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Review

Red meat and colon cancer: A review of mechanistic evidence for heme in the context of risk assessment methodology

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ABSTRACT

On October 26, 2015, IARC published a summary of their findings regarding the association of cancer with consumption of red meat or processed meat (IARC 2015; The Lancet Oncology 2015). The Working Group concluded that there is limited evidence in human beings for carcinogenicity from the consumption of red meat and inadequate evidence in experimental animals for the carcinogenicity of consumption of red meat. Nevertheless, the working group concluded that there is strong mechanistic evidence by which ingestion of red meat can be linked to human colorectal cancer and assigned red meat to Group 2A "probably carcinogenic to humans". The Working Group cited supporting mechanistic evidence for multiple meat components, including those formed from meat processing, such as *N*-nitroso compounds (NOC) and heterocyclic aromatic amines, and the endogenous compound, heme iron. The mechanism of action for each of these components is different and so it is critical to evaluate the evidence for each component separately. Consequently, this review critically examined studies that investigated mechanistic evidence associated with heme iron to assess the weight of the evidence associating exposure to red meat with colorectal cancer. The evidence from *in vitro* studies utilized conditions that are not necessarily relevant for a normal dietary intake and thus do not provide sufficient evidence that heme exposure from typical red meat consumption would increase the risk of colon cancer. Animal studies utilized models that tested promotion of preneoplastic conditions utilizing diets low in calcium, high in fat combined with exaggerations of heme exposure that in many instances represented intakes that were orders of magnitude above normal dietary consumption of red meat. Finally, clinical evidence suggests that the type of NOC found after ingestion of red meat in humans consists mainly of nitrosyl iron and nitrosothiols, products that have profoundly different chemistries from certain N-nitroso species which have been shown to be tumorigenic through the formation of DNA adducts. In conclusion, the methodologies employed in current studies of heme have not provided sufficient documentation that the mechanisms studied would contribute to an increased risk of promotion of preneoplasia or colon cancer at usual dietary intakes of red meat in the context of a normal diet.

1. Introduction

On October 26, 2015, IARC published a summary of their findings regarding the assessment of the association of cancer with consumption red meat or processed meat (IARC, 2015; The Lancet Oncology, 2015). Data on the association of red meat consumption with colorectal cancer were available from 14 cohort studies. IARC concluded that chance, bias, and confounding could not be ruled out for the data on red meat consumption, since no clear association was seen in several of the high-quality studies and residual confounding from other diet and lifestyle risk is difficult to exclude. The Working Group concluded that: 1) there is limited evidence in human beings for the carcinogenicity of the consumption of red meat, 2) the strongest, but still limited, evidence for an association with eating red meat is for colorectal cancer, and 3) there is inadequate evidence in experimental animals for the carcinogenicity of consumption of red meat.

The Working Group cited supporting mechanistic evidence for multiple meat components, including those formed from meat processing, such as NOC and heterocyclic aromatic amines and the endogenous compound, heme iron, to evaluate an association between red meat intake and colorectal cancer. They concluded that studies support the role of heme iron from red meat in nitrosamine formation, genotoxicity and oxidative stress as mechanisms by which ingestion of red meat can be linked to human colorectal cancer. Based on this mechanistic evidence, the Working Group classified consumption of red meat as "probably carcinogenic to humans (Group 2A).

IARC, in the preamble to the Monographs, defines a cancer 'hazard' as an agent that is capable of causing cancer under some circumstances,

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Abbreviations

8-Iso-PGF2α	8-*iso*-prostaglandin-F2α
8-oxo	8-hydroxy-2-deoxyguanosine
ACF	aberrant crypt foci
APC	adenomatous polyposis coli
ATNC	apparent total *N*-nitroso compounds
CRC	Colon rectal cancer
DHN-MA	1,4-dihydroxynonane mercapturic acid
DGAC	dietary guidelines advisory committee
DMPO	5,5-dimethyl-l-pyrroline-*N*-oxide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTPA	diethylenetriaminepentaacetic acid
EPR	electron paramagnetic resonance
HHE	4-hydroxyhexenal
HNE	4-hydroxynonenal
HPLC	high-performance liquid chromatography
IARC	international agency for research on cancer
IQ	2-aminion-3-methylimidazo [4,5-f] quinolone
KRAS	Kirsten ras
LAOOH	linoleic acid hydroperoxides
LOO·	lipid peroxyl radical
LOOH	lipid hydroperoxide
MDA	malondialdehyde
MDF	mucin-depleted foci
Min	multiple intestinal neoplasia
N-NO-IQ	^{14}C-2-nitrosoamino-3-methylimidazo[4,5-f]quinolone
NOC	*N*-nitroso compounds
Nrf2	nuclear factor (erythroid derived 2)-like 2
O^6MeG	O^6-methyldeoxyguanosine
OECD	The Organisation for Economic Co-operation and Development
PUFA	polyunsaturated fatty acid
satHNA	saturated 4-hydroxynonanoic acid
SSB	single strain breakage
TBARS	thiobarbituric acid reactive substances
t-BuOOH	*tert*-butylhydroperoxide
USDA	The United States Department of Agriculture
U.S. FDA	The United States Food and Drug Administration

while a cancer 'risk' is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The Monographs are an exercise in evaluating cancer hazards, despite the historical presence of the word 'risks' in the title. The distinction between hazard and risk is important, and the Monographs identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher (IARC, 2006).

Because there is limited evidence of carcinogenicity in humans and insufficient evidence of carcinogenicity in animals, it is important to thoroughly examine mechanistic evidence cited by IARC as well as evidence from recent related publications on this topic. Therefore, studies that investigated the role of heme iron from red meat in nitrosamine formation, genotoxicity and oxidative stress, as mechanisms by which ingestion of red meat might be linked to human colorectal cancer, were critically reviewed. Pivotal to the assessment of the strength of the mechanistic evidence is an evaluation of the methodology employed in relevant studies as well as the consistency of the response across studies to determine the weight of the evidence. In addition, it is critical to appreciate the risk assessment process in order to evaluate the role of mechanistic data in identifying both potential for hazard and expression of risk under the conditions of a real world dietary exposure.

Risk assessments consists of hazard identification, followed by a dose-response or characterization, exposure assessment and, finally, risk characterization. When the full risk assessment process is not completed, there is the danger that hazard can be confused with risk. An identified hazard does not necessarily mean an identified risk. Hazard is defined as intrinsic toxicity whereas risk is the probability of manifesting that hazard under the conditions of the exposure (Kruger, 2016). As the first step in the risk assessment process, hazard assessment relies on the information gleaned from many sources including structure -toxicity analysis, *in vitro* testing, animal bioassays, and well conducted clinical trials. Hazard identification elucidates target organs, assesses the severity and reversibility of the intrinsic toxicity. Dose response allows the determination of the quantitative relationship between the dose and toxic effect and establishes a threshold for manifestation of that effect. Exposure assessment is critical as it describes the amount, intensity, frequency, duration, and route of exposure to the compound of interest. In the last step of a risk assessment, characterization integrates hazard identification, dose-response information and exposure assessment into an estimation of the adverse effects likely to occur in a specific population (Hayashi, 2009).

It is important to note that as part of the hazard assessment, it is critical to examine not only the results reported by the investigators, but the methodology, as different methodologies may not be appropriately used for extrapolation to human health risk assessments. The relevance of animal testing and the extrapolation of testing results to humans are the subject of continuing deliberation (Barlow et al., 2002). It is important to note that, for example, although carcinogenicity bioassays are intended to relate the relevance of certain tumor types and their causation to human risk, it is known that there are neoplasms that are rodent specific (Dybing et al., 2002) and may be induced by mechanisms that are not relevant to human risk assessment. In addition, for substances in the diet, there is continuing debate regarding the relevance of bioassays that use exposure to high doses of a single substance. Importantly, it has been suggested that it may be more appropriate to evaluate many food components as part of a whole food approach in which the chemical is tested in its usual food matrix rather than admixed to the diet (Barlow et al., 2002). Thus, in a hazard identification, study methodology is critical to interpreting the results.

The objective of this review is to identify those studies available in the public literature that explore the mechanisms of action whereby it has been suggested that heme could play a role in initiation or promotion of colorectal cancer. The methodology employed in these studies and the relevance of extrapolation from the results to human health risk assessment is presented.

2. Methods

2.1. Identification of literature

A search of the public literature referenced in PubMed over the last 20 years (1998 – present) was completed using the search terms colon cancer, colorectal cancer, *in vitro*, animal, clinical, red meat, iron, heme, hemin, lipid peroxidation, genotoxicity, nitrosamine and *N*-nitroso. The inclusion criteria applied were: (1) clinical trials, animal models or *in vitro* studies, (2) used red meat or heme or hemin as treatments, (3) were original research papers and (4) were in English. Exclusion criteria included: (1) review papers, editorials, book chapters, meeting abstracts, proceeding papers, or news items, (2) epidemiology studies, (3) studied processed meat or components of meat other than heme, heme iron or hemin (such as heterocyclic amines or nitrites). This was followed by a review of the references cited in the retrieved papers to identify relevant clinical, animal and *in vitro* studies that addressed the weight of the evidence available investigating proposed mechanisms of dietary heme ingestion on initiating or promoting colorectal cancer.