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FREE RADICALS MEDICINE (X SHI, SECTION EDITOR)

Nitrogen Oxides and Their Roles in Cancer Etiology

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Abstract
Purpose of Review The term "nitrogen oxides" refers to a group of molecules composed of nitrogen and oxygen. Sources of human exposure are varied ranging from environmental (acid rain), occupational (combustion of fossil fuels), and dietary to endogenously generated. Nitrogen oxides have a long association with human diseases including cancer. The goal of this review is to give a brief overview of the biological sources, relevant biological reactions, and the associations with cancer of several biologically relevant nitrogen oxides categorized based on the oxidation state of the nitrogen.
Recent Findings The chemical reactivity of nitrogen oxides as well as their cellular distribution and microenvironmental characteristics determine the downstream biological or pathobiological effects of nitrogen oxides. Some species may react directly at the site of exposure or production, while others can be transported systemically and become metabolized to more reactive species at distant locations. The roles of nitrogen oxides in cancer biology are often contradictory due to the complexity and diversity of their biological effects.
Summary Nitrogen oxides can participate in both causative and curative mechanisms of tumor biology. The appreciation of the high complexity and diversity of the biological effects of these reactive species in biological system will prompt our fundamental understanding of their roles in cancer and our search for improved and novel therapeutic strategies.

Keywords Reactive nitrogen species · Cancer · Free radicals · Nitric oxide · Epigenetics

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Introduction

Under biological conditions, the term reactive nitrogen species (RNS) is often used in general to describe a collection of reactive nitrogen oxides. The most biologically active and relevant RNS are listed in Table 1 and Fig. 1a. Human exposure to nitrogen oxides occurs from various sources including environmental (air, water), dietary, or endogenous synthesis. These inorganic molecules exist in the forms of gasses, ions, and free radicals with various degrees of reactivity (Fig. 1b). The source of the RNS will partially dictate the anatomical and cellular locations of human exposure (lung, stomach vs. cytosol, nuclear). In terms of human health, exposure to RNS can have both beneficial and deleterious consequences. Physiologic and pathologic effects of RNS result from their ability to directly chemically modify macromolecules via oxidation/reduction reactions or indirectly through the induction of signal transduction pathways. In many cases, it is not the initiating RNS that is responsible for downstream phenotypic effects but rather it is due to the formation of adducts on macromolecules containing nitrogen oxide functional groups. Although RNS have been associated with the etiologies of numerous diseases, herein we will focus on their roles in cancer. Like most pathologies associated with RNS, the role(s) of RNS in cancer is multifaceted and often contradictory. The functional consequences of RNS depend on

Table 1 Reactive nitrogen species and the oxidation state of the nitrogen

Chemical name	Formula	Oxidation state of the nitrogen
Nitrate	NO_3^-	+5
Peroxynitrite	$ONOO^-$	+5
Nitrogen dioxide	$\bullet NO_2$	+4
Nitrite	NO_2^-	+3
Nitric oxide	$\bullet NO$	+2
Nitrous oxide	N_2O	+1
Nitrosothiol	RSNO	+1
Nitroxyl	HNO	+1

microenvironmental conditions and the phenotypic makeup of the cell as well as the duration and concentration of RNS exposure.

Nitrate (NO_3^-, Oxidation State +5)

Biological Sources

The major endogenous source of nitrate (NO_3^-) is nitric oxide (NO) [1]. In fact nitrate formation represents the dominant metabolic route for NO removal in the vascular compartment [2]. In the lumen of blood vessel, NO reacts with hemoglobin at the ferrous heme center and undergoes a diffusion-controlled two-electron oxidation to yield nitrate (Rxn. 1) [3]. Other nitrogen oxide derivatives can be converted to nitrate too. The oxidation of nitrite by oxyhemoglobin in red blood cells (Rxn. 2), the decomposition of peroxynitrite at lower pH (Rxn. 3), and the decomposition of dinitrogen tetraoxide (N_2O_4) formed by the dimerization of nitrogen dioxide radical ($\bullet NO_2$, Rxn. 4), all give rise to nitrate [4–6]. Considerable amount of our daily nitrate exposure comes from diet. Green leafy vegetables account for the major dietary source of nitrate [7].

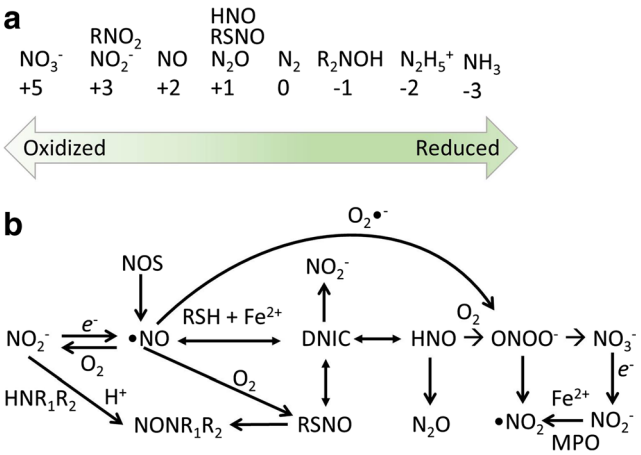

Fig. 1 The relationship and interconversion of various reactive nitrogen oxide species. (**a**) The oxidation states of reactive nitrogen species. (**b**) Mechanisms of formation of reactive nitrogen species

$$\textit{Reaction } 1: \bullet NO + Fe(II)O_2 \rightarrow NO_3^- + Fe(III)$$

$$\textit{Reaction } 2: 4NO_2^- + 4Fe(II)O_2 + 4H^+ \rightarrow 4NO_3^- + 4Fe(III) + 2H_2O + O_2$$

$$\textit{Reaction } 3: ONOO^- + H^+ \rightarrow [ONOOH] \rightarrow NO_3^- + H^+$$

$$\textit{Reaction } 4: 2\bullet NO_2 \leftrightarrow N_2O_4, N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+$$

Relevant Biological Reactions

Nitrate has long been viewed as inert end product of NO metabolism. We now know that nitrate can be reduced back to nitrite by commensal bacteria in the oral cavity and the gastrointestinal tract (Rxn. 5), which represents the bioactivation pathway of dietary nitrate. The reduction of nitrate may also be carried out by xanthine oxidoreductase (XOR) in liver to a lesser extent [8].

$$\textit{Reaction } 5: NO_3^- + e^- + H^+ \rightarrow NO_2^- + H_2O$$

Association with Cancer

Health concerns regarding dietary nitrate were raised largely because of its intragastric conversion to nitrosamines (R_2NNO), which could be carcinogenic and lead to gastric carcinoma [9]. For decades, numerous animal and in-human studies have been conducted to explore the potential link between nitrate and cancer, yet the results are still inconclusive [10]. No significant evidence suggests the correlation of nitrate intake with increased risk of cancer. A recent meta-analysis of 49 epidemiological studies on the association of dietary nitrate with cancer risk revealed that nitrate intake is inversely associated with gastric cancer risk [11]. Furthermore, numerous beneficial effects of nitrate (and nitrite) in lowering blood pressure and protecting against cardiac and liver ischemia–reperfusion injury have been discovered [12–14]. This is consistent with the fact that fruits and vegetables rich in nitrates are associated with lower risk in developing cardiovascular diseases [15].

Peroxynitrite ($ONOO^-$, Oxidation State +5)

Biological Sources

Peroxynitrite, a strong oxidant and nucleophile, is the product of the bimolecular reaction between two biologically relevant

free radicals; nitric oxide (•NO) and superoxide ($O_2^{\cdot -}$) [16]. With a rate constant of 6×10^{10} M^{-1} s^{-1}, this reaction is extremely rapid and diffusion-controlled. Maximal $ONOO^-$ formation occurs when the flux rates of the two reactants •NO and $O_2^{\cdot -}$ are at a ratio of 1:1 [17–19]. At physiological pH (7.4), $ONOO^-$ will exist in instantaneous equilibrium with its conjugate acid peroxynitrous acid (ONOOH, pK_a = 6.8) since protonation is rapid (Rxn. 6).

$$\textit{Reaction } 6: \bullet NO + O_2^{\cdot -} \rightarrow ONOO^-, ONOO^- + H^+ \leftrightarrow ONOOH$$

When the ratio of •NO to $O_2^{\cdot -}$ is not 1:1, the species in excess can further react with $ONOO^-$ itself resulting in the formation of other nitrosating or oxidizing species. The reaction of •NO with $ONOO^-$ tends to form nitrosating species such as dinitrogen trioxide (N_2O_3) [20], whereas the reaction of $O_2^{\cdot -}$ with $ONOO^-$ forms less well-characterized species.

Relevant Biological Reactions

Once formed, there are two routes for ONOOH decomposition: isomerization to form the stable and unreactive nitrate anion (NO_3^-) (Rxn. 3), and hemolysis to yield the oxidizing radical nitrogen dioxide (•NO_2) and hydroxyl (•OH) (Rxn. 7).

$$\textit{Reaction } 7: ONOOH \rightarrow \bullet NO_2 + \bullet OH$$

Another important reaction of $ONOO^-$ is with carbon dioxide (CO_2). Carbon dioxide is relatively ubiquitous in cellular environments undergoing active metabolism, and when concentrations are sufficiently high, the predominant reaction of $ONOO^-$ is with CO_2. Again, there are two outcomes from this reaction: the formation of the strong oxidants •NO_2 and carbonate anion radical ($CO_3^{\cdot -}$) (Rxn. 8) and the catalyzed isomerization to form nitrate (Rxn. 9) [21, 22].

$$\textit{Reaction } 8: ONOO^- + CO_2 \rightarrow CO_3^{\cdot -} + \bullet NO_2$$

$$\textit{Reaction } 9: ONOO^- + CO_2 \rightarrow CO_2 + NO_3^-$$

An important point about the formation of $ONOO^-$ is that both of the reactants (•NO and $O_2^{\cdot -}$) are neither strong oxidants nor strong reductants. Peroxynitrite and peroxynitrous acid, however, are strong oxidants that can further decompose into three other highly oxidizing species (•NO_2, $CO_3^{\cdot -}$, and •HO). In addition to these species being strong oxidants, •NO_2 is a nitrating species associated with the formation of 3-nitrotyrosine (3-NT, see the "Nitrogen dioxide (•NO_2, Oxidation State +4)" section). Due to the generation of numerous highly reactive intermediates, the reaction of •NO with $O_2^{\cdot -}$ to form $ONOO^-$ is considered to have mostly deleterious biological consequences [23].

Association with Cancer

Since generation of $ONOO^-$ results in the formation of strong oxidizing species, the main outcome is damage to macromolecules such as nucleic acids and proteins. Peroxynitrite, therefore, is thought to contribute to the etiology of cancer which is largely a disease of genetic mutations. In addition to causing oxidative stress, however, another effect of $ONOO^-$ formation is the savaging of NO. The reaction of NO with $O_2^{\cdot -}$ is more rapid than the reaction of NO with almost any other biological target. Therefore, when there is sufficient $O_2^{\cdot -}$ generation, the steady-state concentration of NO will be dramatically diminished. As NO is an important signaling molecule, reduction in its bioavailability due to $ONOO^-$ formation can have significant phenotypic and pathological consequences [24].

Studies on cancer cells have shown that peroxynitrite can activate signaling cascades such as nuclear factor kappa beta (NF-κB) via nitration of its inhibitory protein kappa B alpha (IκBα) [25]. In patients with colorectal carcinoma, both 3-nitrotyrosine and nitrate/nitrite levels were significantly higher in the plasma and tumor tissues suggesting peroxynitrite had been generated [26]. Another study on human metastatic colorectal carcinoma in the liver concluded that the cancer proliferation was due to high measured NOS2 expression and 3-NT adducts in the hepatocytes adjacent to metastatic tumor [27]. Samples from human malignant glinoma patients showed elevated levels of 3-NT as well as nitration of the tumor suppressor protein p53 which were suggested to contribute to tumor progression [28]. In human melanoma patients, high levels of 3-NT in primary tumors correlated to an advanced disease progression (Breslow thickness ≥ 2 mm) [29]. Another study demonstrated that NOS2 and 3-NT expression in melanoma cells strongly correlated with poor survival in patients with stage 3 disease [30].

Nitrogen Dioxide (•NO_2, Oxidation State +4)

Biological Sources

Nitrogen dioxide is a highly reactive reddish-brown gas whose color gives rise to the characteristic appearance of urban smog. Nitrogen dioxide is a free radical derived from environmental contaminants such as tobacco smoke, indoor smoke from cooking, and emissions from vehicle exhaust, power plants, and other industrial processes. Although inhalation of •NO_2 constitutes the major route for human exposure, other mechanisms exist. In addition to pulmonary exposure, •NO_2 can arise from dietary nitrite (NO_2^-) and endogenously from nitric oxide (•NO) synthesis [31]. Nitrate and nitrite can occur naturally in the diet from both animal and vegetable sources. Nitrite, under acidic conditions as found in

the stomach, can be reduced to $\bullet NO_2$ and NO (Rxn. 10).

Reaction 10 : $2NO_2^- + 2H^+ \rightarrow 2HNO_2 \rightarrow N_2O_3 + H_2O \rightarrow \bullet NO + \bullet NO_2$

Another mechanism for NO_2 formation is via the hemolysis reactions of $ONOO^-$/ONOOH in the presence or absence of CO_2 (Rxns. 3 and 4) [32]. A third mechanism for $\bullet NO_2$ generation is by peroxidase or metal-mediated nitrite oxidation. Whether nitrite is formed from NO metabolism or derived from dietary sources, it can be enzymatically oxidized to $\bullet NO_2$ by myeloperoxidase (MPO) or eosinophil peroxidase (EPO) (Rxn. 11).

Reaction 11 : $NO_2^- + MPO + H_2O_2 \rightarrow \bullet NO_2$

It has also been demonstrated that H_2O_2 in the presence of heme or free metals (Fe^{2+}, Cu^+) forms hypervalent oxo species that readily oxidize NO_2^- to $\bullet NO_2$ [33, 34] (Rxns. 12 and 13).

Reaction 12 : $Fe(II) + H_2O_2 \rightarrow Fe(IV)O, Fe(IV)O + NO_2^- \rightarrow Fe(III) + \bullet NO_2$

Reaction 13 : $Fe(III) + H_2O_2 \rightarrow Fe(V)O, Fe(V)O + NO_2^- \rightarrow Fe(IV) + \bullet NO_2$

Relevant Biological Reactions

The biological significance of $\bullet NO_2$ formation lies in its unique chemical reactivity. Nitrogen dioxide radical is a strong oxidant that can oxidize (primarily one electron oxidation) proteins, lipids, and nucleic acids, resulting in antioxidant depletion, genetic mutations, and enzyme inhibition. Although highly reactive toward lipids, $\bullet NO_2$ is uncharged and has been shown to diffuse through cell membrane to oxidize intracellular proteins [35]. One of the most important reactions of $\bullet NO_2$ is with tyrosine residues in proteins. In this reaction, $\bullet NO_2$ can both oxidize and add to tyrosine residues to form 3-nitrotyrosien (Rxns. 15 and 16). 3-NT has become an important biomarker associated with numerous pathologies, and its presence is generally considered to be evidence of peroxynitrite formation or $\bullet NO_2$ exposure.

Reaction 15 : $\bullet NO_2 + Tyr \rightarrow Tyr{-}NO_2$

Reaction 16: $\bullet NO_2 + Tyr \rightarrow \bullet Tyr + HNO_2, \bullet Tyr + \bullet NO_2 \rightarrow Tyr{-}NO_2$

Association with Cancer

Despite its central role in numerous pathologic conditions, very little is known about the biological fate of $\bullet NO_2$ and the mechanisms by which it induces disease-associated phenotypic changes. A variety of studies have been conducted in cell culture systems with various $\bullet NO_2$ exposure paradigms and methodologies. These studies measured a variety of end points such as cell death, inflammatory responses, oxidation, and signaling pathways. When cultured human bronchial epithelial cells (HBEC) were treated with 7520–15,040 $\mu g/m^3$ of $\bullet NO_2$, cell membrane damage was observed and increased membrane permeability occurred [36]. Another study using more physiologically relevant concentrations of $\bullet NO_2$ (200–800 $\mu g/m^3$) observed histamine release and other markers of inflammation upon exposure [37]. One study demonstrated that cells exposed to $\bullet NO_2$ (18,800 $\mu g/m^3$) resulted in cell death in a Fas- and JNK-dependent manner and that log-phase cells were more sensitive than confluent cells [38]. $\bullet NO_2$ has also been shown to suppress NF-κB activation, a pathway essential for survival, after a variety of cellular stresses [39]. When a variety of redox agents ($\bullet NO_2$, $O_2^{\cdot -}$, CO_3^-) were tested for their ability to activate Ras, it was found that $\bullet NO_2$ was able to promote Ras guanine nucleotide dissociation independent of its redox potential [40]. A large meta-analysis concluded that there is a weak association between exposure to $\bullet NO_2$ in ambient air and breast cancer at the individual level and a significant association at the aggregate level [41, 42]. Epidemiologic studies have shown that long-term residential exposure to air pollution and specifically $\bullet NO_2$ was strongly correlated to lung cancer incidence [43, 44]. In another analysis, significant correlation between $\bullet NO_2$ concentrations in urban air pollution and lung, breast, prostate, bladder, cervical, and ovarian cancer incidences was observed [45].

Nitrite (NO_2^-, Oxidation State +3)

Biological Sources

Similar to nitrate, nitrite is mainly formed via the oxidation of NO. This process can be an auto-oxidation (Rxn. 17) or be catalyzed by multicopper oxidase or ceruloplasmin (Rxn. 18) [46]. Nevertheless, the major oxidation product of NO remains to be nitrate, as circulating nitrate concentration is in 20–40 μM range while nitrite concentration is about 100- to 10,000-fold lower. Nitrite can also be generated from other nitrogen oxide species such as nitrate via its reduction (described above). The dimerization of nitrogen dioxide radical yields both nitrate and nitrite (Rxn. 4). Dietary nitrite intake comes from processed meat products as nitrite has long been used as a food preservative for cured meats.

Reaction 17 : $4\bullet NO + O_2 + 2H_2O \rightarrow 4NO_2^- + 4H^+$

Reaction 18 : $\bullet NO + Cu(II) \rightarrow NO^{+} + Cu(I)$
$NO^{+} + H_2O \rightarrow HNO_2 + H^{+}, HNO_2 \leftrightarrow H^{+} + NO_2$

Relevant Biological Reactions

Nitrite can be converted back to NO through multiple reductive pathways. The enzymatic pathways for the reduction of nitrite involve a broad range of proteins [47]. In a low pH environment, such as stomach, nitrite is quickly protonated to yield HNO_2, which subsequently dimerizes and decomposes to give NO and other nitrogen oxides with nitrosating and nitrating properties (Rxn. 10). One of the products, N_2O_3, is a potent nitrosating agent that can transfer a NO^{+} equivalent to secondary amines and form nitrosamines (Rxn. 19).

Reaction 19 : $N_2O_3 + HNR_1R_2 \rightarrow NONR_1R_2 + NO_2^{-} + H^{+}$

Association with Cancer

A number of human diet intervention studies and prospective cohort studies have shown correlation between the consumption of nitrite preserved meat and elevated risk in several types of cancer [10]. The same meta-analysis referred to above in the nitrate section also analyzed 51 studies for nitrites and concluded that "dietary nitrite intake was positively associated with adult glioma and thyroid cancer risk", while no significant association was found with cancers of breast, bladder, and colorectal [11]. Another meta-analysis of studies published from 1985 to 2013 linked the increased consumption of nitrite with higher gastric cancer risk [48]. Again controversial evidence was presented showing no significant association of dietary nitrite with cancer [49, 50]. The mechanisms for nitrite to cause cancer are not completely understood, but the conversion of nitrite to carcinogenic nitrosoamines is considered to be a major cause of increased risk.

Nitric Oxide (•NO, Oxidation State +2)

Biological Sources

Within the human body, there are three main mechanisms for the generation of NO. The primary mechanism is enzymatic from one of the tree isoforms of nitric oxide synthase: NOS1 (nNOS), NOS2 (iNOS), and NOS3 (eNOS). The substrates for these enzymes are molecular oxygen and the amino acid L-arginine, and the products are NO and L-citrulline. Necessary cofactors include FAD, FMN, NADPH, and BH4. The three enzymes differ in their expression levels, cell-type distributions, mechanisms of regulation, K_ms for O_2, and amounts of NO produced [51]. The second mechanism of NO production is from the reduction of nitrite under hypoxic or acidic conditions (Rxn. 10). A third, albeit minor, source of NO is pharmacological. Organic nitrates such as glyceryl trinitrate (GTN; nitroglycerin) and nitric oxide-releasing non-steroidal antiinflammatory drugs (NO-NSAIDs [52]) can either generate free NO or liberate nitrite which can be further activated to NO. Although a minor contribution to total body NO production under most circumstances, the biological importance of pharmacological NO production can be significant.

Relevant Biological Reactions

Despite the numerous purported biological functions of NO, under biological conditions, it only reacts with two types of molecules: metals and other free radicals. The majority of the reactions of NO with metals are with ferrous iron (Fe^{2+}). Superoxide and molecular oxygen (a diradical) are the most common free radicals to react with NO. Reactions of NO can be divided into two main categories: (1) the formation of other nitrogen oxides (i.e., $ONOO^{-}$) and (2) the formation of protein adducts containing nitrogen oxide functional groups (i.e., RSNO, see the "Nitrosothiols (RSNO, Oxidation State +1)" section) [23, 53–55]. Of all biological targets, one of the potentially most significant and least studied is the chelatable iron pool (CIP). This small, but chemically significant, fraction of total cellular iron (0.2–3.0%, low M range) [56, 57] is methodologically defined because it is accessible to chemical iron chelators [58]. When cells are exposed to NO, the CIP is quantitatively converted into paramagnetic dinitrosyliron complexes with thiol-containing ligands (DNIC) [59, 60]. Quantitative measurements have demonstrated that cellular concentrations of DNIC are proportionally the largest of all NO-derived adducts, much greater than RSNOs [61, 62]. Despite the potential biological importance of DNIC, their functional significance and phenotypic consequences are largely unknown.

Association with Cancer

Active investigations have led to an increasing list of functional roles for NO in cancer biology. The influence of NO spans the spectrum from cancer initiation (being mutagenic), promotion, and progression to even being used therapeutically as an anticancer agent [63]. One of the long standing conundrums in the field of NO and cancer is the fact that NO seems to play dichotomous roles under seemingly similar circumstances [64]. In some

cases, NO has been shown to be tumorigenic, and in other circumstances, NO is associated with better patient prognosis. Mechanisms of action of NO in cancer are diverse and include activation of cell signaling cascades, increasing migration/invasion, [59, 65], apoptosis, and recently epigenetics [66, 67]. Biochemical mechanisms of NO result from the participation of many of the reactive nitrogen species discussed herein such as $ONOO^-$, RSNO, and DNIC. Much of the information, however, linking NO to various aspects of cancer etiology in humans is correlative. This is largely based on gene expression data demonstrating that NOS enzymes, most notably NOS2, are upregulated in a variety of more aggressive cancers (breast, lung, prostate, gastrointestinal) [68–70]. Given the statistical significance of NOS as a negative prognostic indicator, small molecule NOS inhibitors have been developed and are in various stages of preclinical and clinical developments [71]. In general, the various functions of NO in cancer biology are largely context-specific as phenotypic consequences are uniquely associated with specific tumor types.

Nitrous Oxide (N_2O Oxidation State +1)

Biological Sources

Nitrous oxide (N_2O), commonly known as "laughing gas", is a colorless, almost odorless gas first discovered in eighteenth century. More than 60% of the nitrous oxide in atmosphere is liberated from fugal and bacterial respiratory processes as N_2O is an intermediate involved in denitrification of the nitrogen cycle [72]. In mammalian cells, it is thought to be formed from the dimerization of HNO and subsequent dehydration (Rxn. 20).

$$\textit{Reaction } 20: 2\mathrm{HNO} \rightarrow [\textit{HONNOH}] \rightarrow \mathrm{N_2O} + \mathrm{H_2O}$$

Relevant Biological Reactions and the Association with Cancer

Nitrous oxide has been used as an anesthetic or analgesic agent in surgical procedures and dentistry for centuries. The mechanism of its anesthetic action is likely a result of noncompetitive inhibition of the NMDA subtype of glutamate receptors [73], while the analgesic effect is induced by the activation of opioidergic neurons [74]. The toxicity of nitrous oxide arises from its inhibitory effect on methionine synthase that can lead to genetic and protein aberrations [75–77]. A number of studies investigated the effect of nitrous oxide on cancer, yet the results were inconclusive [78]. No strong evidence has been found to associate the exposure of nitrous oxide at the concentration of its clinical use with an increased risk of cancer [75, 78].

Nitrosothiols (RSNO, Oxidation State +1)

Biological Sources

The three most discussed routes yielding RSNO are as follows: (1) NO oxidation to N_2O_3 followed by reaction with thiols (Rxn. 21), (2) thiyl radicals formed from the reaction of thiols with other oxidants or radicals directly reacting with NO to form RSNO (Rxn. 22), and (3) transition metal ion-catalyzed pathway (although the same transition metals are often good catalysts for RSNO decomposition) [79]. It was reported that thiyl radicals can be formed from small molecular weight thiols during the detoxification of reactive nitrogen/oxygen species or other xenobiotics [80]. The direct reaction of thiyl radical and NO occurs at a diffusion-controlled rate constant ($k = 2.7 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$) representing one important route for in vivo RSNO formation [81, 82].

$$\textit{Reaction } 21: \mathrm{RSH} + \mathrm{N_2O_3} \rightarrow \textit{RSNO} + \mathrm{NO_2^-} + \mathrm{H^+}$$

$$\textit{Reaction } 22: \mathrm{RSH} + \textit{oxidant} \rightarrow \mathrm{RS\bullet} + \textit{reductant} + \mathrm{H^+}, \bullet\mathrm{NO_2} + \mathrm{RSH} \rightarrow \mathrm{NO_2^-} + \mathrm{RS\bullet} + \mathrm{H^+}$$

$$\mathrm{RS\bullet} + \bullet\mathrm{NO} \rightarrow \textit{RSNO}$$

In the presence of transition metal ions like Fe(III) or Cu(II), NO-metal ion complexes are often formed resulting in the reduction of the metal ion and the oxidation of NO to NO^+ [83]. The resulting NO^+ can either be converted to nitrite in an aqueous environment or form an RSNO when a thiol is also coordinated to the metal center. DNIC formed from the coordination of NO with non-heme iron-sulfur cluster may deliver NO^+ to low molecular weight thiols (GSH and cysteine) or protein thiols [84, 85]. In the presence of low level of NO, the reduction of ferric cytochrome *c* to ferrous cytochrome *c* and concomitant formation of nitrosoglutathione (GSNO) was observed [86]. Ceruloplasmin, the multicopper-containing protein was shown to catalyze the formation of GSNO and nitrosoalbumin (Rxn. 23) [87]. NO can also interact with methemoglobin (Hb(III)), at a much slower rate than that of the reaction with hemoglobin (Hb(II)), to form Hb(III)NO complex in which NO has NO^+ character (Rxn. 24). The NO^+ moiety can either transfer to β-93 cysteine to form nitrosohemoglobin (SNOHb) or react with water to form nitrite (Rxn. 25) [88].

$$\textit{Reaction } 23: \mathrm{Cu(II)} + \bullet\mathrm{NO} \rightarrow \mathrm{Cu(I)NO^+}, \mathrm{Cu(I)NO^+} + \mathrm{H_2O} \rightarrow \mathrm{Cu(I)} + \mathrm{NO_2^-} + 2\mathrm{H^+}$$

$$\mathrm{Cu(I)NO^+} + \mathrm{RSH} \rightarrow \mathrm{Cu(I)} + \textit{RSNO} + \mathrm{H^+}$$

Reaction 24 : $Hb(III) + \bullet NO \rightarrow Hb(III)-NO \rightarrow Hb(II)NO^{+} \rightarrow SNOHb(II)$

Reaction 25 : $Hb(II)NO^{+} + H_2O \rightarrow Hb(II) + NO_2^{-} + 2H^{+}$

Relevant Biological Reactions

The most important reaction of nitrosothiols is transnitrosation in which the nitroso functional group is transferred to another thiol susceptible to the electrophilic attack (Rxn. 26). Transnitrosation represents another possible route to generate new RSNOs although there is no net gain in total nitrosothiols.

Reaction 26 : $RSNO + R'SH \leftrightarrow RSH + R'SNO$

When transnitrosation results in the formation of a protein nitrosothiol, this can have significant downstream effects, and this mechanism represents another proposed pathway for the signaling actions of NO. For example, protein *S*-nitrosation (or nitrosylation) has been widely studied and considered as a critical signaling event in modulating protein function and cellular process, ubiquitous across all organisms [89, 90]. The reverse process of nitrosylation, noted as denitrosylation may occur spontaneously in the presence of transition metals or other nucleophiles or catalyzed by several enzymes like GSNO reductase, thioredoxin, and protein disulfide isomerase [91–93]. Another major consequence of RSNO formation is the oxidation of thiols yielding disulfide and hydroxyl (Rxn. 27) [94, 95].

Reaction 27 : $RSNO + R'SH \rightarrow RSSR' + HNO$

Association with Cancer

Like most diseases associated with NO, there are numerous examples of RSNOs participating in the etiology of cancer. Although too numerous to list, RSNOs have been implicated in diverse aspects of cancer biology [90, 96, 97]. The consequences of protein nitrosothiol formation usually stem from a gain or loss of protein activity/function which results in downstream phenotypic effects. These can include inhibition of enzyme catalytic activity or modulation of transcription factor function. In prostate cancer, increased NO levels were linked to androgen receptor (AR) *S*-nitrosation and inactivation that lead to growth inhibition [98]. A study on breast cancer cells revealed that the estrogen receptor status may dictate tumor responses to different nitrosative stresses and participate in the development of hormonal resistance [99]. Looking at the role of NO in cancer therapy, it was noted that NO concentration increased significantly in cells that survived cisplatin treatment. The development of resistance to cisplatin was attributed to *S*-nitrosation of caspase-3 and prolyl-hydroxylase-2, the enzymes responsible for targeting the prosurvival transcription factor hypoxia-inducible factor-1α for proteasomal degradation [100]. A positive role of nitrosothiol formation in cancer therapy was demonstrated in colon cancer cells treated with NO-releasing NSAIDS. These drugs inhibited colon cancer growth by suppressing NF-κB signaling through *S*-nitrosation [101].

Nitroxyl (NO^{-} or HNO, Oxidation State +1)

Biological Sources

Nitroxyl can be viewed as a one-electron reduction product of NO [102]. Under physiological condition, the predominate form of nitroxyl is the conjugate acid HNO, as the pKa of HNO is 11.5 [103, 104]. Despite the lack of evidence for the endogenous generation of HNO, biochemical mechanisms for in vivo formation of HNO do exist [105, 106]. HNO can be a direct product of NO synthase-catalyzed arginine oxidation [107]. Superoxide dismutase (SOD), mitochondrial cytochrome c, and hemoglobin may all be capable of catalyzing the reduction of NO to HNO [108, 109]. The decomposition of nitrosothiols in the presence of free thiol may also lead to the formation of HNO (Rxn. 27).

Relevant Biological Reactions

HNO is highly unstable, and several reaction routes have been elucidated [110]. The first important reaction of HNO is with metalloproteins. Unlike NO's preference for ferrous heme, HNO reacts predominately with ferric heme (Fe(III)) to form Fe(II)NO complex through reductive nitrosylation as both metmyoglobin and methemoglobin have been used as efficient traps for HNO in solution (Rxn. 28). Fe(II)NO in the presence of O_2 reacts giving rise to NO_3^{-} and Fe(III) (Rxn. 29). Formation of other nitroxyl metal complex with metalloproteins CuZnSOD or MnSOD was reported as well [111]. Another target of HNO is thiols. HNO reacts with thiols (RSH) to form N-hydroxysulfenamide (RSN(H)OH) which can either rearrange to sulfonamide or react with another molecule of RSH to yield RSSR and hydroxylamine (R_2NOH) (Rxn. 30 R_2NOH = R′SNHOH) [112]. Reactions of HNO with protein thiols have been proposed as a major mechanism for HNO's pharmacological effect [113, 114]. The reaction rates with thiols can vary, depending on the pKa of the thiol and the hydrophobicity of the thiol environment. In addition to the reactions described above, dimerization of HNO followed by dehydration also occurs at a relatively fast rate ($k = 1.8 \times 10^{9}\ M^{-2}\ s^{-1}$) yielding nitrous oxide (Rxn. 20). The direct reaction of HNO with O_2 to form a

"peroxynitrite like" species occurs at a much slower rate, and its biological relevance has yet to be elucidated.

$$\textit{Reaction } 28 : \mathrm{Fe(III)} + \mathrm{HNO} \rightarrow \mathrm{Fe(II)NO} + \mathrm{H^+}$$

$$\textit{Reaction } 29 : \mathrm{Fe(II)NO} + \mathrm{O_2} \rightarrow \mathrm{Fe(III)} + \mathrm{NO_3^-}$$

$$\textit{Reaction } 30 : \mathrm{R'SH} + \mathrm{HNO} \rightarrow \mathrm{R'}\textit{SNHOH} \rightarrow \mathrm{R'S(O)NH_2R'}\textit{SHNOH} + \mathrm{R\hat{}SH} \rightarrow \mathrm{R'SSR\hat{}} + \mathrm{NH_2OH}$$

Association with Cancer

Evidence for a causative role of HNO in cancer biology has not been presented. However, therapeutically, the anticancer effect of HNO donor compounds has been investigated. HNO has been shown to suppress the proliferation of breast cancer cells and tumor growth in mouse xenograft model, presumably through the initial inhibition of GAPDH and the resulting impairment of energy pathway [115]. The inhibitory effect of HNO donors on PARP, one major enzyme involved in DNA repair, may be utilized to increase the efficacy of chemotherapies and radiation therapy in treating malignancies by inducing DNA damage [116]. Distinct gene expression profile of HNO-donor treated breast cancer cells was observed, providing insights into targets and pathways that can be taken advantage of to develop HNO donor for cancer therapy [117].

Conclusion

Nitrogen oxides are an important class of reactive species involved in the etiology of various diseases and have been implicated, in some form, in almost all stages of cancer development and progression. The biology and pathobiology of nitrogen oxides are largely dictated by the chemical reactions they participate in. The magnitude of the effect of nitrogen oxides on cancer biology is a function of the type of tissue being exposed, as well as the microenvironmental and subcellular characteristics that will determine the types of target molecules. Herein, examples were given for eight major types of RNS that are relevant to cancer (Fig. 1). Although to fully describe the roles of each of these nitrogen oxides in various aspects of cancer is out of the scope of the short review, pertinent examples were given for each to demonstrate their important contributions. In summary, nitrogen oxides can participate in both causative and curative mechanisms of tumor biology. Future research into the complexity and diversity of these reactive species in biological systems will advance our understanding and uncover potential novel therapeutic strategies for cancer.

Compliance with Ethical Standards

Conflict of Interest There are no conflicts of interest to declare.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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