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Mini-Review

Health implications of nitrates and nitrites

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ABSTRACT

Although nitrates and nitrites have been approved for use as food preservatives and curing agents in meat products for decades, their health implications remain a subject of intense debate. Recently the International Agency for Research on Cancer of the World Health Organization has classified nitrate/nitrite-treated processed meats as Group 1 carcinogenic to humans. However, as processed meats account for only a minor portion of the total human nitrates/nitrites exposure, other factors are more likely responsible for the increased cancer risk observed in the subjects of a number of epidemiological studies. Also, nitrates and nitrites can serve both as the precursors and end products of nitric oxide, an important endogenous signal molecule. By helping in maintaining nitric oxide homeostasis, nitrates and nitrites may provide potential benefits beyond their need as food preservatives, and clinical trials for possible medical uses of nitrites and nitrates are in progress. Thus, a reassessment of the health implications of nitrites and nitrates is appropriate and needed.

KEYWORDS: nitrate, nitrite, nitric oxide, benefit, potential risk

INTRODUCTION

Nitrates and nitrites, commonly referring to their sodium salts, have long been employed as preservatives and curing agents in meat products. Nitrite is capable of inhibiting the growth of *Clostridium botulinum* and *Clostridium perfringens*, and slowing the growth of many other pathogenic bacteria. Its effectiveness in inhibiting the growth of *Clostridium botulinum*, which is responsible for the potentially deadly disease botulism, depends on residual nitrite level, pH, salt concentration, reductants present, iron content, type of bacteria and other factors [1]. Nitrite may also react with myoglobin to form an appealing pinkish red color and taste in meat products. The formation of nitrosylating agents, by nitrite, that subsequently react with myoglobin seems to be responsible for producing the cured meat color, while the mechanism involved in the change in taste is not yet clear [1]. In addition to inhibiting the growth of disease-causing microorganisms, and giving desirable taste and color to meat products, nitrite also seems to slow lipid oxidation that leads to rancidity [2]. Sodium nitrite may act as an antioxidant by reacting with heme proteins and metal ions, and terminating the free radical-induced lipid peroxidation that leads to rancidity [1]. Nitrate, on the other hand, is inert and must be converted to nitrite before it can exert its function as a food preservative or curing agent.

Sodium nitrite has been approved for use as a food preservative and curing agent in meat products for decades. After exhaustive review of a large number of studies, the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) ruled that nitrates and nitrites are safe ingredients, and are not associated with cancers in humans when used at the levels recommended [3]. However, the health risk of nitrates and nitrites remains a subject of intensive debate.

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Health risk of nitrates/nitrites

The major health risk of nitrites and nitrates is the possible formation of methemoglobin and nitrosamines. Formation of methemoglobin in the red blood cells is the most recognizable and detectable sign of nitrite toxicity in humans. *In vitro*, one molecule of nitrite can react with two molecules of hemoglobin to form two molecules of methemoglobin [4]. As the iron in the heme group of methemoglobin is in the Fe^{+++} state, rather than the Fe^{++} state as in hemoglobin, it cannot bind or carry oxygen. Spontaneously formed methemoglobin is normally reverted to hemoglobin by such protective systems as nicotinamide adenine dinucleotide (NADH) methemoglobin reductase (cytochrome-b5 reductase), which is a major pathway, nicotinamide adenine dinucleotide phosphate (NADPH) methemoglobin reductase, and to a lesser extent, the ascorbic acid and glutathione enzyme systems. Disruptions of these protective systems can lead to methemoglobinemia. Methemoglobinemia, either congenital or acquired, can occur when the level of methemoglobin in the red cell is >1%. When methemoglobin concentration in the red cells exceeds 10%, the skin or blood color of patients may change to blue color, and neurologic and cardiac symptoms may arise due to hypoxia. As methemoglobin interferes with the ability of the red cell hemoglobin to carry oxygen, high methemoglobin concentration can be fatal. Toxic methemoglobinemia resulting from high nitrate in drinking water and occupational exposure has been reported [5, 6].

N-nitrosamines are found in a number of foods, water, latex, tobacco products and polluted air. A number of nitrosamines have been shown to be carcinogenic in experimental animals. The formation of malignant primary hepatic tumors in the rat by feeding dimethyl-nitrosamine was first reported sixty years ago [7]. Since then many nitrosamines have been reported to cause organ-specific cancers in a wide variety of animal species. Also, carcinogenic nitrosamines can be formed from drug and nitrite interaction [8]. Nitrites may react with certain amines in the food under acidic conditions (e.g., human stomach) to produce carcinogenic nitrosamines [9, 10]. Volatile nitrosamines, for example, have been detected following ingestion of bacon, spinach, tomato sandwich and beer [11]. A high intake of nitrite/nitrate has been implicated as a risk factor for human cancer decades ago [12, 13].

According to the Delaney Clause, a provision in the 1958 amendment to the Food, Drugs, and Cosmetic Act of 1938, if a substance is found to cause cancer in man or animal, it is not permitted for use as a food additive. However, FDA has stipulated that nitrates/nitrites can be safely used in or on specified foods in accordance with the prescribed conditions by FDA [3, 14]. Sodium nitrite, for example, can be used as a color fixative in smoked, cured tuna fish products if the level is <10 ppm (mg/kg) in the finished product. Also, when used as a preservative and color fixative, with or without sodium nitrate, in smoked, cured sablefish, smoked, cured salmon, and smoked, cured shad, as well as in meat-curing preparations for home curing of meat and meat products, the nitrite level should be <200 ppm and nitrate <500 ppm in finished products.

One of the reasons FDA has continuously allowed nitrates and nitrites to be used as food preservatives in meat products is the absence of other preservatives that can safely and effectively replace nitrates and nitrites in inhibiting botulism. Recent information concerning the overall human exposure, metabolic fate and health impact of nitrate and nitrite has also undoubtedly played a role in this decision.

Exposure to nitrates/nitrites

Food is the primary source of nitrates in humans. Nitrates in plants largely originate from soil and water. Water in most areas contains only a small amount of nitrate. It varies greatly depending on ground conditions and fertilizer used, and can be a significant source. Air has negligible amounts of nitrate.

Vegetables such as lettuce, spinach, celery, carrots, red beets, radishes, cabbage and broccoli are the primary sources (>80%) of dietary nitrites [15]. When compared to vegetables, only a small portion of the total nitrite intake comes from processed meats such as bacon, hot dogs, hams, pastrami, corned beef, cured sausages, and cured fish. In Canada, approximately 200 ppm to 2500 ppm nitrate and 10 ppm nitrite are present in vegetables and fruits, and about 35 ppm - 42 ppm nitrate and 11 ppm - 15 ppm nitrite are found in processed meats [16]. In the United States, an average of 37 ppm nitrate and 4.5 ppm nitrite are found in meat products.

Studies have shown that endogenous formation or conversion from nitrate internally is the major

source of nitrite [17, 18]. The sites of endogenous formation of nitrite include mouth, gastrointestinal tract and body cells. A portion of the ingested nitrate is converted to nitrite by the salivary bacteria, and some of the absorbed nitrate is stored in the body [19]. In gastrointestinal tract, *de novo* synthesis of nitrate can occur *via* heterotrophic nitrification of nitrogenous sources by bacteria, and the so-formed nitrate can also be converted to nitrite [20].

Nitric oxide-nitrite-nitrate cycle

Recently, nitrites and nitrates have been shown to be both the precursors and products of nitric oxide, a small-molecule free radical [17, 18, 21]. Nitric oxide is an important intermediate in the chemical industry, and is also known as the endothelium-derived relaxation factor [22]. It is an important cellular signaling molecule involved in many physiological and pathological processes in mammals. The journal 'Science' had proclaimed nitric oxide as "The Molecule of the Year" in 1992.

Biosynthesis of nitric oxide from L-arginine is catalyzed by a family of nitric oxide synthases that operate in different parts of the body [23]. There are several forms of nitric oxide synthases: neuronal nitric oxide synthase, endothelial nitric oxide synthase, inducible nitric oxide synthase, and bacterial nitric oxide synthase. Under hypoxic conditions as in ischemia, nitric oxide can be formed by a nitrite reductase activity of xanthine oxidase [24-26]. Drugs such as sildenafil (*Viagra*), vardenafil (Levitra or Staxyn), tadalafil (*Cialis*), nitroglycerine and amyl nitrite, are found to act through the mechanism of being precursors of nitric oxide. Also, the long-recognized vasodilating properties of nitrite [27] can now be attributed to its ability to release nitric oxide.

As stated above, nitrate and nitrite can be converted to nitric oxide, which in turn may combine with the meat pigment myoglobin to give the cured-meat color. On the other hand, nitric oxide can be oxidized to nitrite and nitrate. A portion of the nitrate consumed is recycled back to the mouth *via* the salivary glands, and some ingested nitrate is reduced to nitrite by the symbiotic bacteria in the oral cavity. As nitric oxide, nitrates and nitrites are inter-convertible, this nitric oxide-nitrite-nitrate cycle may account for the majority of total human exposure to nitrite [17, 18, 28]. Sodium nitrite is capable of scavenging superoxide *via* the release of nitric oxide [29]. Also, similar to nitrite, nitric oxide can convert hemoglobin to methemoglobin, and react with hydrogen peroxide and/or superoxide to produce reactive peroxynitrite. Peroxynitrite may alter cellular redox state and cause oxidative tissue damage [30, 31].

Health risks associated with processed meat products

During the manufacture, storage and cooking of processed meat products, nitrates/nitrites may combine with naturally occurring amines in the meat to form carcinogenic N-nitroso compounds. When ingested, these compounds may be carcinogenic. In a multisite case-control study, for example, processed meat consumption has been shown to be positively associated with cancer incidence of the colon, rectum, stomach, oesophagus, and lung [32]. Also, a meta-analysis of epidemiological observational studies shows an association of increased consumption of processed meat with higher gastric cancer risk [33]. Similarly, prospective studies show a positive association between consumption of processed meat and risk of several chronic diseases [34]. Also, the results of the European Prospective Investigation into Cancer and Nutrition show a moderate positive association between processed meat consumption and mortality and cancer [35]. The World Cancer Research Fund recommends eliminating processed meat consumption [36], and the American Institute for Cancer Research suggests limiting red meat and avoiding processed meat lowers cancer risk.

More recently, the International Agency for Research on Cancer (IARC), the cancer agency of the World Health Organization, evaluated more than 800 studies that investigated associations of more than a dozen types of cancer with the consumption of red meat or processed meat in many countries and populations with diverse diets. Based on the evidence in humans that the consumption of processed meat is positively associated with colorectal cancer and stomach cancer, the IARC has classified processed meat as Group 1 carcinogenic to humans [37]. Those experts concluded that each 50 gram portion of processed meat consumed daily increases the risk of colorectal cancer by 18%. Also, Inoue-Choi *et al.* [38] report that high consumption of red meat and processed meat products is associated with increased risk of postmenopausal breast cancer.

On the other hand, Larsson and Wolk [39] analyzed the results obtained from 7 studies and found no evidence of a non-linear association between the consumption of processed meat and risk of pancreatic cancer. Also, the results from a meta-analysis of 15 studies on red meat consumption and 11 studies on processed meat consumption do not support an independent association between red or processed meat intake and prostate cancer risk [40]. Similarly, Bryan *et al*. [41] report that, in the absence of co-administration of a carcinogenic nitrosamine precursor, there is no evidence for nitrite carcinogenesis. Also, newly published prospective epidemiological cohort studies indicate that there is no association between estimated intake of nitrite and nitrate in the diet and stomach cancer.

As processed meat products contribute to only a small portion of the total nitrate/nitrite exposure for most subjects, it is likely that factors other than nitrate/nitrite are responsible for the higher cancer risk observed in subjects who consume more processed meat products [32-35]. Based on the lifestyle characteristics of subjects who participated in the processed meat consumption and cancer risk studies, those who consume more processed meats also have higher body mass index and total energy intake, and lower vegetable/fruit intake and physical activity than those consuming less processed meats [42, 43]. Thus, higher energy intake and obesity, and low fruit/vegetable intake and physical activity may play a bigger role than processed meat intake in increasing cancer risk.

Dietary nitrate/nitrite and health risk

Although FDA has continuously permitted the use of nitrates and nitrites as food preservatives, their health risk remains a subject of intensive debate. Central to the health risk of nitrate and nitrite is the possible causation of methemoglobinemia and more critically, the increase in cancer risk. In 2003, the World Health Organization reviewed dozens of research papers on the safety of nitrites, and concluded that dietary nitrate has no association with oral, oesophageal, gastric, or testicular cancer, and the greatest health risk of nitrites and nitrates seems to be from severely polluted drinking water. On the other hand, the 2006 International Agency for Research on Cancer Working Group concludes that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans.

Nevertheless, except for a risk of developing methemoglobinemia in certain populations, nitrate/nitrite-containing vegetables/fruits or processed meat products do not appear to pose a health risk. Infants <4 months, for example, are susceptible to nitrate/nitrite-contaminated water, and the methemoglobinemia risk can be exacerbated in infants/children with gastroenteritis [14, 44]. Also, individuals with glucose-6-phosphate dehydrogenase deficiency have a greater susceptibility to nitrite.

Potential health benefits of nitrates and nitrites

As a precursor of nitric oxide, an endogenous signaling molecule and regulator of gene expression, nitrite may serve as a potential regulator of nitric oxide homeostasis and contribute to health maintenance and disease prevention [17, 18, 45, 46]. Lidder and Webb [47] examined the vascular effects of dietary nitrate, and found that dietary nitrate has a range of beneficial vascular effects, including reducing blood pressure, inhibiting platelet aggregation, preserving or improving endothelial dysfunction, and enhancing exercise performance in healthy individuals and patients with peripheral arterial disease. By improving nitric oxide bioavailability, dietary nitrite and nitrate supplements may help in the treatment and prevention of cardiovascular diseases [48] and arterial aging, and in the prevention of age-associated cardiovascular disease in humans [49].

Also, as vegetables are the richest sources of nitrates/nitrites, the concern over the cancer risks of dietary nitrates/nitrites is inconsistent with the Dietary Guidelines for Americans issued by the U.S. Department of Health and Human Services (DHHS) and USDA. Based on the available evidence, the DHHS, USDA, and the National Academy of Sciences recommend increased consumption of vegetables/fruits, with a diet that is low in fat, saturated fat and cholesterol and that containing plenty of whole-grain breads and cereals, to decrease the risk of heart disease and cancer. The U.S. National Cancer Institute (NCI) has published dietary guidelines geared towards cancer prevention for the public. The guidelines are consistent with the USDA/DHHS Dietary Guidelines for Americans which include recommendations on increasing fiber intake to

20-30 grams/day, and inclusion of a variety of fruits and vegetables in the daily diet. Food labeling regulations of FDA correspond to NCI's Five-A-Day guidance and the government's Dietary Guidelines for Americans [50, 51]. Thus, the concern over the health risk of nitrate and nitrite in processed meat products needs to be reassessed.

In a recent study to investigate the effects of sodium nitrite supplementation on vascular function and related small metabolite signatures in middle-aged and older adults, DeVan *et al*. [52] reported that sodium nitrite supplementation is well tolerated in the studied subjects. The supplementation increases plasma nitrite concentrations, improves endothelial function, and lessens carotid artery stiffening in middle-aged and older adults, perhaps by altering multiple metabolic pathways. A phase 2 clinical trial to evaluate the safety and efficacy of sodium nitrite injection for the prevention of ischemia-reperfusion injury associated with acute myocardial infarction is scheduled to complete in 2016. The purpose of this study is to determine whether the intravenous infusion of sodium nitrite safely prevents ischemia-reperfusion injury in subjects with acute myocardial infarction resulting in improved left ventricular function. The randomized interventional trial is led by investigators from the Johns Hopkins University in collaboration with Hope Pharmaceuticals.

CONCLUSION

Nitrates and nitrites have long been approved for use as food preservatives and curing agents in meat products, despite their possible health risks. Vegetables are the major dietary source of nitrate, and conversion of consumed nitrate in the mouth is the major source of nitrite. Experimental evidence linking increased consumption of processed meat products to higher cancer risk is inconsistent. As processed meat products contribute to only a minor portion of the total nitrate/nitrite exposure, factors other than nitrite/nitrate present in processed meats are likely responsible for the increased cancer risk observed in the study subjects. Nitrates and nitrites pose little or no health risk when used according to the guidelines specified by FDA. Nitrites and nitrates may serve both as precursors and products of nitric oxide, an endogenous cell signaling molecule. By helping in maintaining nitric oxide homeostasis, nitrates and nitrites may provide some health benefits beyond their need as food preservatives. Thus, a reassessment of the health implications of nitrite and nitrate is needed.

CONFLICT OF INTEREST STATEMENT

The author does not have any financial or other agreements that may cause a conflict of interest.

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