer, and aging.[62–65]

Mitochondria might be a preferred target for cooked food mutagens because mitochondrial DNA is more sensitive than nuclear DNA to many types of mutagenic insults.[61] *Figure 2* shows ultrastructure aspects of neonatal rat cardiac myocytes, untreated and exposed to activated mutagen. *Figure 2A* shows a cross section of an untreated myocyte with a prominent lipid droplet and large numbers of mitochondria. *Figure 2B* is a higher magnification of a control myocyte, showing normal mitochondria and dispersed myofilaments. A 2-hr exposure of myocytes to 100 μM N-OH-PhIP was moderately cytotoxic as judged by lactic acid dehydrogenase leakage (25% to 30% over 24 hr), whereas myocytes cultured in 50 μM mutagen showed only background levels of 5% to 7% enzyme loss.[40,43] However, myocytes exposed to 50 μM N-OH-PhIP for 2 hr, then fixed 24 hr later, revealed a very high proportion of dense bodies, consistent with cellular damage and increased cellular degradation processes (*Figure 2C*). In addition, treated myocytes seemed to have fewer mitochondria and contained swollen mitochondria marked by abnormal cristae (*Figure 2D*). In contrast to control myocytes, Davis et al.[40] found that only 40% of cells exposed to 100 μM N-OH-PhIP contained myofilaments, and many cells exhibited decreased numbers of mitochondria, Golgi membranes, and glycogen. These results indicate that significant myocyte damage was incurred by a single acute exposure, and that mitochondria seem to be a sensitive target for N-OH-PhIP toxicity. It has been shown that IQ and PhIP formed adducts with mitochondrial DNA in vivo, and rats given 10 doses of PhIP (100 mg/kg, p.o.) over 2 weeks had significantly more mitochondrial DNA adducts than nuclear DNA adducts.[66] One possible explanation for the accumulation of adducts in

mitochondrial DNA with repetitive mutagen exposure is that there are differences in repair capacities between the nuclear and mitochondrial compartments. The studies of LeDoux et al.[67–69] have indicated that mitochondria perform some types of base excision repair. However, nucleotide excision repair that removes bulky adducts, such as those produced by heterocyclic amines, has not been demonstrated for this organelle.

Conclusions

An association between regular intake of cooked meats and cardiac damage in humans has not been established. However, cooked food mutagens are relevant to human nutrition and health because heterocyclic amines are present in our diet and the human liver is capable of metabolizing the ingested compounds to DNA-reactive species that form DNA adducts in cultured human cells.[19,70,71] Furthermore, PhIP-DNA adducts have been detected in human tissue samples.[72] Human daily exposure levels to cooked food mutagens are estimated to be comparable to those of environmental carcinogens such as *N*-nitrosodimethylamine and benzo[a]pyrene.[3] DNA adduct levels are colinear with dietary concentrations and might be extrapolated down to amounts contained in a single meal.[73] Therefore, conventional consumption of heterocyclic amines may lead to an accumulation of DNA damage in the hearts of humans.

Mechanisms through which cardiac toxicity could be expressed include metabolic pathways, membrane receptors/properties, and chemical interactions with various subcellular targets. None of these variables have been elucidated for cooked food mutagens in cardiac myocytes. Characterization of the metabolic pathways leading to activation and detoxification of IQ, MeIQx and PhIP in myocardial cells will determine cellular parameters that affect the outcome of mutagen exposures and the potential risks associated with dietary exposure to these compounds. However, many (perhaps most) biologic changes will result from what happens to the genes involved in maintaining differentiated cell function. The effects of heterocyclic amine DNA adducts on gene regulation and expression in vivo are not known, and there is a gap in our knowledge concerning toxic effects of, and cellular defense mechanisms against, heterocyclic amines in cardiac cells.

Results from studies performed in the past few years suggest a link between eating well-done meats/fish and cellular damage. The long-term outcomes of such damage to myocytes has not been elucidated, but food mutagen exposure could be involved in the pathogenesis of chronic human diseases, such as atherosclerosis, ischemic heart disease, and cardiomyopathies.[74–76] The contribution of diet to the prevention and treatment of diabetes, hypertension, heart diseases, cancer, and kidney diseases is significant. Improved understanding of the causes, rates of progression, and interaction of variables, such as diet, may lead to preventive and therapeutic interventions designed to reduce the incidence of cardiovascular diseases in the population. Thus, future cooked-meat mutagen studies that correlate heterocyclic amine structure, dose, and metabolism with cardiac pathophysiology will advance our understanding of the association between dietary mutagens and heart disease.

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