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Exposure to meat-derived carcinogens and bulky DNA adduct levels in normal-appearing colon mucosa

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ABSTRACT

Introduction: Meat consumption is a risk factor for colorectal cancer. This research investigated the relationship between meat-derived carcinogen exposure and bulky DNA adduct levels, a biomarker of DNA damage, in colon mucosa.

Methods: Least squares regression was used to examine the relationship between meat-derived carcinogen exposure (PhIP and meat mutagenicity) and bulky DNA adduct levels in normal-appearing colon tissue measured using ^{32}P-postlabelling among 202 patients undergoing a screening colonoscopy. Gene-diet interactions between carcinogen exposure and genetic factors relevant to biotransformation and DNA repair were also examined. Genotyping was conducting using the MassARRAY® iPLEX® Gold SNP Genotyping assay.

Results: PhIP and higher meat mutagenicity exposures were not associated with levels of bulky DNA adducts in colon mucosa. The XPC polymorphism (rs2228001) was found to associate with bulky DNA adduct levels, whereby genotypes conferring lower DNA repair activity were associated with higher DNA adduct levels than the normal activity genotype. Among individuals with genotypes associated with lower DNA repair (XPD, rs13181 and rs1799179) or detoxification activity (GSTP1, rs1695), higher PhIP or meat mutagenicity exposures were associated with higher DNA adduct levels. Significant interactions between the XPC polymorphism (rs2228000) and both dietary PhIP and meat mutagenicity on DNA adduct levels was observed, but associations were inconsistent with the *a priori* hypothesized direction of effect.

Conclusion: Exposure to meat-derived carcinogens may be associated with increased DNA damage occurring directly in the colon among genetically susceptible individuals.

1. Introduction

The colon and rectum are among the most common sites for cancer development in both men and women. In fact, despite advances in risk factor reduction, screening, and treatment, colorectal cancer (CRC) continues to account for the majority of cancer deaths worldwide [1]. In 2015, based largely on an association with CRC, IARC classified red meat consumption as probably carcinogenic to humans (Group 2A) [2]. Exposure to carcinogens present in cooked meats like heterocyclic aromatic amines (HAAs), is likely an important contributor to the carcinogenicity of meats. As exposure to meat-derived carcinogens like HAAs is modifiable, increasing our understanding of their role in

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colorectal carcinogenesis will help in the development of prevention strategies aimed at reducing the burden of this important disease.

Over 20 HAAs have been identified in cooked foods; the most abundant carcinogenic HAA formed in meats is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) [3,4]. Dietary exposure to PhIP is considered to be a potential cancer risk factor because PhIP has been demonstrated to be a potent mutagen in bacterial and mammalian cell genotoxicity assays and has been shown to induce colon tumours in rats [5,6]. In general, an increase in the quantity and frequency of meat consumption and the use of high-temperature cooking methods (pan-frying, oven-broiling and grilling/barbecuing) produce the highest HAA concentrations [3]. In addition, the degree of doneness, which is often closely related to surface browning and total cooking time, are key determinants of the level of HAA production in cooked meats [3,7]. Additionally, in cooked meats there are other classes of carcinogens including polycyclic aromatic hydrocarbons and nitrosamines; another metric commonly used in epidemiological research is therefore the level of meat mutagenicity, which incorporates the mutagenic potential of all classes of carcinogens found in consumed meats including HAAs [8].

The mutagenicity and, presumably, the carcinogenicity of HAAs like PhIP, involve bioactivation by cytochrome P450 s (CYP). CYP-mediated bioactivation of PhIP involves *N*-oxidation of the exocyclic amine group producing *N*-hydroxy-PhIP that can be subsequently conjugated by N-acetyltransferases (NATs) to highly unstable esters. Alternate fates of the *N*-hydroxy-PhIP include detoxification by pathways such as those mediated by glutathione S-transferases (GSTs) possibly through a formation of an unstable glutathione conjugate back to its parent amine [9]. Reactive metabolites of HAAs preferentially bind to the carbon-8 position of the guanine residue of DNA resulting in the formation of adducts that tend to be more promutagenic than those bound to other sites on DNA [10]. DNA adducts can then either persist or be repaired by nucleotide excision repair (NER) [11]. As the persistence of a DNA adduct increases, the probability that tumours will develop also increases [12]. It is expected that DNA adducts achieve a steady-state level that reflects the balance between exposure to a carcinogen, adduct formation and adduct elimination by DNA repair.

This research examined the influence of dietary exposures to PhIP and total dietary meat mutagenicity on bulky DNA adduct levels measured in normal-appearing colon tissue using ^{32}P-postlabelling. We also assessed potential gene-environment interactions in determining bulky DNA adduct levels in colon mucosa.

2. Materials and methods

This research is nested within a cross-sectional study that recruited 342 male and female consenting patients, aged 40 to 65, undergoing a screening colonoscopy at a regional endoscopy centre at Hotel Dieu Hospital in Kingston, Ontario, Canada between 2009 and 2012 [13]. Briefly, to obtain the study sample of 342 participants, several study exclusion criteria were applied including: 1) diagnosis of inflammatory bowel disease (ulcerative colitis or Crohn's disease) or; 2) known genetic disorders that predispose to CRC (hereditary nonpolyposis CRC, familial adenomatous polyposis) or; 3) any gastrointestinal abnormality (adenoma, hyperplastic polyps or cancer) detected at a previous colonoscopy. As well, patients with a diagnosis of cancer in the last five years (except non-melanoma skin cancer) were not recruited. Out of the 342 participants, 205 had adequate colon tissue DNA yields (5 μg) for adduct quantification and thus, contributed to this analysis. Indications for a screening colonoscopy in this population included a positive family history of CRC or adenoma in a first- or second-degree relative, a positive fecal occult blood test result and/or average risk screening.

2.1. Assessment of dietary PhIP and meat mutagenicity

Prior to the colonoscopy visit, a self-administered questionnaire that included a meat consumption module was administered. It assessed average exposure to meat-derived carcinogens as well as other personal, lifestyle and dietary factors. The collection of dietary exposure to PhIP and meat mutagenicity have been previously described [13]. Briefly, the meat consumption module collected information on the number of times per month that different meat items were consumed over the previous summer (April to October) and winter (November to March) months, according to the type of cooking method and the usual preferred level of doneness. Dietary exposure to PhIP and meat mutagenicity over the previous year was then estimated by linking the responses to the meat consumption items to the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) mutagen database developed by the National Institutes of Health [8]. In the CHARRED mutagen database, meat mutagenicity (total mutagenic activity) was quantified using the Ames test [14].

For each participant, the intake of dietary PhIP was derived for each meat item by multiplying the specific PhIP (ng/g) by the serving size (g) and by the frequency of intake per day [13]. For meat mutagenicity, exposure was calculated by multiplying the specific mutagenic activity (expressed in number of revertant colonies per gram of meat) by the serving size (g) and by the frequency of intake per day for each meat item. Serving size information was not collected in this study; thus, the medium serving size for each meat item provided in the CHARRED mutagen database was utilized in all calculations. Total dietary PhIP intake (expressed in ng per day) and meat mutagenicity exposure (expressed in number of revertant colonies per day measured using the Ames test [8]) were estimated by summing intake across meat items separately for summer and winter months. Average PhIP and meat mutagenicity exposure over the previous year was then calculated by taking an average of the measures derived from summer and winter months.

2.2. Assessment of bulky DNA adducts in normal-Appearing colon mucosa via ^{32}P-Postabelling

During the colonoscopy, two pinch biopsies of healthy, normal appearing mucosa were obtained from the descending colon, 5 cm apart and at least 5 cm away from any lesion, polyp or other mucosal abnormality [15]. Specimens were placed in lysis solution and stored at −20 °C until DNA extraction using the 5-PRIME DNA isolation kit (Inter Medico, ON, Canada). DNA concentration and purity were determined using NanoDrop. Bulky DNA adduct levels in normal appearing colon tissue were quantified via ^{32}P-Postlabelling using the butanol enrichment procedure [16]. ^{32}P-Postlabelling analysis is a sensitive method for the detection of carcinogen-DNA adducts [17]. The ^{32}P-postlabelling assay had a detection limit of approximately 1 adduct per 10^9 nucleotides and the reliability of this assay has been previously demonstrated (intra-assay coefficient of variation was 17%) [18]. The procedure involves four main steps: enzymatic digestion of DNA into single nucleotides; enrichment of the carcinogen-adducted nucleotides; radiolabelling of the adducts using ^{32}P and chromatographic separation of labelled adducts for quantification measured by their radioactive decay [17]. Bulky DNA adduct levels in normal-appearing colon tissue were successfully quantified for all 205 participants.

2.3. Genetic polymorphisms

Polymorphisms in genes involved in the biotransformation of PhIP and DNA repair were genotyped utilizing DNA isolated from blood leukocytes using the MassARRAY® iPLEX® Gold − SNP Genotyping assay by Genome Québec, Québec, Canada. Selection of polymorphisms for genotyping was based on identifying functional polymorphisms in genes involved in the metabolism of PhIP or those which are important components of NER. A general schema was developed for prioritization to ensure the selection of polymorphisms with the strongest biologic plausibility and high exposure prevalence. First, due to the limited sample size of this study, candidate polymorphisms were only