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Chapter 11

Nitric Oxide Synthase and Nitric Oxide Involvement in Different Toxicities

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Abstract

Nitric oxide (NO) is known to have a very short half-life, and it is oxidized to nitrate (NO_3^-) and nitrite (NO_2^-). The activity and/or expression of nitric oxide synthases (NOSs) can change in response to toxins or therapeutic medications. For example, in recent studies in our laboratory and others, it has been reported that the amount of NO was increased in the serum of N-nitroso compounds-treated animals. N-nitroso compounds, which are found in different types of foodstuffs, including meat, salted fish, alcoholic beverages, agricultural drugs, insecticides, cigarettes, and several vegetables, are known to have carcinogenic effects. In addition, it is experimentally used to induce liver carcinoma to study the mechanisms of liver cytotoxic injury. Uncontrolled, prolonged, and/or massive production of NO by inducible NOS may cause liver damage, inflammation, and even tumor development during N-nitroso compound toxicity. In this chapter, we explain the roles of NOS and NO in various toxicity conditions, such as toxicity in environment pollutant or food additive, and present the evaluation of the toxicity and the importance of NOSs in human health.

Keywords: nitric oxide synthase, nitrate, nitrite, nitric oxide, N-nitroso compounds, toxicity

1. Introduction

Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) (EC: 1.14.13.39) through oxidation of L-arginine to L-citrulline [1–5]. NO is a biologically significant molecule for many species from bacteria to mammals. Mechanisms for NO synthesis in an organism are extremely limited. Nitric oxide synthase enzyme is the only source of endogenous NO, except NO formed by metabolism of the nitro compounds entering the organism [6]. Three

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different types of NOS isoforms have been isolated from different tissues, such as vascular endothelium, brain, macrophage, and urinary system, of mammals, including (a) neuronal NOS (nNOS), (b) inducible NOS (iNOS), and (c) endothelial NOS (eNOS) [5, 7]. Neuronal NOS (nNOS, NOS1) is a Ca^{+2}-dependent, ~160 kDa enzyme that is found in the central and peripheral nervous system cells and striated muscle [4, 8]. Inducible NOS (iNOS, NOS2) is a calcium-insensitive, ~130 kDa enzyme that was first isolated from activated macrophages and that can be activated by some cytokines (IL-1, TNF, IF-γ) or bacterial endotoxins [4, 9, 10]. Endothelial NOS (eNOS, NOS3) is a Ca^{+2}/calmodulin-dependent, ~135 kDa enzyme that is localized in vascular endothelial cells, hippocampal neural cells, pulmonary and renal epithelial cells, and cardiac myocytes [4, 9, 11].

Level of NO can be determined indirectly by measuring the concentration of nitrate (NO_3^-) and nitrite (NO_2^-) using an acidic Griess reaction. In recent studies in our laboratory and others, it has been reported that the amount of NO can change in response to various toxicity conditions [12–18] which are closely associated with animal and human disease conditions. In this chapter, we mention NO_3^- and NO_2^-, the molecules which are naturally found in foods as NO sources, agricultural activities such as the use of artificial fertilizers, polluted water, curing process to give a natural smell and taste to meat. The conversion of NO_3^- and NO_2^- into NO in the gastrointestinal tract, the effect of NOS, and possible mechanisms for how it is converted back into NO_3^- and NO_2^- in the bloodstream will also be covered. Studies on N-nitrosamines, which are formed by reaction of NO_3^- and NO_2^- with amines, and which can be seen in cured meats, cigarette smoke, and rubber industry, reveal the carcinogenic effects of these molecules (**Figure 1**).

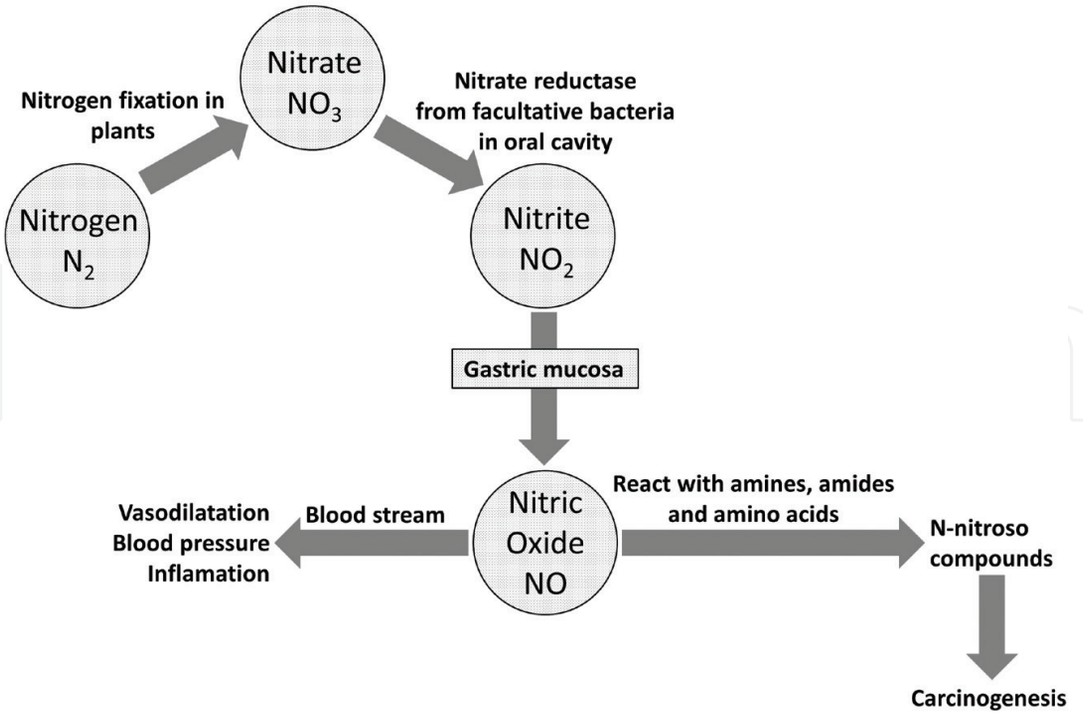


Figure 1. Formation of N-nitroso compounds from NO_3^-, NO_2^-, NO, and their effects on human health.

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2. NO_3^- and NO_2^- as environment pollutants and food additives

Nitrogen is the basic element for essential micromolecules, such as amino acids, proteins, and nucleic acids. Nitrogen is the most abundant gas in the atmosphere; however, it has to be fixated before it is taken by plants and animals. Fixation is an important part of the nitrogen cycle. In this cycle, N_2 is converted into ammonium and various nitrogen oxides. These higher nitrogen oxides are eventually gradually reduced. The nitrogen is then freed into the atmosphere and the cycle is completed. Bacteria play an important role in the cycle as they can catalyze each step, including the interconversion of different nitrogen oxides. NO_3^-, NO_2^-, and NO are all necessary intermediate products in the denitrification process and are catalyzed by NO_3^-, NO_2^-, and NO reductases, respectively [19]. Bacteria use these molecules as terminal electron acceptors in the absence of oxygen. The production and metabolism of nitrogen oxides also occur in mammals. NO_3^- is easily converted into NO_2^- in mammals by the activity of enzymes in both bacteria and mammals. The NO_2^- then later can react with different molecules, such as amines, amides, and to form N-nitroso compounds which can be carcinogenic [20]. Potentially carcinogenic or inert oxidizing molecules, such as NO_3^- and NO_2^-, occur as a result of endogenous NO metabolism during the food chain (**Figure 1**) [21].

Although nitrogen is found naturally in surface waters, its amount increases in many parts of the world. The reason for this is the pollution caused by commonly used inorganic fertilizers, soil drainage, or contamination of water resources by sewage [22]. The main causes of water pollution are pollution from industrial and agricultural activities. Chemical fertilizers used in agricultural production have an important role. NO_3^- is applied in increasing amounts in the fertilizers for agricultural production, and they accumulate in the soil. This accumulated NO_3^-, in varying amounts depending on the conditions, moves toward the deeper parts of the ground especially with rainwater, and some of it reaches underground and some to surface waters. High NO_3^- concentrations in water resources pose a potential risk to human health, because sunlight and some bacteria can easily convert NO_3^- into NO_2^- [23]. NO_3^- and NO_2^- can also occur spontaneously in vegetables and fruits consumed by humans and especially in animal feed [24]. Vegetables and fruits usually receive NO_3^- and NO_2^- from the soil [25]. As a result of nitrogenous fertilizers being used in excess to increase appearance and yield in the plants, plants store NO_3^- in excess of their need. When the amount of received NO_3^- is high, the reduction to ammonia is limited and NO_2^- accumulates as an intermediate metabolism product [26, 27].

Excess NO_3^- and NO_2^- can also be utilized to cure meats. In order to improve the taste, protection, appearance, and quality of the meat, NO_3^- and NO_2^- are used for curing purposes. NO_2^- can also be used as a preservative against the proliferation of microorganisms, especially *Clostridium botulinum*. It also inhibits lipid peroxidation and prevents putrefaction [28].

Dietary NO_3^- and NO_2^-, which are taken by the organisms, could cause various physiological and pathological outcomes.

3. Conversion of NO_3^- and NO_2^- into NO in the gastrointestinal system: possible role of NOSs

In an organism, NO_2^- is converted into NO in three ways:

(a) It is enzymatically (via NOSs) reduced to NO in the circulation and tissues.

(b) It is non-enzymatically reduced to NO in acidic stomach environment, capillary beds, and at low pH and hypoxic conditions that occur during intense exercise.

(c) NO can be produced by acidification of NO_2^- in the oral cavity.

In this section, the conversion of NO_3^- and NO_2^-, taken from foods in the gastrointestinal tract, into NO will be mentioned. At the end of this pathway, NO_3^- and NO_2^- are synthesized again from NO. This synthesis in the tissues is catalyzed by NOSs. With the identification of the NO_3^--NO_2^--NO pathway, the importance of the diet, rather than the biological significance of systemic NO_3^- and NO_2^-, in the physiological regulation of NO has arisen. NO_3^-, rich in green leafy vegetables such as beetroot, is reduced to NO_2^- by bacterial NO_3^- reductase in the commensal anaerobic microflora in the oral cavity by saliva secretion and is reduced to NO in the stomach [29–31]. The highest NO concentration is obtained from an acidic stomach pH after a NO_3^--rich meal (**Figure 1**) [29].

The rapid postprandial increase in gastric NO is directly proportional to many actions, such as mucus production in the gastrointestinal tract, increased vascular tone, antimicrobial effect, and immunomodulation. It has also been shown that this increased NO is related to many physiological mechanisms, such as the prevention of ischemia-reperfusion injury and increased cerebral blood flow [21, 29].

How endogenous NO_3^- and NO_2^- can be synthesized in the body if NO_3^- and NO_2^- are not taken into the body with nutrients? The inorganic NO_3^- and NO_2^-, which cannot be taken up with nutrients in starving mammals, are mainly derived from NOSs. These enzymes form NO by using L-arginine and oxygen, and then this NO is rapidly degraded to NO_3^- and NO_2^-. Endogenous NO_3^- and NO_2^- are synthesized in this way [30]. Endogenous NO can also be produced by NOSs using NO_3^- and NO_2^- in our daily nutrients [30, 32].

NO is a highly diffusible free radical that participates in various in vivo signal pathways and is involved in critical physiological events, such as regulation of vascular tone and immune response [31]. NO also exhibits antimicrobial activity [33] other than its regulatory role in vascular tone. Numerous intracellular pathogenic parasites [34] and bacteria [35] are susceptible to NO.

NO_2^- in saliva is converted non-enzymatically into NO and some other nitrogen oxide species when it enters into the stomach with low pH [30]. Increasing number of studies on cardiovascular, inflammatory, and gastrointestinal diseases reported that the NO-related effects of dietary NO_3^- and NO_2^- are protective and preventive. Recent data suggest that these anions are beneficial to gastrointestinal cancer [36] and cardiovascular diseases rather than having harmful effects. Dietary NO_3^- and salivary NO_2^- have been shown to protect gastric mucus from experimentally induced gastric damage by increasing gastric mucus thickness and mucosal blood flow [30].

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NO plays an important role for the intestine. It is produced from arginine by eNOS and iNOS in a reaction catalyzed in the intestine. eNOS is structurally expressed in low levels in intestinal microcapillaries and is responsible for the initial levels of NO. Low NO levels regulate vascular tone and mucosal blood flow in cyclic guanosine monophosphate and neuron-dependent manner and are also crucial for mucosal homeostasis. Additionally, NO can protect from oxidative stress by diminishing oxygen radicals. eNOS-derived NO facilitates leucocyte uptake by supporting endothelial adhesion of leukocytes. iNOS is upregulated during inflammation and increases NO synthesis. NO also allows dilatation of capillary vessels. Excess NO secreted during inflammation has harmful effects on the intestinal barrier [37, 38].

NO reacts with the superoxide ion to form a reactive oxygen and nitrogen type, peroxynitrite, which can be harmful for epithelial cells. It can induce enterocyte cell apoptosis and inhibit proliferation. iNOS is expressed in intestinal smooth muscle cells, endothelial and epithelial cells [38]. During inflammatory conditions in the intestine, such as necrotizing enterocolitis [39], ulcer [29], and colon cancer [40], expression of iNOS mRNA increases.

Numerous studies have shown that NO_3^- and NO_2^- obtained from pharmacologic supplements or diet have obvious effects on gastrointestinal function [29, 39, 40]. However, it is still unclear whether endogenous NO_3^- and NO_2^- derived from NOSs in the endothelium and elsewhere affect gastric function. This situation has been tried to be illuminated in germ-free and starved animals [30, 41].

The gastric NO levels are very low in germ-free animals lacking microflora even after dietary NO_3^- load. A significant amount of NO_2^- is produced in the saliva even in the case of fasting, indicating NO_3^- production due to endogenous NOS production [30]. Petersson et al. [30] reported that three doses of NO_2^- given to germ-free rats not only increased stomach mucus thickness by more than fourfold but also unexpectedly had an effect in the non-NO_2^- group. This suggests that endogenous NO_3^- from NOSs also plays a role in the regulation of gastric physiology. In a similar study in humans, individuals were given a low NO_3^- diet with an antibacterial mouthwash containing chlorhexidine to lower the reduction of oral NO_3^-, and it was determined that the levels of circulating NO_2^- lowered, and this then increased the blood pressure [42]. These studies show that NO_3^- and NO_2^- have a NO_3^--NO_2^--NO pathway that starts from the mouth and ends in the mouth through digestive and circulatory system. It is possible that NO_3^- and NO_2^- in the circulation and saliva may originate from the endogenous NOS pathway.

In addition to NO_3^-, NO_2^-, NO, sodium nitrite ($NaNO_2$) is an inorganic compound taken by endogenous sources. $NaNO_2$ may have some beneficial and undesirable effects. $NaNO_2$ is a preservative used in processed meats, such as salami and bacon. $NaNO_2$ is synthesized by several chemical reactions, including the reduction of sodium nitrate. $NaNO_2$ is used as an additive in foods. There are some suspects about the safety of use in foods, but it is still being used, and, on the contrary, there is information that $NaNO_2$ may actually be healthy [43]. There are studies on the effects of $NaNO_2$ on human health from 1945 [44] to present date [45].

NO_3^- salts are used as a cheap nitrogen source in fertilizers. Therefore, with the widespread use of nitrogenous fertilizers in agriculture and inappropriate disposal of nitrogenous wastes,

humans are exposed to high NO_3^-/NO_2^- levels at an alarming rate, especially through contaminated food and water [46]. Infants and individuals with deficiency of glucose-6-phosphate dehydrogenase are particularly sensitive to high levels of NO_3^-/NO_2^- [45]. The digestive system of newborn babies convert NO_3^- to NO_2^-, and NO_2^- reacts with hemoglobin and prevents oxygen transport to the tissues. As a result, "methemoglobinemia" known as "blue baby syndrome" occurs in infants [47].

Prolonged non-lethal exposure to high levels of NO_3^-/NO_2^- may cause respiratory failure, growth failure, diabetes, neurological disorders, and cancer [48]. $NaNO_2$ causes oxidative stress in human erythrocytes in vitro by increasing lipid and protein oxidation, osmotic fragility, and membrane damage [49].

Despite the fact that $NaNO_2$ has not been reliable in the past and has the potential to cause many cancers, it has recently been reported that it can prevent myocardial ischemia-reperfusion injury in diabetic rats by regulating eNOS and iNOS expression and inhibiting lipid peroxidation in the heart [50]. It also prevents hypertension and increases endothelium-dependent relaxation and total NO by regulating eNOS activity [51].

Serum malondialdehyde, NO, arginase, and glutathione S-transferase activities were increased, and glutathione and catalase activities were decreased in $NaNO_2$-treated rats [52]. In another study, decrease in GSH and catalase activity was reported in $NaNO_2$-intoxicated rats [53]. In their study investigating the histopathological, biochemical, and genotoxic effects of low dose $NaNO_2$ administration for 8 months, Ozen et al. [54] reported that the liver and kidney NO levels were decreased in rats. The reduction in NO levels may be explained by the rapid and/or efficient removal of this molecule from these tissues, resulting in an increase in serum levels due to reduced NO by metabolic depletion. Therefore, the investigators reported that the increased serum NO level did not contradict with the decreasing NO level in the tissues [54]. In addition, these and other investigators indicated that chronic administration of $NaNO_2$ increased iNOS activity in experimental animals [54, 55].

Peroxynitrite can interact with tyrosine residues to form nitrotyrosine. Ozen et al. [54] showed that the expression of iNOS and nitrotyrosine was increased in the liver and kidney tissues of $NaNO_2$-treated mice, and it caused tissue degeneration in both organs. Peroxynitrite can be decomposed to form NO_3^- and NO_2^- which can cause DNA damage, as well [56, 57].

The mechanisms of $NaNO_2$ are still not fully understood; this suggests that further work needs to be performed in the future.

4. N-nitroso compounds

4.1. The chemical structure and sources of N-nitroso compounds

NO_2^- is the precursor of N-nitroso compounds that have carcinogenic effect [58, 59]. NO_2^- is converted into nitrous acid in acidic environment, and nitrous acids react with secondary amines to form nitrosamine compounds (**Figure 2**) [60].

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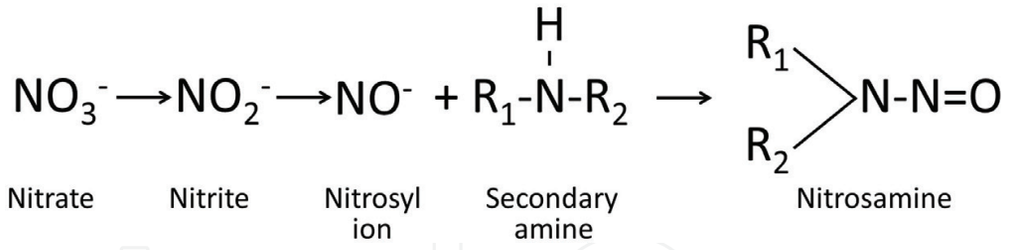


Figure 2. Formation of nitrosamine from NO_3^-.

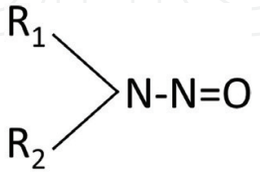


Figure 3. Chemical formula for nitrosamine.

Nitrosamines are chemical compounds with general formulas as shown in (**Figure 3**).

Nitrosamines are used in the manufacture of some cosmetics, pesticides, and most rubber products [61, 62]. Nitrosamines are found in latex products, cereal, tea, many foods, cigarettes, and cigarette smoke [60]. They are also formed by the reduction of NO_3^-, which is abundant in nature, into NO_2^- by bacteria [27].

The most commonly used N-nitroso compounds for the purpose of toxicity are N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosopyrrolidine, and N-nitrosopiperidine [63, 64].

Dimethylnitrosamine (also called N-nitrosodimethylamine, DMN, DMNA, NDMA—$C_2H_6N_2O$) is found in wheat flour, cheese, smoked meat, fish, and other food products (**Figure 4**) [65]. It can be formed by reaction of dimethylamine with NO_2^-. In addition, it can be formed by nitrozation and decarboxylation of amino acids, such as glycine and alanine [66].

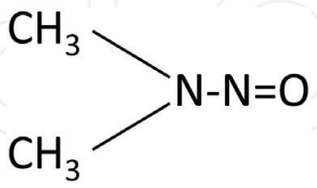


Figure 4. Chemical formula for dimethylnitrosamine.

Diethylnitrosamine (also called N-nitrosodiethylamine, DEN, DENA, NDEA—$C_4H_{10}N_2O$) is found in chemicals used in agriculture and rubber industry, cigarette smoke, alcoholic beverages, and processed meat products (**Figure 5**) [67]. It can be formed by reaction of diethylamine with NO_2^-. Additionally, it can be formed by nitrozation and decarboxylation of amino acids, such as glycine alanine [66].

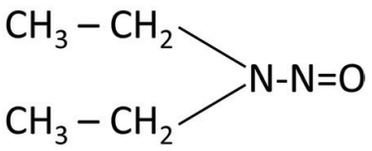


Figure 5. Chemical formula for diethylnitrosamine.

N-nitrosopyrrolidine (also called NPYR—$C_4H_8N_2O$) is found in cigarette smoke, meat and fish products (**Figure 6**) [68]. In meat products, this compound is formed by the nitrozation and decarboxylation of L-proline [69].

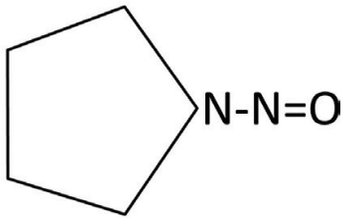


Figure 6. Chemical formula for N-nitrosopyrrolidine.

N-nitrosopiperidine (also called NPIP—$C_5H_{10}N_2O$) formation follows these steps: decarboxylation of lysine results in cadaverine; cadaverine is converted into piperidine by heat and the reaction of the resulting piperidine with NO_2^- (**Figure 7**) [69, 70].

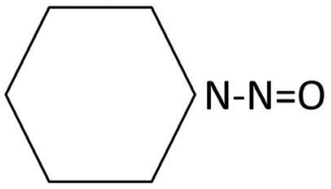


Figure 7. Chemical formula for N-nitrosopiperidine.

N-nitrosamines can be found in food products and might cause serious health problems for humans.

4.2. N-nitrosamines in meat and dairy products

N-nitrosamines taken with food and found in the environment have been found to cause serious health risks above a certain level, even though they have both food processing and protection functions as additives.

Nitrosamines occur mostly in meat and dairy products [71]. Since meat, an important nutrient, is easily decomposed by different factors, it is necessary to protect it with various methods and to increase its durability. For this purpose, some ingredients are added to meat and

meat products. Cured meat products, unlike fresh meat or salted meat with only table salt, have a pleasant smell, flavor, and a natural looking but a heat-resistant color. Today, this process is applied to most of the meat products consumed [72, 73]. It has been reported that the degradation products of NO_3^- and NO_2^- result in the formation of carcinogenic nitrosamines by combining with amino acids, such as putrescine, thiamine, piperidine, pyrrolidine, histamine, cadaverine, trimethylamine, β-phenylethylamine, n-propylamine, and isopropylamine [74–77].

The most important sources of nitrosamines in dairy products, such as cheese and butter, are NO_3^-, NO_2^-, and amine compounds [78]. The metabolic activities of some microorganisms in milk and dairy products result in the formation of histamine and tyramines, and nitrosamines are formed by the reaction of the secondary amines, which are the degradation products of these biogenic amines in various ways, with NO_2^- [65, 69].

The first formation of these nitrosamines in meat and dairy products is seen in the oral cavity [79]. The salivary secretion contains abundant NO_3^- and this NO_3^- is reduced to NO_2^- by nitrate reductase enzyme [72]. This NO_2^- causes the formation of nitrosamines [80]. These compounds can be taken into the stomach in various ways, such as ingestion or smoking, or can also be formed by the reaction of NO_2^- and amines in acidic conditions [81]. Some bacteria in the stomach and intestines increase the formation of nitrosamines by facilitating the conversion of NO_3^- into NO_2^-. NO_2^- transforms into nitrous acid in the acidic environment of the stomach, and nitrous acid reacts with amines in the environment to form nitrosamines [60, 82]. Nitrosamines are usually excreted through urine [83, 84].

5. The roles of NOS isoforms and *N*-nitrosamine compounds in liver toxicity or carcinogenesis

NO_3^- can easily be formed in mammalian systems through bacterial and mammalian enzymes. The resulting NO_3^- can then react with amines, amides, and amino acids to form N-nitroso compounds. While NO_3^- has relatively low toxicity, NO_2^- and N-nitroso compounds have higher toxicity in mammals. For this reason, there are many studies investigating the toxicity of these two molecules, as well as studies investigating ways to decrease the detrimental effects of these two molecules [20]. It has been suggested in long-term experimental animal studies that nitrosamines cause cancer in many tissues, but the role of nitrosamines in the formation of cancers is still being investigated.

5.1. NOS isoforms in carcinogenesis

Various studies have indicated that three NOS isoforms both trigger and prevent cancer etiology. Nitric oxide synthase activity has been detected in a variety of tumor cells, and it has been shown to be closely related to tumor grade, proliferation rate, and cancer development. High NOS expression can be cytotoxic for cancer cells. On the other hand, low NOS expression may

have an adverse effect and may increase tumor development [85, 86]. For this reason, NOS can be both genotoxic and angiogenic. High NO production leads to angiogenesis by increasing the VEGF gene, especially in p53 mutant cells. In addition, NO can alter the expressions of DNA repair proteins, such as poly (ADP-ribose) polymerase and DNA-protein kinase in tumor cells. NO may exhibit carcinogenic effect by the production of different NO metabolites. For example, NO may rapidly react with intracellular environment to form N-nitroso compounds. These metabolites, for example, can cause genotoxic effects by creating DNA damage [86]. In some other studies, N-nitroso compounds have been reported to alter the activity of creatine kinase, lactate dehydrogenase [87], pyruvate kinase [88], and Na/K-ATPase [89] enzymes.

5.2. *N*-nitrosamine compounds in liver toxicity and cancer

Certain levels of nitrosamines taken in the body with any food ingredient are less likely to cause cancer in the human body alone. However, different types of nitrosamines, which are continuously taken from different sources, such as air and cigarettes, increase the risk of developing cancer [65, 90]. Although NO_3^- and NO_2^- create a toxicological problem, the main problem is that they turn into nitrosamines, which are carcinogenic. Nitrosamines are known to exhibit carcinogenic effect through binding to proteins and nucleic acids [91]. Nitrosamines also have mutagenic and teratogenic effects [61]. Because many organ-specific nitrosamines are metabolized in the same way in human and animal tissues, humans are very sensitive to the carcinogenic properties of nitrosamines [67]. N-nitroso compounds are potent alkylating agents that can form endogenously and can cause cancer in surrounding animals [64]. Bacterial decarboxylation of amino acids in NO_3^- taken with nutrients results in amines and amides [58]. There is a relationship between the formation of N-nitroso compounds by bacterial catalysis and increased risk for liver, stomach, esophagus, nasopharynx, chronic urinary tract infections, and bladder squamous cell carcinoma [77, 92].

Metabolic activations of the nitrosamines occur primarily in the liver, and this transformation can occur in all cells. Dimethylnitrosamine is a potent carcinogen that can induce malignant tumors in various animal species in various tissues, including the liver, lungs, and stomach [79]. Various studies on different species of mice have shown that adenomas and adenocarcinomas in the lungs and hepatocarcinoma in the liver are formed by dimethylnitrosamine. The target organs of dimethylnitrosamine are the liver, lungs, and kidneys [65, 79].

It is suggested that diethylnitrosamine metabolism is catalyzed by the enzymes of the multifunctional cytochrome P-450 monooxygenase system and toxic effects are initiated by its metabolic activation, and that the resulting reactive intermediate products have little affinity for the catalytic domains of the binding enzymes, so that instead of being excreted with urine, they stimulate the onset of mutation, cancer, and necrosis by forming covalent bonds with important cellular components [93, 94]. Low concentrations of diethylnitrosamine cause mutations and cancer which was shown by the Ames assay [94]. Many studies suggest that the harmful effects of diethylnitrosamine may be reduced by various antioxidant molecules. The administration of molecules such as α-lipoic acid [95], omega-3 [96], blueberry [97], and beta-carotene [98] were stated to reduce the carcinogenic effect of nitrosamines in experimental animals.

N-nitrosopiperidine and N-nitrosopyrrolidine are structurally cyclic nitrosamines with different carcinogenic activities. Comparative carcinogenicity studies of these two nitrosamines in rats revealed that N-nitrosopiperidine caused esophagus, liver, and stomach tumors, and N-nitrosopyrrolidine caused tumors mainly in the liver [63].

6. Conclusion

NO-mediated responses are cell specific, and they depend on the existence of different NOS isoforms at different concentrations, and their regulations at pre- and post-transcriptional levels are quite complex. The latest developments on strategies for treating or preventing pathological events in association with the stimulation or inhibition of excessive production of NO and N-nitroso compounds present a crucial importance in medicine.

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