Dietary meat, endogenous nitrosation and colorectal cancer

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Abstract
Colorectal cancer is the third most common cancer in developed countries such as the U.K., but incidence rates around the world vary approx. 20-fold. Diet is thought to be a key factor determining risk: red and processed meat, but not white meat or fish, are associated with an increased risk of colorectal cancer. The endogenous formation of N-nitroso compounds is a possible explanation because red and processed meat, but not white meat or fish, cause a dose-dependent increase in faecal ATNCs (apparent total N-nitroso compounds) and the formation of nitroso-compound-specific DNA adducts in humans. Red meat is particularly rich in haem which has been found to promote the endogenous formation of ATNC. Nitrosyl haem and nitroso thiols have been identified as major constituents of both faecal and ileal ATNC with a significant increase in the formation of these compounds following a diet rich in red meat. In vitro incubations show that, under simulated gastric conditions, nitroso thiols are the main species of nitroso compound formed, suggesting that acid-catalysed thionitrosation is the initial step in the endogenous formation of nitroso compounds. Nitrosyl haem and other nitroso compounds can then form under the alkaline and reductive conditions of the small and large bowel.

Red meat and colorectal cancer
Colorectal cancer is the third most common cancer (excluding non-melanoma skin cancer) in developed countries such as the U.K., with more than 10^6 annual cases [1]. Incidence rates vary approx. 20-fold around the world [2], and it is thought that diet is a key factor determining risk [3,4]. It has been estimated that up to 70% of all cases could be prevented by changes in diet [5,6], particularly avoiding a diet rich in red and processed meat, which has been identified as a risk factor for colorectal cancer [7]. There are several plausible mechanisms to explain the association between red and processed meat and colorectal cancer, for example the increased amount of fat or ammonia, HCAs (heterocyclic amines) and the endogenous formation of NOCs (N-nitroso compounds) [8].

NOCs such as nitrosamines, nitrosamides or nitrosoguanidines are known to be potent and often organ-specific carcinogens [9,10]. The carcinogenicity of more than 300 nitrosamines has been tested, and over 90% have shown an effect in 20 different animal species [11]. NOCs can act as alkylating agents, either directly or after metabolic activation by cytochrome P450 enzymes; alkylation of the O6-position of guanine appears to be the major mutagenic lesion and can lead to G→A transitions [12].

Endogenous formation of NOCs
The G→A transition is a common mutation in colorectal cancer found in codons 12 or 13 of ki-Ras [13], which is characteristic of alkylating agents such as NOCs. Furthermore, DNA adducts (O6-methyl- and O6-carboxymethyl-deoxyguanosine, O6MeG and O6CMeG) have been detected in human colonic tissue and colonocytes [14,15], suggesting the presence of alkylating agents in the gastrointestinal tract. In vitro studies also showed that the mutation pattern induced by nitrosated glycine (diazoacetate) is similar to the one found in gastrointestinal cancer [16]. An early report about the detection of volatile NOCs in human faeces [17] was not confirmed [18]; however, it was possible to detect the endogenous formation of NOCs in faeces using a group-specific chemical denitrosation method [19]. The analytical method used relies on chemical denitrosation using HBr and/or HCl [20–22] and NO detection by chemiluminescence [23]. Although this method has been developed and refined for the analysis of NOCs [21,24–26], the chemical denitrosation with HBr can also release NO from other compounds such as nitroso thiols (SNO) [24,27], nitriolic acid [26], O-nitroso compounds and nitrosyl iron (FeNO) [28]. Owing to this lack in specificity, the term ATNC (apparent total N-nitroso compound) is used to describe substances measured by this technique [19].

Following the detection of ATNCs in human faeces, it was possible to find a link between ATNCs and red meat [29] and to establish a dose–response relationship [30]. In contrast with red meat, there was no increase in detectable ATNCs following a diet of white meat [31]. The high haem content in red meat compared with white meat (approximate amounts: beef steak, 500 nmol/g; chicken, 20 nmol/g [32]) suggested a
link between faecal NOCs and haem intake, which has been confirmed [33]. Haem can easily become nitrosylated and is known to be able to act as a nitrosating agent and thus promote the formation of NOCs [34–37]. However, the lack of specificity of the analytical method used made it impossible to identify the nitroso compounds formed and to elucidate the underlying mechanisms [38].

Characterization of NOCs

The major classes of nitroso compounds detected by chemical denitrosation with HBr, HCl or iodine/iodide reagent [28] are, apart from nitrite, N-, S-, O-nitroso and iron-nitrosoyl compounds. Although these compounds release NO at different rates, they cannot be discriminated between by the technique used. An alternative method is to use selective de-nitrosation prior to analysis. Nitrosothiols can be denitrosated with mercury(II) in a reaction also used in the Saville assay [39]. Similarly, potassium ferricyanide \([\text{K}_3\text{Fe(CN)}_6]\) can be used to oxidize ferrous iron \((\text{Fe}^{2+} \rightarrow \text{Fe}^{3+})\) [40], thereby reducing its affinity for, and releasing, NO. This allows the indirect determination of nitrosothiols, nitrosyl iron and other nitroso compounds, in particular the latter since most O-nitroso compounds are unstable.

In a controlled study, volunteers were fed on a diet rich in red meat (420 g of beef/day) and a vegetarian control diet. Faecal samples were collected and analysed for haem and nitroso compounds [38]; furthermore, exfoliated colonocytes were isolated and analysed for the NOC-specific DNA adduct O6CMeG [15]. In a similar study, volunteers with an ileostomy were fed on a diet rich in red meat (240 g of beef/day) and a vegetarian diet [41]; ileal output was collected and analysed for haem content and nitroso compounds [38]. The amount of nitroso compounds found following a vegetarian diet was significantly lower compared with the meat diet when compared with the vegetarian conditions, whereby significantly more nitrosothiols were following incubation with 200 pp.m nitrite under gastric incubations to simulate the processes in the stomach. The mechanisms underlying endogenous nitrosation under acidic conditions in relation to gastric cancer have been widely discussed [42], but nitrosation in the lower intestine has received less attention. Of particular interest is the formation of different classes of nitroso compound and their action in vivo, in particular regarding their alkylating activity. Nitrosyl haem and nitroso thiolis are two classes of nitroso compound that contribute significantly to the increase in diet-induced endogenous formation of nitroso compounds. In both ileal and faecal output, the concentration of nitrosyl iron was significantly higher than the concentration of nitroso thiolis, confirming the important role of haem in the formation of faecal ATNCs as reported previously [33]. The difference between ileal and faecal nitroso thiolis/nitrosyl haem ratios was not significant, suggesting that the composition of nitroso compounds does not change during the passage through the large bowel. However, in vitro incubations showed that nitroso thiolis are mainly formed under gastric conditions. This is not surprising since the rate constant, \(k\), for the acid-catalysed nitrosation is much higher for thiol than for other groups \((k = 465\,000\,\text{M}^{-1} \cdot \text{s}^{-1} \text{for cysteine}; \text{rate} = k[\text{HNO}_2][\text{H}_2\text{O}^+][\text{substrate}])\) [43]. Furthermore, haem nitrosation is generally promoted by an anaerobic reductive environment which is more likely to be found in the small intestine or beyond. The initial step of endogenous formation of nitroso compounds is therefore presumably the nitrosation of thiol groups which can be promoted by haem. With increasing pH, while passing through the small intestine [44], nitroso thiolis become more unstable and susceptible to Cu++-catalysed decomposition [45], leading to the formation of disulphides and NO. The reductive and anaerobic environment in the small intestine helps to maintain the haem iron in its ferrous state and facilitates its nitrosylation [46] by nitrite or NO. Haem could therefore salvage NO released from nitroso thiolis and thus increase the total amount of ileal and faecal nitroso compounds. Cross et al. [33] fed haem to healthy volunteers and found the faecal level of ATNCs to increase when compared with the level of inorganic iron. The effect of haemoglobin on faecal nitroso compounds can be 2-fold as haemoglobin contains both accessible thiol and haem groups. In particular, the nitrosation-promoting properties described by Lunn et al. [41] can be attributed to both nitrosation and nitrosylation reactions.

Both nitrosyl haem and nitroso thiolis are known to be able to act as NO donors [47,48] and can act as nitrosating agents [34–36,49]. The presence of these compounds in the ileum...
and in faeces may promote the formation of highly reactive alkylating agents such as diazoacetate, causing the formation of the NOC-specific DNA adducts such as O6CMeG, which have been found previously to increase in colonic cells in response to red meat [15]. It may also be possible that the NO-donating properties of nitrosyl haem and nitroso thiols cause elevated NO levels in the colon which can contribute to the increased cancer risk associated with diets rich in red meat [50].

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References


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