FORUM REVIEW ARTICLE

Nitroxyl as a Potential Theranostic in the Cancer Arena

Hai-Jian Sun,¹ Wei-Thye Lee,¹ Bin Leng,¹ Zhi-Yuan Wu,¹ Yong Yang,² and Jin-Song Bian^{1,3}

Abstract

Significance: As one-electron reduced molecule of nitric oxide (NO), nitroxyl (HNO) has gained enormous attention because of its novel physiological or pharmacological properties, ranging from cardiovascular protective actions to antitumoricidal effects.

Recent Advances: HNO is emerging as a new entity with therapeutic advantages over its redox sibling, NO. The interests in the chemical, pharmacological, and biological characteristics of HNO have broadened our current understanding of its role in physiology and pathophysiology.

Critical Issues: In particular, the experimental evidence suggests the therapeutic potential of HNO in tumor pharmacology, such as neuroblastoma, gastrointestinal tumor, ovarian, lung, and breast cancers. Indeed, HNO donors have been demonstrated to attenuate tumor proliferation and angiogenesis.

Future Directions: In this review, the generation and detection of HNO are outlined, and the roles of HNO in cancer progression are further discussed. We anticipate that the completion of this review might give novel insights into the roles of HNO in cancer pharmacology and open up a novel field of cancer therapy based on HNO. *Antioxid. Redox Signal.* 32, 331–349.

Keywords: nitroxyl, nitric oxide, hydrogen sulfide, anticancer, cancer therapy

Introduction

GIVEN CONTINUOUS PROGRESSION in the field of life science and medicine during the last several decades, the physiological functions of nitric oxide (NO) in mammalian systems have been constantly identified (163). Subsequently, other freely diffusible and small molecules including hydrogen sulfide (H₂S), carbon monoxide (CO), hydrogen peroxide (H₂O₂), and nitroxyl (HNO) in biological system have been discovered (140). Interests in this area have made significant advances in the current understanding of the chemical biology of NO and its derivatives, such as dinitrogen trioxide (N₂O₃), nitrogen dioxide (NO₂), peroxynitrite (ONOO[−]), and HNO (48). Among which, HNO has received enormous interest due to its novel pharmacological potential (112). Over the past few years, the pharmacological actions of HNO on cardiovascular system have been well established (19). HNO donors exert positive inotropy/lusitropy effects in both normal (114) and failing hearts (113), improve cardiac

function in patients with heart failure (122). Like NO, HNO donors are capable of inducing vasorelaxation (3), inhibiting platelet aggregation (15), and attenuating vascular oxidative stress (170). These results support the notion that HNO donors may be developed as attractive drugs for the treatment of cardiovascular ailments.

Besides its cardiovascular protective effects, HNO donors confer a number of anticancer properties (134). HNO donors have been revealed to restrain the proliferation of breast cancer cells (107). HNO donors could also trigger cancer cell apoptosis and retard tumor angiogenesis (107). In a mouse xenograft model of neuroblastoma, HNO remarkably reduces tumor growth (143). Recently, a novel HNO-based diagnostic method for carcinogenesis is developed on the basis of its distinctive redox activity in normal and cancer tissues, and this has been demonstrated by visualization of cell-permeable HNO probes under magnetic resonance imaging (MRI) (180). With redox-sensitive HNO probes, the high spatial and temporal resolution of magnetic resonance

¹Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

²State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, Center for New Drug Safety Evaluation and Research, China Pharmaceutical University, Nanjing, China.

³National University of Singapore (Suzhou) Research Institute, Suzhou, China.

redox imaging (MRRI) technique can estimate tumor redox microenvironment, which can be a potential biomarker to design appropriate strategies for the treatment of cancers (91). These above observations suggest that HNO radicals not only have therapeutic effects but also possess diagnostic utilities in different cancers, and hence potential “theranostics” as well.

In this review, we summarize the generation of HNO and HNO detection methods, and discuss the interactions of HNO with oxidants, thiols, proteins, and metalloproteins. Moreover, we highlight the anticancer properties and diagnostic utilities of HNO-based agents.

Generation of HNO

It has been more than thirty years since the identification of the massive important physiological properties of NO in mammalian systems (140). The metabolism of NO gives rise to other nitrogen oxides such as nitrate, nitrite, ONOO^- , NO_2 , hydroxylamine (NH_2OH), and ammonia, thereby the biology of NO seems to be complex (110). One of the most overlooked, yet chemically simple nitrogen oxide is HNO, which is the reduced form of NO (43). Since the early 1900s, HNO has been of interest primarily to chemists (45), thus a wide spectrum of the important advancements in the area of HNO chemistry have been occurred (43). In the last decades, HNO has emerged as a novel therapy for cardiovascular diseases

and other pathologies including cancer (122, 134). The unique chemical properties and pharmacological utilities of HNO have garnered this molecule a considerable amount of recent interest.

In light of its unique biochemical properties, it may be envisaged that HNO, like other gaseous transmitters including NO or CO, could be regarded as an endogenous molecule. However, the endogenous generation of HNO in mammalian system remains speculative, which may be attributed to the absence of specific detection methods for HNO. Nevertheless, a growing body of *in vitro* evidence supports the endogenous production of HNO in biologically relevant systems (37, 47). In several settings, the endogenous generation of HNO may be developed in nitric oxide synthase (NOS)-dependent or -independent pathways (Fig. 1). Biochemical studies have shown that HNO can be synthesized from NOS itself, especially without tetrahydrobiopterin. The chemical oxidation of the NOS intermediates, such as *N*-hydroxy-L-arginine and NH_2OH , may be an important source for HNO production (36, 37). In addition to NOS-dependent manner, HNO can be generated by reduction of NO in the presence of xanthine oxidase, ubiquinol, mitochondrial cytochrome *c*, hemoglobin, and manganese superoxide dismutase (MnSOD) (12, 47). Moreover, the reaction of S-nitrosothiols with other thiol species gives rise to HNO (37, 141). Some additional pathways for the formation of HNO that are not implicated in the generation and interconversion of nitrogen

FIG. 1. *In vitro* formation of HNO. (A) In the consumption of O_2 , NADPH, and the cofactor tetrahydrobiopterin (BH4), L-arginine was converted by NOS to NO and L-citrulline. In the oxidation of arginine, the intermediate compounds such as NOHA, peroxide, and superoxide (O_2^-) were also generated. (B) When it is uncoupled, NOS-dependent production of HNO is related to the oxidation of NOHA (36, 37). In turn, HNO could be also converted to NO *via* reaction of Fe(II) at the heme site of the enzyme or through SOD. (C) HNO is generated from S-nitrosothiols or *via* enzymatic reduction of NO (37, 141). In addition to NOS-dependent mechanisms, heme protein-mediated oxidation of NH_2OH directly leads to HNO formation (37, 119). Moreover, the generation of HNO is associated with reduction of NO by mitochondrial cytochrome *c*, xanthine oxidase, ubiquinol, hemoglobin, and MnSOD (12, 47). HNO, nitroxyl; MnSOD, manganese superoxide dismutase; NADPH, nicotinamide adenine dinucleotide phosphate; NH_2OH , hydroxylamine; NO, nitric oxide; NOHA, NG-hydroxy-L-arginine; NOS, nitric oxide synthase; SH, thiol; SNO, thionitrites; SOD, superoxide dismutase. Color images are available online.

oxides are available. For instance, the oxidation of NH_2OH by peroxide or heme protein can produce HNO (37, 119). This intriguing mechanism suggests that the possible endogenous signaling molecules, such as H_2O_2 and the oxygenated derivative of ammonia, may lead to the formation of HNO. In the end, recent research has demonstrated that endogenous molecules could act as substrates for HNO biosynthesis. Despite this, whether HNO could function as an endogenous signaling molecule remains undetermined. Regardless of its endogenous generation, HNO holds considerable importance as potential cancer pharmacological modulators (see below).

Formation of HNO by $\text{H}_2\text{S}/\text{NO}$ Interaction

A plethora of evidence suggests that H_2S is a critical biological and pharmacological regulator (23). In mammalian system, the endogenous H_2S primarily comes from the enzyme metabolism of cystathionine γ -lyase (CSE), cystathionine β -synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST) (158). Both CSE and CBS are cytosolic enzymes, while 3-MST is localized in the cytosol and mitochondria (21, 76, 111). NO is a free radical gas that is produced by the oxidation of the amino acid L-arginine using NOS family,

including neuronal NOS, endothelial NOS, and inducible NOS (74, 82). It has been reviewed that H_2S plays a central role in the progression of different types of cancer cells (22). Likewise, numerous studies have shown that NO may be used as cancer therapeutic, especially in cancer cells resistance to conventional treatments (64). Being gaseous molecules, both H_2S and NO are involved in the pathogenesis of various cancer disorders. It is clear that their individual biological characteristics have been extensively studied, while the possible crosstalk between H_2S and NO in cancer biology may be of great importance.

The formation of HNO can be traced from chemical interaction of H_2S with NO in various systems (154, 174). With regard to this, our group provided the first evidence that HNO can be formed in isolated cardiomyocytes *via* a crosstalk between H_2S and NO; this is based on our observations that HNO donor Angeli's salt (AS) shows similar inotropic and lusitropic effects in isolated cardiomyocytes when a H_2S donor sodium hydrosulfide is combined with an NO donor sodium nitroprusside (173, 174). Furthermore, the effects of $\text{H}_2\text{S}/\text{NO}$ interaction on myocyte contractility in isolated cardiomyocytes were abolished by HNO scavenger, L-cysteine (173, 174). The existing evidence suggests that induction of HNO by interaction between H_2S and NO may be of

FIG. 2. Chemical interaction of NO and H_2S . With the aid of three isoforms of NOS including nNOS, eNOS, and iNOS, L-arginine is oxidized into L-citrulline with NO (74, 82). The three isoforms of CBS, CSE, and 3-MST are major contributors to the production of H_2S by oxidizing L-homocysteine and L-cysteine, together with byproducts of pyruvate, α -ketobutyrate, and serine (158). This chemical interaction can produce intermediate products, including HNO, SSNO(-), polysulfides, dinitrososulfite [*N*-nitrosohydroxylamine-*N*-sulfonate (SULFI/NO)], and other compounds (30). 3-MST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine β -synthase; CSE, cystathionine γ -lyase; eNOS, endothelial NOS; iNOS, inducible NOS; H_2S , hydrogen sulfide; nNOS, neuronal NOS; SSNO(-), nitrosopersulfide. Color images are available online.

physiological significance in the heart. Endothelial cell-derived HNO by chemical interaction of H₂S with NO induces relaxation of smooth muscle cells (3). Moreover, CSE-deficient arteries exhibit higher NO levels but lower HNO levels, and pretreatment with exogenous H₂S donor rescues the endothelial dysfunction in CSE-deficient aortas (146). Besides, we identified that H₂S and NO could react with each other to generate HNO, which confers anti-inflammatory effects in BV-2 microglial cells (183). The formation of HNO by the interaction of H₂S with NO may be very slow, but this reaction can be detected in cultured cells (94). In parallel to these findings, a systematic research has uncovered the chemical basis of this interaction (30). In this study, the coexistence of H₂S and NO may trigger a network of chemical reactions that lead to S/N-hybrid species, polysulfides, and HNO at physiological pH (30), thereby offering a fundamental basis for the chemical processes governed by H₂S and NO (Fig. 2). It is agreed now that HNO could be formed through the chemical reaction between the free radicals generated by H₂S and NO individually, and further investigations are needed to explore the therapeutic effects of such chemical interaction during cancer progression.

HNO Donors

The emergence of HNO donors has greatly expanded the understanding of its biological/pharmacological activities in many systems. For biological research, various HNO donors are commonly available (98). The currently used HNO releasing compounds are AS (Na₂N₂O₃; an oxide diazeniumdiolate), isopropylamine NONOate (IPA/NO; a primary amine diazeniumdiolate), Piloty's acid (PhSO₂NHOH), acyloxy nitroso, cyanamide and acyloxy nitroso compounds such as 1-nitrosocyclohexyl acetate (NCA) (69, 96, 99, 112, 127, 154). It appears that the most extensively utilized HNO donor is AS (112). As mentioned previously, the dimerization of HNO may lead to subsequent generation of N₂O and water in most chemical or biological studies (98). Under acidic pH condition, AS may generate NO, and hence precautions must be taken when conducting experiments at acidic or slightly acidic conditions (38). Although fairly stable at high pH, AS would still spontaneously decompose to equivalent amount of HNO and NO₂⁻ in neutral solution (69), which may confound experimental results interpretation. Therefore, the control experiments using fully decomposed AS are required in most studies, and other possible NO₂⁻ effects are also considered. Despite these limitations, AS is still the widely used reagent in the discovery of the biology/pharmacology actions of HNO, and numerous studies will continue to use it as the HNO source in the future studies.

N-hydroxysulfonamides (PhSO₂NHOH its analogs or derivatives) are also served as HNO donors. However, these compounds only release HNO at basic pH condition (112). Thereafter, these compounds prefer to release NO rather than HNO under physiological conditions (176). This might limit the application of these compounds in biological or pharmacological studies. IPA/NO, a primary amine diazenium diolate, can dissociate to HNO at physiological pH and temperature without corelease of NO₂⁻ (97). However, this drug may be a mixed HNO/NO donor at pH < 7 (97). In addition, it should be mentioned that one byproduct of amine diazenium diolate decomposition is nitrosamine, a potential toxic spe-

cies (39, 96). As discussed above, two-electron oxidation of NH₂OH can be a source of HNO, thus NH₂OH might represent another class of HNO donor (98). For this reason, the oxidative conversion of NH₂OH to HNO must be considered when interpreting studies involving NH₂OH. The antialcoholism drug cyanamide produces an intermediate, *N*-hydroxycyanamide, which spontaneously decomposes to HNO and cyanide (34). The acyloxy nitroso compounds are developed as a potentially novel class of HNO donors (127), these compounds are synthesized from oxidation of oximes by excessive carboxylic acid, and they may be able to release HNO under physiological conditions. Overall, the favorable roles of HNO donors are clinically limited due to their highly alkaline properties (120). To overcome these disadvantages, novel HNO donors with variable stability have been continuously synthesized, such as NCA (32, 127). Although NCA replicates the protective actions of classical HNO donors, it also serves as a mixed HNO/NO donor (127, 135). With further studies, the novel HNO donors such as CXL-1020, CXL-1427, and BMS-986231 are gradually identified (115, 122, 153, 184). However, the therapeutic potential of these donors in various diseases remains to be tested. It is clear that the long-lasting and safe HNO donors will be obligatory in the future development of drugs with the proper therapeutic effects.

HNO Detection

Despite that the progression of HNO biology/pharmacology is largely understood, it is still necessary to address whether HNO is an endogenous and regulated signaling molecule. To resolve this, a sensitive and specific detection method for HNO is necessary. The advances in HNO detection strategies and their potential in pathological biology are continuous (93) (Fig. 3). HNO can decompose to N₂O and water, and the level of N₂O is commonly tested by headspace gas chromatography-mass spectrometry; nevertheless, this method is not amenable for studies that attempt to point to HNO as an endogenous species (28). Furthermore, the formation of N₂O may occur through nitrogen oxide decompositions that do not involve free HNO. Detection of the nitrosyl adducts of ferric heme proteins, such as Fe^{II}-NO myoglobin, has been used as an indicator for HNO (93). Other methods based on the binding of metalloporphyrins to HNO, HNO-selective electrodes, are capable of detecting HNO signaling (144). However, the low sensitivity and selectivity of these traditional analytical techniques for HNO detection have been extensively reviewed (44, 112). It is reported that fluorescence probe methods offer high detection sensitivity for HNO detection (166). Over the last decade, the development of fluorogenic probes for HNO detection has been progressed (140).

In recent years, copper(II)-based fluorescent probes, arylphosphine-based fluorescent probes, nitroxide-based fluorescent probes, thiol-based fluorescent probes, and chemiluminescent probes are newly developed (140). These novel HNO probes confer more advantages than the traditional methods, and the newly developed methods hold greater specificity and selectivity for HNO. However, the selective HNO detection is still a challenge, as the fluorescence response of such probe toward HNO in the presence of glutathione (GSH) is uncertain. In addition, the sensitivity

FIG. 3. HNO detection methods. Color images are available online.

and selectivity of these probes toward biological reductants or other nitrogen oxides remain problematic. At present, fluorescence measurements combined with the high-performance liquid chromatography techniques are recommended for detection of HNO-specific products. We believe that the rapid development of specific, sensitive, and biologically compatible HNO detectors is crucial for the future investigations of its pharmacological activities.

Chemical Properties of HNO

Being a molecule with only three atoms, the chemical properties of HNO are complex. The earliest misconception on the acidity of HNO came from the reported pK_a of 4.7, suggesting that NO^- , not HNO, was the predominant species at physiological pH (145). However, more recent works have shown that HNO is actually a very poor acid (pK_a of 11.4), and the revised pK_a of 11.4 indicates that HNO is the most relevant species under physiological conditions (12, 128).

HNO is highly reactive, as reflected by its fast dimerization and dehydration to nitrous oxide (19, 85). Moreover, it is likely that HNO functions as a good electrophile preferring addition reaction to nucleophiles, especially thiols (112, 145). Theoretical prediction of HNO reactivity suggests that HNO may be unresponsive toward hydrate or alcohols (11). HNO is found to be highly reactive toward a number of potential biomolecules, such as ferric heme proteins, CuZn-SOD, MnSOD, and thiols (12, 84). It is documented that NO reacts preferentially with ferrous heme proteins, whereas HNO reacts with ferric heme proteins ~ 100 times faster than ferrous heme proteins (145, 168). Besides reactions with thiols or thiol proteins, the other major biological target for HNO seems to be calcium channels and metalloproteins (145). Further studies have revealed that reactive thiolates, ubiquitin ligases use acidic thiols, fatty acid acyl transferase, and caspases are potentially reactive with HNO (145). Given the high reactivity of HNO with ferric heme proteins, thiols and other targets, this could provide guidance when exam-

ining the biological effects of HNO. Overall, a large body of work is obligatory to investigate the potential biochemical targets of HNO, thus holding great promise for the development of HNO biochemistry and pharmacology.

Oxidation Chemistry and Antioxidant Properties of HNO in Cancer

Oxidative stress and reactive oxygen species (ROS) overproduction are historically considered harmful to the various cell types as they can induce DNA, lipids, and protein damage, which eventually lead to cell destruction (88, 124, 131). This notion leads to numerous clinical trials that attempt to scavenge ROS or reduce oxidative damage. However, mounting evidence suggests that simply attempting to scavenge ROS is potentially harmful (7, 88). The roles of ROS in numerous cancer cells have been intensively reviewed (41, 102, 156) (Fig. 4). In comparison with normal cells, the higher basal ROS levels were detected in cancer cells, and the induction of ROS is closely associated with tumor development, progression, and survival (103). To be expected, moderate intake of antioxidants reduces the risk of cancer development and slows cancer progression (20). However, long-term clinical trials have demonstrated a disappointed efficacy of antioxidants in the prevention of cancer progression (57). On the contrary, high levels of ROS are toxic to cancer cells, thus potentially initiating oxidative stress-mediated cancer cell death (57). This viewpoint renders pro-oxidant cancer therapy as an interesting area of study. It seems that both beneficial and harmful roles of ROS in cancer cells are identified. A reasonable explanation is that the elevated levels of antioxidant proteins in cancer cells may detoxify intensified ROS levels to establish a redox balance, thus preserving tumorigenesis (58). These complicated results demonstrate the complexity of ROS in cancer progression. The appropriate modulation of ROS levels may be a potential anticancer strategy.

FIG. 4. ROS in normal and cancer cells. Healthy cells possess normal production of ROS, enough antioxidant activity, and cellular repair. Low ROS levels are sufficient to guarantee cell normal survival and proliferation (88, 124). The high levels of ROS in tumor cells yield DNA damage, thus enhancing cell survival and proliferation (88, 124). In tumor cells, the DNA damage and genetic instability decrease cellular repair. Cancer cells upregulate antioxidant systems to scavenge high ROS levels, thus maintaining protumorigenic signaling pathway (103). When ROS reach toxic levels, especially in chemotherapy, excessive oxidative stress may result in inadequate adaptations and cancer cell death (57). ROS, reactive oxygen species. Color images are available online.

Similar to NO, the pro-oxidant and antioxidant effects of HNO have been observed (Fig. 5). The unique chemical properties of HNO could affect the oxidation of various biomolecules *via* interacting with other ROS and reactive nitrogen species. HNO is sufficient to induce thiol oxidation,

and this oxidation is occurred in the absence of free radical intermediates (11, 112). Purine DNA bases can also be oxidized by HNO through hydroxyl radical production (42, 109). HNO at the high dose (2–4 mM) is cytotoxic to several tissues, including neurons (61), through DNA oxidation and

FIG. 5. The pro-oxidant and antioxidant effects of HNO. HNO at high concentration might induce hydroxyl radical production, DNA strand breakage, lipid oxidation, DNA bases oxidation, and loss of thiol (11, 42, 70, 109, 112). By contrast, induction of Nrf2/HO-1 signaling pathway, inhibition of membrane lipid peroxidation, and upregulations of oxidative-stress-sensitive genes may contribute to the antioxidant effects of HNO at low dose (29, 67, 83, 106). FTH1, ferritin heavy chain 1; GCLC, glutamate-cysteine ligase catalytic; HO-1, heme oxygenase-1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; TXNRD1, thioredoxin reductase 1. Color images are available online.

thiol loss (112). Such a pro-oxidant effect of HNO may involve the intermediate formation of hydroxyl radical (70). A new HNO radical tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-*O*-1H-imidazole-3-cationic-1-oxyl-2)-pyrrolidine-1-carboxylic ester (L-NNP) initiates a significant increase in lipid oxidation and oxidative damage, thus resulting in MCF-7 and MDA-MB-231 cell death (179). Moreover, the higher cytotoxicity of L-NNP in MDA-MB-231 cells than in MCF-7 cells is related to more free radical production (179).

By contrast, it is expected that low dose of HNO may exhibit therapeutic effects without toxicity. Not surprisingly, the HNO donor cyanamide holds minimal toxicity, and long-term administration of AS is well tolerated in animals (48). HNO treatment induces the expressions of the antioxidant protein heme oxygenase-1 (HO-1) dependent on the redox-sensitive nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which has a protective role against cellular oxidative damage (106). By using the yeast *Saccharomyces cerevisiae* model system, HNO is described as an antioxidant *via* inhibiting membrane lipid peroxidation (86). Moreover, HNO donors display antihypertrophic and superoxide-suppressing effects in the myocardium (67, 83). It is also observed that HNO radical-containing nanoparticles reduce the amount of oxidized lipids and cell apoptosis in human neuroblastoma SH-SY5Y cells (29). Thereafter, HNO donors are cytoprotective at low concentrations. Importantly, HNO is resistant to scavenging by superoxide anions, thus the bioavailability of HNO might be preserved in those diseases associated with oxidative stress (19). The redox chemistry of HNO may be a new area of interest, and further investigations are clearly warranted.

It is established that the pharmacological actions of hydroxamic acids are partially ascribed to their roles as HNO/NO donors under oxidative stress state. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, amplifies tumor radioresponse *via* oxidation of HNO to NO (123). Like quercetin, a HNO derivative pirolin decreases the median number and volume of tumors, together with a restoration of the GSH activity and decreased hydroperoxides in 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary carcinoma model (148). In bladder cancer cell line RT112 and cisplatin-resistant subline RT112-CP, the cisplatin-triggered oxidative DNA damage is eliminated by newly synthesized platinum-HNO complexes (PNCs) (24). This study provides the evidence that PNCs may be more effective for tumors that have developed resistance to cisplatin. The same research group has revealed that platinum(IV)-HNO complex PN149 treatment causes cell cycle arrest and cell apoptosis in the bladder cancer cells and the renal cell carcinoma cells (125). Application of PN149 stimulates the oxidative-stress-sensitive genes, including ferritin heavy chain 1, glutamate-cysteine ligase catalytic, Nrf2, HO-1, and thioredoxin reductase 1 in RT112 cells, but not A498 cells (125), suggesting a cell-type specific activation. In addition, infusion of a HNO derivative pirolin protects the rat brain against the toxic effects of oxidative stress and inflammation response induced by the anticancer drugs doxorubicin (DOX), paclitaxel, and docetaxel (DTX), thus highlighting HNO as a potential drug against these anticancer drug-evoked brain oxidative damage (147). In a rat model of mammary tumor induced by DMBA, the anticancer drugs DOX-DTX chemotherapy triggers considerable oxidative

stress in the plasma, which could be partially ameliorated by the HNO antioxidant pirolin (149). Surprisingly, only pirolin could exhibit both antioxidant and pro-oxidant properties, but its conjunctions with DOX-DTX chemotherapy are capable of mitigating the systematic oxidative stress generated from chemotherapy (149). It is likely that the redox chemistry of pirolin may be dependent on the ratio of this compound and anticancer drugs. Collectively, HNO and its derivatives seem to exert both pro-oxidative and antioxidant activities in different cancer cells. Thereafter, the application of HNO donors or its derivatives in cancer management should be carefully weighted. It should be figured out whether endogenous or exogenous HNO donors can result in beneficial actions when high oxidative stress is occurred under pathophysiological conditions. Furthermore, it is required to understand the underlying mechanisms by which HNO targets ROS pathways for cancer therapy.

Interaction of HNO with Thiols or Thiol Proteins in Cancer

In the body, thiols are predominantly located in the amino acids; thiol redox protein-regulated pathways will provide new therapeutic targets for cancer therapy (55). Within the cells, the most abundant free thiol is GSH, a cysteine-containing tripeptide (117). The antioxidant capacity of GSH is important for regulation of redox status even under basal condition (63, 71) (Fig. 6). Under stress conditions, conjunction of GSH with electrophilic carcinogens may retard tumor initiation and carcinogenic process (63). Deficiency of GSH or decreased GSH/glutathione disulfide ratio is implicated in the progression of cancers, and the GSH and thioredoxin systems are activated in carcinogenesis (157). Depletion of GSH may serve as an effective strategy to sensitize cancer cells to radiation therapy (92). The involvement of GSH in tumor progression and drug resistance is clear. It might be crucial to further unveil the GSH-driven molecular mechanisms, which may be helpful to develop novel therapeutic approaches to fight cancer progression.

It is evident that HNO can interact with specific thiol proteins that modulate fundamental cellular functions (45) (Fig. 7). A comprehensive identification of HNO-mediated derivatization of protein thiols is conducted by mass spectrometric analysis (129). It is reported that HNO donors inhibit glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity *via* forming a reduction reversible active site disulfide and a reduction irreversible sulfinic acid or sulfinamide modification at Cys244 (101). The cyanamide is oxidized by the catalase/H₂O₂ system, followed by generation of *N*-hydroxy intermediate that decomposes to hydrogen cyanide and HNO (35, 104). The formed HNO reacts with cysteine thiolate within the enzyme aldehyde dehydrogenase, resulting in inhibition of this enzyme (34, 133). Incubation of rat hepatocytes with cyanamide inhibits the activity of aldehyde dehydrogenase without altering GSH level (60). Similarly, HNO is found to suppress the activity of another cysteine-based dehydrogenase GAPDH, without altering either GSH levels or GSH/oxidized GSH ratios (85). These results imply that HNO can interact with selected proteins without altering the redox status of GSH within cells. However, another report shows that HNO donor AS promotes macrophage differentiation *via* upregulating the GSH synthesis rate-limiting enzyme

FIG. 6. Antioxidant effects of GSH. In the process of aerobic metabolism, the H_2O_2 can be decomposed by GPx in the cytosol or metabolized by catalase in the peroxisomes (63). In the presence of NADPH, the GSSG is reduced to GSH by GSSG reductase, thus forming a redox cycle and preventing the oxidative damage (157). Under oxidative stress condition, the conversion of GSSG to GSH might be less, thereby leading to the deposition of GSSG in the cytosol (92). To achieve redox equilibrium, the GSSG could be transported out of the cell or react with protein sulfhydryl groups (PSH) to form mixed disulfides (PSSG) (92). GSH, glutathione; GSSG, glutathione disulfide; GPx, GSH peroxidase; H_2O_2 , hydrogen peroxide; ROH, lipid alcohols; ROOH, lipid hydroperoxides. Color images are available online.

gamma-glutamylcysteine synthetase, and incrementing intracellular GSH levels in THP-1 monocyte/macrophages (162). Still, whether HNO affects cysteine thiolate within aldehyde dehydrogenase in THP-1 monocyte/macrophages has yet to be determined. The effects of HNO donors on the

GSH or oxidized GSH levels by modifying certain protein thiols should be fully explored in future studies.

HNO is defined as a pharmacological drug for the treatment of heart failure (112). It is demonstrated that HNO elicits dose-dependent increases in coronary flow in normoxic

FIG. 7. Pathways for the reaction of HNO with thiols. The chemical reactions of HNO with thiols are divided into two steps (45, 129). In the first step, a nucleophilic thiol attacks the electrophilic nitrogen atom of HNO, resulting in the generation of *N*-hydroxysulfenamide. With the aid of additional thiols, further reactions lead to the corresponding disulfide and hydroxylamine (Pathway 1). Without additional thiols, a rearrangement occurs to form a sulfinamide (Pathway 2). RSSR, disulfides. Color images are available online.

hearts, and holds hemodynamic advantages over existing pharmacotherapy in acute heart failure (27). Mechanistically, HNO elicits both inotropy and lusitropy *via* interacting with specific thiol proteins involved in Ca^{2+} release and uptake within the sarcoplasmic reticulum (SR) (50, 52). In failing hearts, HNO can activate cardiac thiol-containing proteins such as the sarcoplasmic ryanodine receptors (RyRs) and sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2a) to increase myocardial contractility (26, 155); this unique property only belongs to HNO, rather than its redox congener NO. RyRs are believed to be modulated through redox control of critical cysteine thiols (45). The evidence that HNO activates RyRs *via* thiol protein modification is supported by a finding that administration of the thiol reductant dithiothreitol (DTT) reverses HNO-induced Ca^{2+} release from the SR (26, 155). Very recently, HNO is implicated to target cysteines 41 and 46 in phospholamban to enhance myocyte/cardiac function (72). Calcitonin gene-related peptide (CGRP) is a vasoactive peptide that exhibits protective effects in several cardiovascular diseases, including heart failure (73). The release of CGRP by HNO occurs *via* binding of HNO to a ferric or thiol site, thus altering calcium channel function (100). These observations suggest that HNO augments cardiac contractile function by interacting with the critical regulatory cysteines in excitation–contraction coupling proteins.

Based on the above findings, the thiols and thiol proteins might be the critical targets for HNO (134). Due to high reactivity toward thiols, HNO may act as an endogenous molecule that is regulated by GSH content (37). For instance, the irreversible thiol modification of GAPDH by HNO affects glycolysis (85, 87), which is a major energy pathway in most solid cancers. Therefore, GAPDH or other thiol proteins may be the pivotal targets for HNO-related anticancer drugs. In consistence with this idea, HNO is indicated to inhibit breast (107) and neuroblastoma cancer development (143). However, it is still uncertain whether HNO influences the targeted thiols and thiol proteins to fight these cancers. Reaction of the Ru(II) complex $[\text{Ru}(\text{bpy})_2(\text{SO}_3)(\text{NO})](+)$ (1) with GSH or *N*-acetyl-L-cysteine produces HNO; the subsequent HNO formation counteracts hypoxia-induced stabilization of hypoxia-inducible factor-1 α (HIF-1 α) in cancer cells (137). Moreover, HNO from this metallonitrosyl complex disrupts neovascularization in an angiogenesis model (137). These results demonstrate that the thiol-dependent modifications of HNO are attractive approaches for cancer therapy. Unfortunately, the studies regarding the interaction of HNO with thiols or thiol proteins in cancer development are very rare. Further studies are necessary to better recognize the underlying molecular mechanisms of HNO-mediated thiol modifications in cancer progression, and these investigations could lead to the development of novel therapeutic strategies for cancer.

Interaction of HNO with Metalloproteins in Cancer

NO maintains normal cardiovascular function by activating the soluble guanylate cyclase (sGC)/cyclic GMP (cGMP) signaling pathway (121). Besides, the roles of the NO/sGC/cGMP signaling axis in the development and progression of cancers are well established (62). HNO is presumed to induce vasorelaxation *via* activation of the sGC/cGMP signaling pathway (46). Since sGC is a heme protein, this may give a possibility that the interaction between HNO and a heme

protein, such as sGC, may exhibit biological responses *via* second messenger cGMP generation. Subsequent studies have found that HNO donor AS activates sGC and voltage-dependent K^+ channels to induce vasorelaxation in resistance vessels (68). More recent work has shown that the purified bovine sGC can be activated by two distinct HNO donors (95). It is speculated that a ferrous–HNO complex may induce activation of sGC, akin to what occurs with NO. However, Zeller *et al.* found that the purified sGC was not activated by HNO donor in the absence of superoxide dismutase (SOD) or DTT (177). The oxidation of HNO to NO by SOD causes biphasic sGC activation (177). It was somewhat surprising since the formation of NO-Fe^{2+} species through binding to the ferrous enzyme could activate sGC. Likewise, the NO-Fe^{2+} species could also be generated by the reaction of HNO with the Fe^{3+} enzyme (142). There is no doubt that the formation of HNO/ferrous heme is complicated because sGC contains multiple regulatory thiols that may be susceptible to HNO. It is reported that HNO could stimulate sGC through interaction with the ferrous heme, as this activation could be eradicated by HNO-mediated protein thiol oxidation (95). It would be expected that the complex formation of HNO/ferrous heme eventually leads to the ferrous–nitrosyl. Thus, it may be a possible pathway for HNO-mediated activation of sGC in biological systems.

Besides sGC, HNO is postulated to coordinately react with many other metalloproteins. However, there is little evidence that this possible interaction exists in biological systems. This is not to say that it is biologically irrelevant. HNO/ferrous heme could be easily oxidized to NO complex through one-electron oxidation reaction. In particular, the interaction of HNO with heme proteins under biological condition might yield the formation of the ferrous/NO complex. It is highly probable that interactions of HNO with metal protein could be involved in their pharmacological properties, including cancer pharmacology.

HNO Radical As a Probe for Cancer Detection

Redox dysregulation is mainly characterized by excessive production of ROS, which is critically involved in cancer initiation and progression (8). Redox chemotherapeutics may target multiple redox-sensitive signaling pathways to modulate numerous oncogenes and tumor suppressor genes (169). Thus, obtaining knowledge of redox status in each tumor should be useful for accurate prognosis for clinical therapeutic methods, including radiation therapy, photodynamic therapy, and chemotherapy (10, 33, 49, 65, 89, 180).

HNO radicals are membrane permeable and less toxic, which mimic SOD to scavenge ROS (108). HNO derivatives and their metal complexes possess potent anticancer activity in a variety of cancer cell lines (5, 126). It has been demonstrated that the rate of nitroxide decay is related to tumor redox environment, which is dependent on intracellular GSH levels and oxygenation in tumor tissues (66). Based on this, nitroxide-radicals-modified gold nanorods function as a dual-mode contrast chemical during computed tomography/MRI in tumors, and the agent also ablates the solid tumor in 4T1 tumor-bearing mice, implying that it may be a possible theranostic drug for cancer imaging and therapy (171). Similarly, HNO radicals are established to be promising agents for evaluation of tissue redox state (4, 151, 159). It is

described that HNO radicals are favorable for measurement of redox status in the circulation (90, 182). In a detailed study, the hydrophobic cell-permeable HNO derivatives are appropriate for estimation of oxidation/reduction status in both cells and tissues, while the hydrophilic HNO derivatives are suitable for determination of redox status of blood by using electron paramagnetic resonance imaging (EPRI) (182). A novel HNO radical compound is developed as an anticancer drug with noninvasive real-time imaging diagnostic value (4). Using HNO radicals as spin labels, the permeability of this radical for blood-brain barrier and blood clearance as well as its MRI signal dynamic in the brain is successfully obtained (181). A bioluminescent probe, BP-HNO, is invented to detect the HNO fluorescence in xenografted mice with fLuc-transfected MDA-MB-231 tumors (80). These published articles substantiate that the redox-sensitive HNO probes are suitable for assessment of tissue redox condition through anatomic imaging techniques, thus offering valuable signals from the region of interest.

EPRI enables us to generate images of tissue oxygenation by using exogenous probes such as trityl radicals or HNO radicals (77). The spatial distribution of such probes could be produced using magnetic field gradients with MRI, thus the oxygen space map and its spectral features could be observed from live cells or animals (77). Low molecular weight cyclic HNO radicals are viewed as useful tools to detect biological redox (9, 150, 151, 160, 161). In agreement with these findings, redox status could be measured by a sensitive HNO probe in a mouse tumor model (17). A comparative study has demonstrated that MRRI technique-based HNO decay rates are much faster than those from the electron paramagnetic resonance spectroscopy (EPRS) and EPRI (91). Using a HNO contrast agent carbamoyl-proxyl (CmP), MRRI technique can effectively map tumor tissue redox status, thus making it a useful biomarker to guide appropriate treatments (91). One should keep in mind that heterogenic structure in tumor tissues must be considered to achieve the reliable tissue redox status from the reduction rates of HNO contrast agents, such as CmP. Furthermore, due to the existence of cellular redox state and radical metabolism in cancer biology and treatment, the methodologies such as EPRS, EPRI, or MRI might provide valuable strategies for the study and visualization of various cancer models.

The pH-sensitive HNO radical-containing nanoparticles are found to be effective against cerebral ischemia-reperfusion-induced oxidative stress injuries (175). Acidity, or low pH, is a key parameter in living organisms (2). The extracellular pH and intracellular pH values are strictly regulated to maintain physiological homeostasis in different organs (56). Aberrant tissue pH value has been shown to act as a pathological factor in the process of cancer (54, 178). As such, the accurate, precise, and reproducible measurements of acidic pH of tumors are highly necessary (18). The recent developments regarding the measurement of cancer pH *via* pH-sensitive positron emission tomography radiotracers, MRI, and magnetic resonance spectroscopy have been systematically reviewed (178). At physiological pH, HNO donors can decompose to ONOO⁻ and nitrite in aqueous solutions (40, 139). Upon exposure to pH 6.0, HNO-dependent production of hydroxyl radical is higher than that at pH 7.4 (70). HNO donor is more toxic to neuroblastoma cells at pH 6.2 than at pH 7.4 (70). Likewise, HNO donor has

powerful toxicity to cancer cells in weakly acidified solutions, and this is prevented by scavenging hydroxyl radical (143). These results provide the evidence that HNO may be sensitive to cancer cells with an acidic intra- or extracellular microenvironment. Taking advantage of its pH-sensitive characteristic, low-frequency EPRS is capable of measuring tumor pH using appropriate exogenous free radical probes, such as HNO (89). In addition to redox sensitivity, a pH-sensitive HNO radical is recently developed as an exogenous paramagnetic probe in different tumor xenograft mouse models (78).

In summary, a dual pH- and redox-sensitive HNO probe confers unsurpassed opportunities for measurement of tumor microenvironment. With the continuous efforts, it is certain that HNO-based probes will be extensively used to monitor pH and redox status in tumor microenvironment. The measurement of cellular redox status and pH by HNO probes may have important directions for in-depth understanding of tumor diagnosis and treatment.

Role of HNO Donors or Its Derivatives in Different Cancers

HNO has been shown to elicit a variety of pharmacological responses, ranging from treatment of cardiovascular diseases to anticancer actions (Table 1) (145). HNO treatment induces cell death in human lymphocytes, human nonsmall cell lung cancer cells, and human colon cancer cells (6). In a subcutaneous xenograft model of pheochromocytoma, HNO donor AS markedly delays tumor growth (143).

Breast cancer is a complex, heterogeneous disease, and it is also the most cause of cancer death among women around the world (105, 132, 167). Glycolysis is the main energy source for most solid tumors under hypoxic environment (130, 164). Inhibition of glycolysis may be an attractive strategy for cancer therapy (1). GAPDH is critically involved in the glycolytic pathway (79). HNO is found to suppress GAPDH activity, a key glycolytic enzyme (85, 87). Moreover, it has been reported that HNO donors possess the ability to repress the proliferation of human breast cancer cell lines (107). The anticancer activity of HNO is associated with reduced blood vessel density in breast cancer samples from xenografts (107). In this study, Norris *et al.* have demonstrated that inhibition of GAPDH activity by HNO decreases glycolysis and induces subsequent downregulation of HIF-1 α protein expression, thus leading to reduced angiogenesis and tumor growth (107). A recent study has revealed that several genes are uniquely expressed in human breast cancer cells in response to HNO donors, such as miR-21, heat shock protein 70, CSE, and interleukin-24 (25). These findings give new insight into the possible targets by HNO in breast cancer cells. In general, HNO might become a forefront drug to treat breast cancer (Fig. 8).

Apart from the direct effects of HNO donors on cancer cells, HNO donors may serve as adjuvant agents for cancer chemotherapy. As a key component in the DNA repair machinery, the poly (ADP-Ribose) polymerase (PARP) is a main target during chemotherapies and radiation therapies in cancer cells (31, 118). The PARP family plays a pivotal role in diverse cellular processes, and their inhibitors are therapeutic agents in the treatment of breast cancer (53, 75). An interesting study has shown that HNO donors elicit strong

TABLE 1. ROLE OF NITROXYL DONORS OR ITS DERIVATIVES IN DIFFERENT TUMORS

<i>HNO donors or its derivatives</i>	<i>Cancer types</i>	<i>Biological activities</i>	<i>References</i>
AS	Human nonsmall cell lung cancer cells A549	HNO induced both apoptotic and necrotic cell death in A549 cells.	(6)
AS	Human colon cancer cells HT29	HNO induced both apoptotic and necrotic cell death in HT29 cells.	(6)
Alicyclic amine diazeniumdiolates	Human MCF-7 cancer cells	Diazeniumdiolate-based HNO donors have been shown to produce HNO and exhibit high cytotoxicity toward human MCF-7 cancer cells.	(16)
Platinum-HNO complex	RT112 bladder cancer cells	This complex is a potential candidate to circumvent cisplatin resistance in RT112 bladder cancer cells.	(24)
IPA/NO	Human breast cancer cell lines MDA-MB-231 cells	After incubation of IPA/NO in MDA-MB-231 cells, several genes were uniquely expressed, such as miR-21, HSP70, CSE, and IL-24. These findings give new insight into targets that could be therapeutically targeted by HNO in breast cancer cells.	(25)
TEMPOL	Human MCF-7 cells	TEMPOL was able to induce cell damage; this may be associated with HNO reactivity.	(51)
L-NNP	Human liver cancer cells HepG-2	L-NNP triggers oxidative stress-dependent cell apoptosis in HepG2 liver cancer cells	(59)
Spin-labeled combretastatin A-4 derivative	Human lung cancer cells A-549; human liver cancer cells HepG-2	The compound conferred cytotoxic activities against human lung cancer cells and human liver cancer cells.	(81)
AS	Human breast cancer cell lines MDA-MB-231 and MCF-7 cells; mouse tumor model	HNO dose dependently inhibited the proliferation of human breast cancer cell lines <i>in vitro</i> , HNO treatment reduced xenograft tumor size and decreased blood vessel density in mouse tumor models. Inhibition of GAPDH activity by HNO decreases glycolysis and induces subsequent down-regulation of HIF-1 α protein expression, thus leading to inhibition of angiogenesis and tumor growth.	(107)
Diazeniumdiolate	Ovarian cancer cells (ES2 and SKOV3); AML monocyte-derived cancer cells (THP-1)	Diazeniumdiolates exert antiproliferative effects on ovarian cancer cells and AML monocyte-derived cancer cells.	(116)
AS	Human breast cancer cell lines MCF-7 cells	HNO donors elicit strong PARP inhibition in intact human MCF-7 cancer cells presumably <i>via</i> impairment of the zinc-finger domains.	(136)
Ruboxyl	Experimental models of colorectal cancer	Ruboxyl reduced the development of liver metastases in experimental models of colorectal cancer.	(138)
AS	Pheochromocytoma	HNO markedly inhibited tumor growth at a concentration that was nontoxic to nude mice.	(143)
HNO derivative pirolin	A rat mammary carcinoma model	A HNO derivative pirolin decreases the tumor volume in a rat mammary carcinoma model.	(148)
HNO-labeled derivatives of podophyllotoxin 4–6	Human nasopharyngeal carcinoma, lung cancer A549, and stomach carcinoma SGC-7901 cells	HNO-labeled derivatives of podophyllotoxin 4–6 inhibit the growth of these cancer cells.	(152, 165)
HNO spin-labeled ester derivatives of podophyllotoxin	Neoplastic cell lines including K562, HL-60, SPCA-1, Lewis, and L-1210 cells	The compound exhibited cytotoxicity against these five neoplastic cell lines.	(172)

AML, acute myeloid leukemia; AS, Angeli's salt; HIF-1 α , hypoxia-inducible factor-1 α ; HNO, nitroxyl; HSP70, heat shock protein 70; IL, interleukin; IPA/NO, isopropylamine NONOate; L-NNP, tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-*O*-1*H*-imidazole-3-cationic-1-oxyl-2)-pyrrolidine-1-carboxylic ester; PARP, poly (ADP-ribose) polymerase; TEMPOL, nitroxide 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl.

FIG. 8. Effect of HNO on breast cancer cells. HNO inhibited GAPDH-mediated glycolysis (85, 87), retarded the HIF-1 α and VEGF protein expression (107), thus resulting in angiogenesis inhibition. HNO is also found to suppress PARP activity (136); this might improve the efficacy of the chemotherapies and radiation therapies. Moreover, in HNO-treated human breast cancer cells, the expression of CSE, TXNIP, and IL-24 was downregulated, while the miR-21 and HSP70 levels were upregulated, all of which may synergistically contribute to its anticancer effects (25). HIF-1 α , hypoxia-inducible factor-1 α ; IL, interleukin; PARP, poly (ADP-ribose) polymerase; VEGF, vascular endothelial growth factor. Color images are available online.

PARP inhibition in human MCF-7 cancer cells presumably *via* impairment of the zinc-finger domains (136), suggesting that inhibition of PARP by HNO might be an effective therapy in breast cancer. Blockade of PARP by HNO might improve the clinical efficacy of chemotherapies and radiation therapies. This hypothesis is awaiting further research.

As reported earlier, three novel HNO-labeled derivatives of podophyllotoxin 4–6 significantly inhibit the growth of human nasopharyngeal carcinoma cells, lung cancer cells, and stomach carcinoma cells (152, 165). A HNO derivative pirolin decreases the tumor volume in a rat mammary carcinoma model; this effect may be mediated by upregulation of the GSH pool and downregulation of hydroperoxide levels (148). In bladder cancer cells, a newly synthesized PNC confers antitumor effect (24). Alicyclic amine diazeniumdiolates have been shown to produce HNO and decrease the viability of human MCF-7 cancer cells (16). A novel spin-labeled combretastatin A-4 derivative from reaction of CA-4 with the HNO radical exhibits more cytotoxic activities against human lung cancer cells and human liver cancer cells than the clinical drug irinotecan (81). In human MCF-7 cells, the stable nitroxide 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxy is able to induce cell damage; this may be associated

with HNO reactivity (51). Intraperitoneal injections ruboxyl, a HNO analog of daunorubicin, and 5-fluorouracil reduces the development of liver metastases in experimental mouse models of colorectal cancer (138). It has been found that diazeniumdiolate-based HNO-releasing aspirin exhibits enhanced cytotoxicity toward nonsmall cell lung carcinoma cells but not endothelial cells (13), suggesting cancer-specific sensitivity. In similarity, this prodrug inhibits the growth of breast adenocarcinoma cells, while it is not cytotoxic to a related nontumorigenic cell line (MCF-10A) (14). These results indicate a therapeutic potential of this compound for breast cancer.

MTT assay results have demonstrated that both HNO/NO donor, diazeniumdiolate, exert antiproliferative effects on ovarian cancer cells and acute myeloid leukemia monocyte-derived cancer cells (116). As discussed above, L-NNP is a stable HNO radical, and L-NNP triggers oxidative stress-dependent cell apoptosis in HepG2 liver cancer cells (59). Thus, it might be developed as a novel drug for the treatment of liver cancer. These above observations indicate that HNO derivatives also possess promise in the realm of cancer therapy, especially for a wide coverage of cancer cell types. However, it is vital to fully consider the possible toxic actions of supraphysiological levels of HNO derivatives, and great care must be taken regarding the development of HNO derivatives-based therapeutic agents.

HNO is thought to be a pharmacologically important agent due to its potential anticancer characteristics. However, future clinical studies are required to evaluate whether HNO is a valuable alternative to currently known anticancer drugs.

Future Directions for HNO Research in the Cancer Arena

As a redox congener, HNO exhibits specific and diverse biological effects. At this juncture, targeting the enzymes containing thiols by HNO can regulate DNA repair, apoptosis, and glycolysis in tumor progression. Given the complicated reactions of HNO with GSH, metalloproteins, and other scavengers, additional studies are needed to further clarify the underlying mechanisms, thus providing the opportunity to use HNO donors as pharmacological drugs for cancer treatment.

The most striking gap in the HNO field is that the transformation of HNO donors as tumor treatment drugs in both animals and patients is almost completely unknown. It is particularly worth noting that the pharmacology and potential toxicology of HNO should be also examined in the future studies. Moreover, little is known about the metabolic pathway of HNO in cancer cells. Therefore, to fully understand the effect of HNO on tumor biology, it will be prerequisite to thoroughly discover where all these “keys” are located.

In mammalian system, if endogenously generated HNO could be specifically detected, and the expression of HNO has cancer-specific characteristics, this may greatly expand the biochemical and pharmacological utilities of HNO in cancer research. However, it is still unknown whether endogenous HNO levels are altered under pathological conditions, particularly in the development and progression of cancer. With the rapid development of specific, sensitive, and biologically compatible HNO probes, the production and metabolism of endogenous HNO in cancer cells may become an overwhelmingly important aspect of cancer pharmacology. Excepting for this,

the unique chemical property of HNO makes it a useful probe in the cancer diagnosis. It will be interesting to know whether HNO could be a potential biomarker or target for the diagnosis and treatment of different cancers.

Concluding Remarks

To date, the unique chemical and biological activities of HNO in cancer system have still been incompletely understood; despite HNO donors possess therapeutic potential to prevent different cancers. Future work in cancer biochemistry/pharmacology of HNO will focus on several urgent areas: (i) Is HNO an endogenously produced molecule in the cancer development? To address this issue, it is indispensable to develop novel approaches for HNO detection in biological systems, thus, confirming its endogenous generation. (ii) If as an endogenous signaling molecule or a pharmacological drug, how does HNO exhibit signaling specificity regarding its actions? It is obvious that more research is required to clarify the regulation of HNO generation, signaling, and metabolism, especially under pathological cancer conditions. More original work in this field will uncover the new characteristics and therapeutic applications of HNO in cancer field.

Taken together, with a deeper understanding of the chemical, biological, and pharmacological characteristics, as well as the underlying molecular mechanisms with respect to the roles of HNO in cancer prevention and treatment, the appropriate dose and time frame of HNO donors could be critical for future preclinical and clinical research.

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References

1. Akram M. Mini-review on glycolysis and cancer. *J Cancer Educ* 28: 454–457, 2013.
2. Anderson M, Moshnikova A, Engelman DM, Reshetnyak YK, and Andreev OA. Probe for the measurement of cell surface pH in vivo and ex vivo. *Proc Natl Acad Sci U S A* 113: 8177–8181, 2016.
3. Andrews KL, Irvine JC, Tare M, Apostolopoulos J, Favalaro JL, Triggie CR, and Kemp-Harper BK. A role for nitroxyl (HNO) as an endothelium-derived relaxing and hyperpolarizing factor in resistance arteries. *Br J Pharmacol* 157: 540–550, 2009.
4. Aoki I. [Nano-theranostics and nitroxyl radical-labeled antitumor agents for magnetic resonance imaging]. *Yakugaku Zasshi* 136: 1087–1091, 2016 (Article in Japanese).
5. Arion VB, Dobrov A, Goschl S, Jakupec MA, Keppler BK, and Rapt A. Ruthenium- and osmium-arene-based paulones bearing a TEMPO free-radical unit as potential anticancer drugs. *Chem Commun (Camb)* 48: 8559–8561, 2012.
6. Augustyniak A, Skolimowski J, and Blaszczak A. Cytotoxicity of nitroxyl (HNO/NO⁻) against normal and cancer human cells. *Chem Biol Interact* 206: 262–271, 2013.
7. Auten RL and Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res* 66: 121–127, 2009.
8. Azad MB, Chen Y, and Gibson SB. Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment. *Antioxid Redox Signal* 11: 777–790, 2009.
9. Bacic G, Nilges MJ, Magin RL, Walczak T, and Swartz HM. In vivo localized ESR spectroscopy reflecting metabolism. *Magn Reson Med* 10: 266–272, 1989.
10. Bakalova R, Zhelev Z, Aoki I, and Saga T. Tissue redox activity as a hallmark of carcinogenesis: from early to terminal stages of cancer. *Clin Cancer Res* 19: 2503–2517, 2013.
11. Bartberger MD, Fukuto JM, and Houk KN. On the acidity and reactivity of HNO in aqueous solution and biological systems. *Proc Natl Acad Sci U S A* 98: 2194–2198, 2001.
12. Bartberger MD, Liu W, Ford E, Miranda KM, Switzer C, Fukuto JM, Farmer PJ, Wink DA, and Houk KN. The reduction potential of nitric oxide (NO) and its importance to NO biochemistry. *Proc Natl Acad Sci U S A* 99: 10958–10963, 2002.
13. Basudhar D, Bharadwaj G, Cheng RY, Jain S, Shi S, Heinecke JL, Holland RJ, Ridnour LA, Caceres VM, Spadari-Bratfisch RC, Paolucci N, Velazquez-Martinez CA, Wink DA, and Miranda KM. Synthesis and chemical and biological comparison of nitroxyl- and nitric oxide-releasing diazeniumdiolate-based aspirin derivatives. *J Med Chem* 56: 7804–7820, 2013.
14. Basudhar D, Cheng RC, Bharadwaj G, Ridnour LA, Wink DA, and Miranda KM. Chemotherapeutic potential of diazeniumdiolate-based aspirin prodrugs in breast cancer. *Free Radic Biol Med* 83: 101–114, 2015.
15. Bermejo E, Saenz DA, Alberto F, Rosenstein RE, Bari SE, and Lazzari MA. Effect of nitroxyl on human platelets function. *Thromb Haemost* 94: 578–584, 2005.
16. Bharadwaj G, Benini PG, Basudhar D, Ramos-Colon CN, Johnson GM, Larriva MM, Keefer LK, Andrei D, and Miranda KM. Analysis of the HNO and NO donating properties of alicyclic amine diazeniumdiolates. *Nitric Oxide* 42: 70–78, 2014.
17. Bobko AA, Eubank TD, Driesschaert B, and Khramtsov VV. In vivo EPR assessment of pH, pO₂, redox status, and concentrations of phosphate and glutathione in the tumor microenvironment. *J Vis Exp* [Epub ahead of print]; DOI: 10.3791/56624, 2018.
18. Bobko AA, Eubank TD, Voorhees JL, Efimova OV, Kirilyuk IA, Petryakov S, Trofimov DG, Marsh CB, Zweier JL, Grigor'ev IA, Samouilov A, and Khramtsov VV. In vivo monitoring of pH, redox status, and glutathione using L-band EPR for assessment of therapeutic effectiveness in solid tumors. *Magn Reson Med* 67: 1827–1836, 2012.
19. Bullen ML, Miller AA, Andrews KL, Irvine JC, Ritchie RH, Sobey CG, and Kemp-Harper BK. Nitroxyl (HNO) as a vasoprotective signaling molecule. *Antioxid Redox Signal* 14: 1675–1686, 2011.
20. Cabello CM, Bair WB, 3rd, and Wondrak GT. Experimental therapeutics: targeting the redox Achilles heel of cancer. *Curr Opin Investig Drugs* 8: 1022–1037, 2007.
21. Cao X and Bian JS. The role of hydrogen sulfide in renal system. *Front Pharmacol* 7: 385, 2016.
22. Cao X, Ding L, Xie ZZ, Yang Y, Whiteman M, Moore PK, and Bian JS. A review of hydrogen sulfide synthesis, metabolism, and measurement: is modulation of hydrogen sulfide a novel therapeutic for cancer? *Antioxid Redox Signal* 31: 1–38, 2019.

23. Cao X, Zhang W, Moore PK, and Bian J. Protective smell of hydrogen sulfide and polysulfide in cisplatin-induced nephrotoxicity. *Int J Mol Sci* 20: pii:E313, 2019.
24. Cetraz M, Sen V, Schoch S, Streule K, Golubev V, Hartwig A, and Koberle B. Platinum(IV)-nitroxyl complexes as possible candidates to circumvent cisplatin resistance in RT112 bladder cancer cells. *Arch Toxicol* 91: 785–797, 2017.
25. Cheng RY, Basudhar D, Ridnour LA, Heinecke JL, Kesarwala AH, Glynn S, Switzer CH, Ambs S, Miranda KM, and Wink DA. Gene expression profiles of NO- and HNO-donor treated breast cancer cells: insights into tumor response and resistance pathways. *Nitric Oxide* 43: 17–28, 2014.
26. Cheong E, Tumbey V, Abramson J, Salama G, and Stoyanovsky DA. Nitroxyl triggers Ca²⁺ release from skeletal and cardiac sarcoplasmic reticulum by oxidizing ryanodine receptors. *Cell Calcium* 37: 87–96, 2005.
27. Chin KY, Michel L, Qin CX, Cao N, Woodman OL, and Ritchie RH. The HNO donor Angeli's salt offers potential haemodynamic advantages over NO or dobutamine in ischaemia-reperfusion injury in the rat heart ex vivo. *Pharmacol Res* 104: 165–175, 2016.
28. Cho JY, Dutton A, Miller T, Houk KN, and Fukuto JM. Oxidation of *N*-hydroxyguanidines by copper(II): model systems for elucidating the physiological chemistry of the nitric oxide biosynthetic intermediate *N*-hydroxyl-L-arginine. *Arch Biochem Biophys* 417: 65–76, 2003.
29. Chonpathompikunlert P, Yoshitomi T, Han J, Toh K, Isoda H, and Nagasaki Y. Chemical nanotherapy: nitroxyl radical-containing nanoparticle protects neuroblastoma SH-SY5Y cells from Abeta-induced oxidative stress. *Ther Deliv* 2: 585–597, 2011.
30. Cortese-Krott MM, Kuhnle GG, Dyson A, Fernandez BO, Grman H, DuMond JF, Barrow MP, McLeod G, Nakagawa H, Ondrias K, Nagy P, King SB, Saavedra JE, Keefer LK, Singer M, Kelm M, Butler AR, and Feelisch M. Key bioactive reaction products of the NO/H₂S interaction are S/N-hybrid species, polysulfides, and nitroxyl. *Proc Natl Acad Sci U S A* 112: E4651–E4660, 2015.
31. D'Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. *DNA Repair (Amst)* 71: 172–176, 2018.
32. Dai T, Tian Y, Tocchetti CG, Katori T, Murphy AM, Kass DA, Paolucci N, and Gao WD. Nitroxyl increases force development in rat cardiac muscle. *J Physiol* 580: 951–960, 2007.
33. Davis RM, Matsumoto S, Bernardo M, Sowers A, Matsumoto K, Krishna MC, and Mitchell JB. Magnetic resonance imaging of organic contrast agents in mice: capturing the whole-body redox landscape. *Free Radic Biol Med* 50: 459–468, 2011.
34. DeMaster EG, Redfern B, and Nagasawa HT. Mechanisms of inhibition of aldehyde dehydrogenase by nitroxyl, the active metabolite of the alcohol deterrent agent cyanamide. *Biochem Pharmacol* 55: 2007–2015, 1998.
35. DeMaster EG, Shirota FN, and Nagasawa HT. Catalase mediated conversion of cyanamide to an inhibitor of aldehyde dehydrogenase. *Alcohol* 2: 117–121, 1985.
36. Donzelli S, Espey MG, Flores-Santana W, Switzer CH, Yeh GC, Huang J, Stuehr DJ, King SB, Miranda KM, and Wink DA. Generation of nitroxyl by heme protein-mediated peroxidation of hydroxylamine but not *N*-hydroxy-L-arginine. *Free Radic Biol Med* 45: 578–584, 2008.
37. Donzelli S, Espey MG, Thomas DD, Mancardi D, Tocchetti CG, Ridnour LA, Paolucci N, King SB, Miranda KM, Lazzarino G, Fukuto JM, and Wink DA. Discriminating formation of HNO from other reactive nitrogen oxide species. *Free Radic Biol Med* 40: 1056–1066, 2006.
38. Dutton AS, Fukuto JM, and Houk KN. Mechanisms of HNO and NO production from Angeli's salt: density functional and CBS-QB3 theory predictions. *J Am Chem Soc* 126: 3795–3800, 2004.
39. Dutton AS, Suhrada CP, Miranda KM, Wink DA, Fukuto JM, and Houk KN. Mechanism of pH-dependent decomposition of monoalkylamine diazeniumdiolates to form HNO and NO, deduced from the model compound methylamine diazeniumdiolate, density functional theory, and CBS-QB3 calculations. *Inorg Chem* 45: 2448–2456, 2006.
40. Espey MG, Miranda KM, Thomas DD, and Wink DA. Ingress and reactive chemistry of nitroxyl-derived species within human cells. *Free Radic Biol Med* 33: 827–834, 2002.
41. Fang J, Seki T, and Maeda H. Therapeutic strategies by modulating oxygen stress in cancer and inflammation. *Adv Drug Deliv Rev* 61: 290–302, 2009.
42. Fedeli D, Damiani E, Greci L, Littarru GP, and Falcioni G. Nitroxide radicals protect against DNA damage in rat epithelial cells induced by nitric oxide, nitroxyl anion and peroxynitrite. *Mutat Res* 535: 117–125, 2003.
43. Fukuto JM. A recent history of nitroxyl chemistry, pharmacology and therapeutic potential. *Br J Pharmacol* 176: 135–146, 2019.
44. Fukuto JM, Bartberger MD, Dutton AS, Paolucci N, Wink DA, and Houk KN. The physiological chemistry and biological activity of nitroxyl (HNO): the neglected, misunderstood, and enigmatic nitrogen oxide. *Chem Res Toxicol* 18: 790–801, 2005.
45. Fukuto JM and Carrington SJ. HNO signaling mechanisms. *Antioxid Redox Signal* 14: 1649–1657, 2011.
46. Fukuto JM, Chiang K, Hszieh R, Wong P, and Chaudhuri G. The pharmacological activity of nitroxyl: a potent vasodilator with activity similar to nitric oxide and/or endothelium-derived relaxing factor. *J Pharmacol Exp Ther* 263: 546–551, 1992.
47. Fukuto JM, Dutton AS, and Houk KN. The chemistry and biology of nitroxyl (HNO): a chemically unique species with novel and important biological activity. *ChemBiochem* 6: 612–619, 2005.
48. Fukuto JM, Switzer CH, Miranda KM, and Wink DA. Nitroxyl (HNO): chemistry, biochemistry, and pharmacology. *Annu Rev Pharmacol Toxicol* 45: 335–355, 2005.
49. Gallez B, Baudalet C, and Jordan BF. Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications. *NMR Biomed* 17: 240–262, 2004.
50. Gao WD, Murray CI, Tian Y, Zhong X, DuMond JF, Shen X, Stanley BA, Foster DB, Wink DA, King SB, Van Eyk JE, and Paolucci N. Nitroxyl-mediated disulfide bond formation between cardiac myofilament cysteines enhances contractile function. *Circ Res* 111: 1002–1011, 2012.
51. Gariboldi MB, Lucchi S, Caserini C, Supino R, Oliva C, and Monti E. Antiproliferative effect of the piperidine nitroxide TEMPOL on neoplastic and nonneoplastic mammalian cell lines. *Free Radic Biol Med* 24: 913–923, 1998.
52. Ge Y and Moss RL. Nitroxyl, redox switches, cardiac myofilaments, and heart failure: a prequel to novel therapeutics? *Circ Res* 111: 954–956, 2012.

53. Geenen JJJ, Linn SC, Beijnen JH, and Schellens JHM. PARP inhibitors in the treatment of triple-negative breast cancer. *Clin Pharmacokinet* 57: 427–437, 2018.
54. Gerweck LE. Tumor pH: implications for treatment and novel drug design. *Semin Radiat Oncol* 8: 176–182, 1998.
55. Giles GI. The redox regulation of thiol dependent signaling pathways in cancer. *Curr Pharm Des* 12: 4427–4443, 2006.
56. Gillies RJ and Lynch RM. Frontiers in the measurement of cell and tissue pH. *Novartis Found Symp* 240: 7–19; discussion 19–22, 152–153, 2001.
57. Glasauer A and Chandel NS. Targeting antioxidants for cancer therapy. *Biochem Pharmacol* 92: 90–101, 2014.
58. Gorrini C, Harris IS, and Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov* 12: 931–947, 2013.
59. Guo J, Zhang Y, Zhang J, Liang J, Zeng L, and Guo G. Anticancer effect of tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-O-1H-imidazole-3-cationic-1-oxy 1-2)-pyrrolidine-1-carboxylic ester on human hepatoma HepG2 cell line. *Chem Biol Interact* 199: 38–48, 2012.
60. Hammond AH and Fry JR. Effect of cyanamide on toxicity and glutathione depletion in rat hepatocyte cultures: differences between two dichloropropanol isomers. *Chem Biol Interact* 122: 107–115, 1999.
61. Hewett SJ, Espey MG, Uliasz TF, and Wink DA. Neurotoxicity of nitroxyl: insights into HNO and NO biochemical imbalance. *Free Radic Biol Med* 39: 1478–1488, 2005.
62. Hirst DG and Robson T. Nitrosative stress as a mediator of apoptosis: implications for cancer therapy. *Curr Pharm Des* 16: 45–55, 2010.
63. Huber WW and Parzefall W. Thiols and the chemoprevention of cancer. *Curr Opin Pharmacol* 7: 404–409, 2007.
64. Huerta S. Nitric oxide for cancer therapy. *Future Sci OA* 1: FSO44, 2015.
65. Hyodo F, Soule BP, Matsumoto K, Matusmoto S, Cook JA, Hyodo E, Sowers AL, Krishna MC, and Mitchell JB. Assessment of tissue redox status using metabolic responsive contrast agents and magnetic resonance imaging. *J Pharm Pharmacol* 60: 1049–1060, 2008.
66. Ilangovan G, Li H, Zweier JL, and Kuppusamy P. In vivo measurement of tumor redox environment using EPR spectroscopy. *Mol Cell Biochem* 234–235: 393–398, 2002.
67. Irvine JC, Cao N, Gossain S, Alexander AE, Love JE, Qin C, Horowitz JD, Kemp-Harper BK, and Ritchie RH. HNO/cGMP-dependent antihypertrophic actions of isopropylamine-NONOate in neonatal rat cardiomyocytes: potential therapeutic advantages of HNO over NO. *Am J Physiol Heart Circ Physiol* 305: H365–H377, 2013.
68. Irvine JC, Favalaro JL, and Kemp-Harper BK. NO- activates soluble guanylate cyclase and Kv channels to vasodilate resistance arteries. *Hypertension* 41: 1301–1307, 2003.
69. Irvine JC, Ritchie RH, Favalaro JL, Andrews KL, Widdop RE, and Kemp-Harper BK. Nitroxyl (HNO): the Cinderella of the nitric oxide story. *Trends Pharmacol Sci* 29: 601–608, 2008.
70. Ivanova J, Salama G, Clancy RM, Schor NF, Nylander KD, and Stoyanovsky DA. Formation of nitroxyl and hydroxyl radical in solutions of sodium triiodonitrate: effects of pH and cytotoxicity. *J Biol Chem* 278: 42761–42768, 2003.
71. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal* 8: 1865–1879, 2006.
72. Keceli G, Majumdar A, and Thorpe CN. Nitroxyl (HNO) targets phospholamban cysteines 41 and 46 to enhance cardiac function. *J Gen Physiol* 151:758–770, 2019.
73. Kee Z, Kodji X, and Brain SD. The role of calcitonin gene related peptide (CGRP) in neurogenic vasodilation and its cardioprotective effects. *Front Physiol* 9: 1249, 2018.
74. Keshet R and Erez A. Arginine and the metabolic regulation of nitric oxide synthesis in cancer. *Dis Model Mech* 11: pii:dmm033332, 2018.
75. Keung MYT, Wu Y, and Vadgama JV. PARP inhibitors as a therapeutic agent for homologous recombination deficiency in breast cancers. *J Clin Med* 8: pii: E435, 2019.
76. Kimura H. Hydrogen sulfide: its production, release and functions. *Amino Acids* 41: 113–121, 2011.
77. Kishimoto S, Matsumoto KI, Saito K, Enomoto A, Matsumoto S, Mitchell JB, Devasahayam N, and Krishna MC. Pulsed electron paramagnetic resonance imaging: applications in the studies of tumor physiology. *Antioxid Redox Signal* 28: 1378–1393, 2018.
78. Komarov DA, Ichikawa Y, Yamamoto K, Stewart NJ, Matsumoto S, Yasui H, Kirilyuk IA, and Khramtsov VV. In vivo extracellular pH mapping of tumors using electron paramagnetic resonance. *Anal Chem* 90: 13938–13945, 2018.
79. Krasnov GS, Dmitriev AA, Snezhkina AV, and Kudryavtseva AV. Deregulation of glycolysis in cancer: glyceraldehyde-3-phosphate dehydrogenase as a therapeutic target. *Expert Opin Ther Targets* 17: 681–693, 2013.
80. Li JB, Wang Q, Liu HW, Yin X, Hu XX, Yuan L, and Zhang XB. Engineering of a bioluminescent probe for imaging nitroxyl in live cells and mice. *Chem Commun (Camb)* 55: 1758–1761, 2019.
81. Li XJ, Yang L, Liu YQ, and Li C. Synthesis of novel spin-labelled combretastatin A-4 derivatives as potential anti-neoplastic agents. *Nat Prod Res* 26: 1271–1277, 2012.
82. Liao W and Ye T. Prognostic value of inducible nitric oxide synthase (iNOS) in human cancer: a systematic review and meta-analysis. *Biomed Res Int* 2019: 6304851, 2019.
83. Lin EQ, Irvine JC, Cao AH, Alexander AE, Love JE, Patel R, McMullen JR, Kaye DM, Kemp-Harper BK, and Ritchie RH. Nitroxyl (HNO) stimulates soluble guanylyl cyclase to suppress cardiomyocyte hypertrophy and superoxide generation. *PLoS One* 7: e34892, 2012.
84. Liochev SI and Fridovich I. The mode of decomposition of Angeli's salt ($\text{Na}_2\text{N}_2\text{O}_3$) and the effects thereon of oxygen, nitrite, superoxide dismutase, and glutathione. *Free Radic Biol Med* 34: 1399–1404, 2003.
85. Lopez BE, Rodriguez CE, Pribadi M, Cook NM, Shinyashiki M, and Fukuto JM. Inhibition of yeast glycolysis by nitroxyl (HNO): mechanism of HNO toxicity and implications to HNO biology. *Arch Biochem Biophys* 442: 140–148, 2005.
86. Lopez BE, Shinyashiki M, Han TH, and Fukuto JM. Antioxidant actions of nitroxyl (HNO). *Free Radic Biol Med* 42: 482–491, 2007.
87. Lopez BE, Wink DA, and Fukuto JM. The inhibition of glyceraldehyde-3-phosphate dehydrogenase by nitroxyl (HNO). *Arch Biochem Biophys* 465: 430–436, 2007.
88. Magder S. Reactive oxygen species: toxic molecules or spark of life? *Crit Care* 10: 208, 2006.
89. Matsumoto K, Hyodo F, Matsumoto A, Koretsky AP, Sowers AL, Mitchell JB, and Krishna MC. High-resolution mapping of tumor redox status by magnetic

- resonance imaging using nitroxides as redox-sensitive contrast agents. *Clin Cancer Res* 12: 2455–2462, 2006.
90. Matsumoto K, Krishna MC, and Mitchell JB. Novel pharmacokinetic measurement using electron paramagnetic resonance spectroscopy and simulation of in vivo decay of various nitroxyl spin probes in mouse blood. *J Pharmacol Exp Ther* 310: 1076–1083, 2004.
 91. Matsumoto KI, Mitchell JB, and Krishna MC. Comparative studies with EPR and MRI on the in vivo tissue redox status estimation using redox-sensitive nitroxyl probes: influence of the choice of the region of interest. *Free Radic Res* 52: 248–255, 2018.
 92. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacol Ther* 51: 155–194, 1991.
 93. Miao Z and King SB. Recent advances in the chemical biology of nitroxyl (HNO) detection and generation. *Nitric Oxide* 57: 1–14, 2016.
 94. Miljkovic J, Kenkel I, Ivanovic-Burmazovic I, and Filipovic MR. Generation of HNO and HSNO from nitrite by heme-iron-catalyzed metabolism with H(2)S. *Angew Chem Int Ed Engl* 52: 12061–12064, 2013.
 95. Miller TW, Cherney MM, Lee AJ, Francoleon NE, Farmer PJ, King SB, Hobbs AJ, Miranda KM, Burstyn JN, and Fukuto JM. The effects of nitroxyl (HNO) on soluble guanylate cyclase activity: interactions at ferrous heme and cysteine thiols. *J Biol Chem* 284: 21788–21796, 2009.
 96. Miranda KM, Dutton AS, Ridnour LA, Foreman CA, Ford E, Paolucci N, Katori T, Tocchetti CG, Mancardi D, Thomas DD, Espey MG, Houk KN, Fukuto JM, and Wink DA. Mechanism of aerobic decomposition of Angeli's salt (sodium trioxodinitrate) at physiological pH. *J Am Chem Soc* 127: 722–731, 2005.
 97. Miranda KM, Katori T, Torres de Holding CL, Thomas L, Ridnour LA, McLendon WJ, Cologna SM, Dutton AS, Champion HC, Mancardi D, Tocchetti CG, Saavedra JE, Keefer LK, Houk KN, Fukuto JM, Kass DA, Paolucci N, and Wink DA. Comparison of the NO and HNO donating properties of diazeniumdiolates: primary amine adducts release HNO in vivo. *J Med Chem* 48: 8220–8228, 2005.
 98. Miranda KM, Nagasawa HT, and Toscano JP. Donors of HNO. *Curr Top Med Chem* 5: 649–664, 2005.
 99. Miranda KM, Nims RW, Thomas DD, Espey MG, Citrin D, Bartberger MD, Paolucci N, Fukuto JM, Feelisch M, and Wink DA. Comparison of the reactivity of nitric oxide and nitroxyl with heme proteins. A chemical discussion of the differential biological effects of these redox related products of NOS. *J Inorg Biochem* 93: 52–60, 2003.
 100. Miranda KM, Paolucci N, Katori T, Thomas DD, Ford E, Bartberger MD, Espey MG, Kass DA, Feelisch M, Fukuto JM, and Wink DA. A biochemical rationale for the discrete behavior of nitroxyl and nitric oxide in the cardiovascular system. *Proc Natl Acad Sci U S A* 100: 9196–9201, 2003.
 101. Mitroka S, Shoman ME, DuMond JF, Bellavia L, Aly OM, Abdel-Aziz M, Kim-Shapiro DB, and King SB. Direct and nitroxyl (HNO)-mediated reactions of acyloxy nitroso compounds with the thiol-containing proteins glyceraldehyde 3-phosphate dehydrogenase and alkyl hydroperoxide reductase subunit C. *J Med Chem* 56: 6583–6592, 2013.
 102. Moloney JN and Cotter TG. ROS signalling in the biology of cancer. *Semin Cell Dev Biol* 80: 50–64, 2018.
 103. Montero AJ and Jassem J. Cellular redox pathways as a therapeutic target in the treatment of cancer. *Drugs* 71: 1385–1396, 2011.
 104. Nagasawa HT, Lee MJ, Kwon CH, Shirota FN, and DeMaster EG. An N-hydroxylated derivative of cyanamide that inhibits yeast aldehyde dehydrogenase. *Alcohol* 9: 349–353, 1992.
 105. Nagini S. Breast cancer: current molecular therapeutic targets and new players. *Anticancer Agents Med Chem* 17: 152–163, 2017.
 106. Naughton P, Hoque M, Green CJ, Foresti R, and Motterlini R. Interaction of heme with nitroxyl or nitric oxide amplifies heme oxygenase-1 induction: involvement of the transcription factor Nrf2. *Cell Mol Biol (Noisy-le-grand)* 48: 885–894, 2002.
 107. Norris AJ, Sartippour MR, Lu M, Park T, Rao JY, Jackson MI, Fukuto JM, and Brooks MN. Nitroxyl inhibits breast tumor growth and angiogenesis. *Int J Cancer* 122: 1905–1910, 2008.
 108. Offer T, Russo A, and Samuni A. The pro-oxidative activity of SOD and nitroxide SOD mimics. *FASEB J* 14: 1215–1223, 2000.
 109. Ohshima H, Glibert I, and Bianchini F. Induction of DNA strand breakage and base oxidation by nitroxyl anion through hydroxyl radical production. *Free Radic Biol Med* 26: 1305–1313, 1999.
 110. Oliveira C, Benfeito S, Fernandes C, Cagide F, Silva T, and Borges F. NO and HNO donors, nitrones, and nitroxides: past, present, and future. *Med Res Rev* 38: 1159–1187, 2018.
 111. Olson KR. Hydrogen sulfide as an oxygen sensor. *Antioxid Redox Signal* 22: 377–397, 2015.
 112. Paolucci N, Jackson MI, Lopez BE, Miranda K, Tocchetti CG, Wink DA, Hobbs AJ, and Fukuto JM. The pharmacology of nitroxyl (HNO) and its therapeutic potential: not just the Janus face of NO. *Pharmacol Ther* 113: 442–458, 2007.
 113. Paolucci N, Katori T, Champion HC, St John ME, Miranda KM, Fukuto JM, Wink DA, and Kass DA. Positive inotropic and lusitropic effects of HNO/NO- in failing hearts: independence from beta-adrenergic signaling. *Proc Natl Acad Sci U S A* 100: 5537–5542, 2003.
 114. Paolucci N, Saavedra WF, Miranda KM, Martignani C, Isoda T, Hare JM, Espey MG, Fukuto JM, Feelisch M, Wink DA, and Kass DA. Nitroxyl anion exerts redox-sensitive positive cardiac inotropy in vivo by calcitonin gene-related peptide signaling. *Proc Natl Acad Sci U S A* 98: 10463–10468, 2001.
 115. Parissis J, Bistola V, Ikonomidis I, and Triposkiadis F. Nitroxyl donors for acute heart failure: promising newcomers. *Eur J Heart Fail* 19: 1333–1334, 2017.
 116. Puglisi MP and Bradaric MJ. Novel primary amine diazeniumdiolates-chemical and biological characterization. *Drug Dev Res* 79: 136–143, 2018.
 117. Pullar JM, Thomson SJ, King MJ, Turnbull CI, Midwinter RG, and Hampton MB. The chemopreventive agent phenethyl isothiocyanate sensitizes cells to Fas-mediated apoptosis. *Carcinogenesis* 25: 765–772, 2004.
 118. Ramakrishnan Geethakumari P, Schiewer MJ, Knudsen KE, and Kelly WK. PARP inhibitors in prostate cancer. *Curr Treat Options Oncol* 18: 37, 2017.
 119. Reisz JA, Bechtold E, and King SB. Oxidative heme protein-mediated nitroxyl (HNO) generation. *Dalton Trans* 39: 5203–5212, 2010.
 120. Ritchie RH, Drummond GR, Sobey CG, De Silva TM, and Kemp-Harper BK. The opposing roles of NO and oxidative stress in cardiovascular disease. *Pharmacol Res* 116: 57–69, 2017.

121. Rogers NM, Seeger F, Garcin ED, Roberts DD, and Isenberg JS. Regulation of soluble guanylate cyclase by extracellular thrombospondins: implications for blood flow. *Front Physiol* 5: 134, 2014.
122. Sabbah HN, Tocchetti CG, Wang M, Daya S, Gupta RC, Tunin RS, Mazhari R, Takimoto E, Paolucci N, Cowart D, Colucci WS, and Kass DA. Nitroxyl (HNO): a novel approach for the acute treatment of heart failure. *Circ Heart Fail* 6: 1250–1258, 2013.
123. Samuni Y, Wink DA, Krishna MC, Mitchell JB, and Goldstein S. Suberoylanilide hydroxamic acid radiosensitizes tumor hypoxic cells in vitro through the oxidation of nitroxyl to nitric oxide. *Free Radic Biol Med* 73: 291–298, 2014.
124. Schieber M and Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol* 24: R453–R462, 2014.
125. Schoch S, Sen V, Gajewski S, Golubev V, Strauch B, Hartwig A, and Koberle B. Activity profile of the cisplatin analogue PN149 in different tumor cell lines. *Biochem Pharmacol* 156: 109–119, 2018.
126. Sen VD, Golubev VA, Volkova LM, and Konovalova NP. Synthesis and antitumor activity of platinum(II) complexes with trans-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl. *J Inorg Biochem* 64: 69–77, 1996.
127. Sha X, Isbell TS, Patel RP, Day CS, and King SB. Hydrolysis of acyloxy nitroso compounds yields nitroxyl (HNO). *J Am Chem Soc* 128: 9687–9692, 2006.
128. Shafirovich V and Lymar SV. Nitroxyl and its anion in aqueous solutions: spin states, protic equilibria, and reactivities toward oxygen and nitric oxide. *Proc Natl Acad Sci U S A* 99: 7340–7345, 2002.
129. Shen B and English AM. Mass spectrometric analysis of nitroxyl-mediated protein modification: comparison of products formed with free and protein-based cysteines. *Biochemistry* 44: 14030–14044, 2005.
130. Shestov AA, Liu X, Ser Z, Cluntun AA, Hung YP, Huang L, Kim D, Le A, Yellen G, Albeck JG, and Locasale JW. Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife* 3: [Epub ahead of print]; DOI: 10.7554/eLife.03342, 2014.
131. Shinde A and Tsai CSJ. Toxicity mechanism in fetal lung fibroblast cells for multi-walled carbon nanotubes defined by chemical impurities and dispersibility. *Toxicol Res* 5: 248–258, 2016.
132. Shinde A, Wilmanski T, Chen H, Teegarden D, and Wendt MK. Pyruvate carboxylase supports the pulmonary tropism of metastatic breast cancer. *Breast Cancer Res* 20: 76, 2018.
133. Shoeman DW, Shiota FN, DeMaster EG, and Nagasawa HT. Reaction of nitroxyl, an aldehyde dehydrogenase inhibitor, with N-acetyl-L-cysteine. *Alcohol* 20: 55–59, 2000.
134. Shoman ME and Aly OM. Nitroxyl (HNO): a possible strategy for fighting cancer. *Curr Top Med Chem* 16: 2464–2470, 2016.
135. Shoman ME, DuMond JF, Isbell TS, Crawford JH, Brandon A, Honovar J, Vitturi DA, White CR, Patel RP, and King SB. Acyloxy nitroso compounds as nitroxyl (HNO) donors: kinetics, reactions with thiols, and vasodilation properties. *J Med Chem* 54: 1059–1070, 2011.
136. Sidorkina O, Espey MG, Miranda KM, Wink DA, and Laval J. Inhibition of poly(ADP-RIBOSE) polymerase (PARP) by nitric oxide and reactive nitrogen oxide species. *Free Radic Biol Med* 35: 1431–1438, 2003.
137. Silva Sousa EH, Ridnour LA, Gouveia FS, Jr., Silva da Silva CD, Wink DA, de Franca Lopes LG, and Sadler PJ. Thiolo-activated HNO release from a ruthenium anti-angiogenesis complex and HIF-1 α inhibition for cancer therapy. *ACS Chem Biol* 11: 2057–2065, 2016.
138. Sirovich I, Konovalova N, Codacci-Pisanelli G, Volkova LM, Giuliani A, Cicconetti F, Seminara P, Mazzocconi G, and Franchi F. Activity of ruboxyl, a nitroxyl derivative of daunorubicin, on experimental models of colorectal cancer metastases. *Tumour Biol* 20: 270–276, 1999.
139. Smulik R, Debski D, Zielonka J, Michalowski B, Adamus J, Marcinek A, Kalyanaraman B, and Sikora A. Nitroxyl (HNO) reacts with molecular oxygen and forms peroxynitrite at physiological pH. Biological implications. *J Biol Chem* 289: 35570–35581, 2014.
140. Smulik-Izydorczyk R, Debowska K, Pieta J, Michalski R, Marcinek A, and Sikora A. Fluorescent probes for the detection of nitroxyl (HNO). *Free Radic Biol Med* 128: 69–83, 2018.
141. Spencer NY, Patel NK, Keszler A, and Hogg N. Oxidation and nitrosylation of oxyhemoglobin by S-nitrosoglutathione via nitroxyl anion. *Free Radic Biol Med* 35: 1515–1526, 2003.
142. Stone JR, Sands RH, Dunham WR, and Marletta MA. Spectral and ligand-binding properties of an unusual hemoprotein, the ferric form of soluble guanylate cyclase. *Biochemistry* 35: 3258–3262, 1996.
143. Stoyanovsky DA, Schor NF, Nylander KD, and Salama G. Effects of pH on the cytotoxicity of sodium trioxodinitrate (Angeli's salt). *J Med Chem* 47: 210–217, 2004.
144. Suarez SA, Bikiel DE, Wetzler DE, Marti MA, and Doctorovich F. Time-resolved electrochemical quantification of azanone (HNO) at low nanomolar level. *Anal Chem* 85: 10262–10269, 2013.
145. Switzer CH, Flores-Santana W, Mancardi D, Donzelli S, Basudhar D, Ridnour LA, Miranda KM, Fukuto JM, Paolucci N, and Wink DA. The emergence of nitroxyl (HNO) as a pharmacological agent. *Biochim Biophys Acta* 1787: 835–840, 2009.
146. Szijarto IA, Marko L, Filipovic MR, Miljkovic JL, Tabeling C, Tsvetkov D, Wang N, Rabelo LA, Witzentrath M, Diedrich A, Tank J, Akahoshi N, Kamata S, Ishii I, and Gollasch M. Cystathionine gamma-lyase-produced hydrogen sulfide controls endothelial NO bioavailability and blood pressure. *Hypertension* 71: 1210–1217, 2018.
147. Tabaczar S, Czepas J, Koceva-Chyla A, Kilanczyk E, Piasecka-Zelga J, and Gwozdziński K. The effect of the nitroxide pirolin on oxidative stress induced by doxorubicin and taxanes in the rat brain. *J Physiol Pharmacol* 68: 295–308, 2017.
148. Tabaczar S, Domeradzka K, Czepas J, Piasecka-Zelga J, Stetkiewicz J, Gwozdziński K, and Koceva-Chyla A. Anti-tumor potential of nitroxyl derivative Pirolin in the DMBA-induced rat mammary carcinoma model: a comparison with quercetin. *Pharmacol Rep* 67: 527–534, 2015.
149. Tabaczar S, Koceva-Chyla A, Czepas J, Pieniazek A, Piasecka-Zelga J, and Gwozdziński K. Nitroxide pirolin reduces oxidative stress generated by doxorubicin and docetaxel in blood plasma of rats bearing mammary tumor. *J Physiol Pharmacol* 63: 153–163, 2012.
150. Takeshita K, Hamada A, and Utsumi H. Mechanisms related to reduction of radical in mouse lung using an L-band

- ESR spectrometer. *Free Radic Biol Med* 26: 951–960, 1999.
151. Takeshita K and Ozawa T. Recent progress in in vivo ESR spectroscopy. *J Radiat Res* 45: 373–384, 2004.
 152. Tian X, Wang YG, Yang MG, and Chen YZ. Synthesis and antitumor activity of spin labeled derivatives of podophyllotoxin. *Life Sci* 60: 511–517, 1997.
 153. Tita C, Gilbert EM, Van Bakel AB, Grzybowski J, Haas GJ, Jarrah M, Dunlap SH, Gottlieb SS, Klapholz M, Patel PC, Pfister R, Seidler T, Shah KB, Zielinski T, Venuti RP, Cowart D, Foo SY, Vishnevsky A, and Mitrovic V. A phase 2a dose-escalation study of the safety, tolerability, pharmacokinetics and haemodynamic effects of BMS-986231 in hospitalized patients with heart failure with reduced ejection fraction. *Eur J Heart Fail* 19: 1321–1332, 2017.
 154. Tocchetti CG, Stanley BA, Murray CI, Sivakumaran V, Donzelli S, Mancardi D, Pagliaro P, Gao WD, van Eyk J, Kass DA, Wink DA, and Paolocci N. Playing with cardiac “redox switches”: the “HNO way” to modulate cardiac function. *Antioxid Redox Signal* 14: 1687–1698, 2011.
 155. Tocchetti CG, Wang W, Froehlich JP, Huke S, Aon MA, Wilson GM, Di Benedetto G, O'Rourke B, Gao WD, Wink DA, Toscano JP, Zaccolo M, Bers DM, Valdivia HH, Cheng H, Kass DA, and Paolocci N. Nitroxyl improves cellular heart function by directly enhancing cardiac sarcoplasmic reticulum Ca²⁺ cycling. *Circ Res* 100: 96–104, 2007.
 156. Tong L, Chuang CC, Wu S, and Zuo L. Reactive oxygen species in redox cancer therapy. *Cancer Lett* 367: 18–25, 2015.
 157. Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, Marinari UM, and Domenicotti C. Role of glutathione in cancer progression and chemoresistance. *Oxid Med Cell Longev* 2013: 972913, 2013.
 158. Untereiner A and Wu L. Hydrogen sulfide and glucose homeostasis: a tale of sweet and the stink. *Antioxid Redox Signal* 28: 1463–1482, 2018.
 159. Utsumi H. [Novel redox molecular imaging “ReMI” with dual magnetic resonance]. *Yakugaku Zasshi* 133: 803–814, 2013 (Article in Japanese).
 160. Utsumi H, Muto E, Masuda S, and Hamada A. In vivo ESR measurement of free radicals in whole mice. *Biochem Biophys Res Commun* 172: 1342–1348, 1990.
 161. Utsumi H and Yamada K. In vivo electron spin resonance-computed tomography/nitroxyl probe technique for non-invasive analysis of oxidative injuries. *Arch Biochem Biophys* 416: 1–8, 2003.
 162. Vaananen AJ, Salmenpera P, Hukkanen M, Rauhalta P, and Kankuri E. Cathepsin B is a differentiation-resistant target for nitroxyl (HNO) in THP-1 monocyte/macrophages. *Free Radic Biol Med* 41: 120–131, 2006.
 163. Vanhoutte PM, Zhao Y, Xu A, and Leung SW. Thirty years of saying NO: sources, fate, actions, and misfortunes of the endothelium-derived vasodilator mediator. *Circ Res* 119: 375–396, 2016.
 164. Wang D, Moothart DR, Lowy DR, and Qian X. The expression of glyceraldehyde-3-phosphate dehydrogenase associated cell cycle (GACC) genes correlates with cancer stage and poor survival in patients with solid tumors. *PLoS One* 8: e61262, 2013.
 165. Wang Y, Pan J, Shi J, and Chen Y. New spin labeled analogues of podophyllotoxin as potential antitumor agents. *Life Sci* 61: 537–542, 1997.
 166. Wardman P. Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. *Free Radic Biol Med* 43: 995–1022, 2007.
 167. Wilmanski T, Zhou X, Zheng W, Shinde A, Donkin SS, Wendt M, Burgess JR, and Teegarden D. Inhibition of pyruvate carboxylase by 1 α ,25-dihydroxyvitamin D promotes oxidative stress in early breast cancer progression. *Cancer Lett* 411: 171–181, 2017.
 168. Wink DA, Miranda KM, Katori T, Mancardi D, Thomas DD, Ridnour L, Espey MG, Feelisch M, Colton CA, Fukuoto JM, Pagliaro P, Kass DA, and Paolocci N. Orthogonal properties of the redox siblings nitroxyl and nitric oxide in the cardiovascular system: a novel redox paradigm. *Am J Physiol Heart Circ Physiol* 285: H2264–H2276, 2003.
 169. Wondrak GT. Redox-directed cancer therapeutics: molecular mechanisms and opportunities. *Antioxid Redox Signal* 11: 3013–3069, 2009.
 170. Wynne BM, Labazi H, Carneiro ZN, Tostes RC, and Webb RC. Angeli's salt, a nitroxyl anion donor, reverses endothelin-1 mediated vascular dysfunction in murine aorta. *Eur J Pharmacol* 814: 294–301, 2017.
 171. Xia L, Zhang C, Li M, Wang K, Wang Y, Xu P, and Hu Y. Nitroxide-radicals-modified gold nanorods for in vivo CT/MRI-guided photothermal cancer therapy. *Int J Nanomed* 13: 7123–7134, 2018.
 172. Yang L, Liu YQ, Tan H, Li WG, and Tian X. Design, synthesis, and biological evaluation of novel spin-labeled derivatives of podophyllotoxin as potential antineoplastic agents. Part XII. *Nat Prod Res* 21: 998–1008, 2007.
 173. Yong QC, Cheong JL, Hua F, Deng LW, Khoo YM, Lee HS, Perry A, Wood M, Whiteman M, and Bian JS. Regulation of heart function by endogenous gaseous mediators-crosstalk between nitric oxide and hydrogen sulfide. *Antioxid Redox Signal* 14: 2081–2091, 2011.
 174. Yong QC, Hu LF, Wang S, Huang D, and Bian JS. Hydrogen sulfide interacts with nitric oxide in the heart: possible involvement of nitroxyl. *Cardiovasc Res* 88: 482–491, 2010.
 175. Yoshitomi T and Nagasaki Y. Nitroxyl radical-containing nanoparticles for novel nanomedicine against oxidative stress injury. *Nanomedicine (Lond)* 6: 509–518, 2011.
 176. Zamora R, Grzesiok A, Weber H, and Feelisch M. Oxidative release of nitric oxide accounts for guanylyl cyclase stimulating, vasodilator and anti-platelet activity of Pi-loty's acid: a comparison with Angeli's salt. *Biochem J* 312 (Pt 2): 333–339, 1995.
 177. Zeller A, Wenzl MV, Beretta M, Stessel H, Russwurm M, Koesling D, Schmidt K, and Mayer B. Mechanisms underlying activation of soluble guanylate cyclase by the nitroxyl donor Angeli's salt. *Mol Pharmacol* 76: 1115–1122, 2009.
 178. Zhang X, Lin Y, and Gillies RJ. Tumor pH and its measurement. *J Nucl Med* 51: 1167–1170, 2010.
 179. Zhang Y, Guo J, Zeng L, Zhang J, Hui Y, Liu J, Qing X, Sun X, and Guo G. Tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-O-1H-imidazole-3-cationic-1-oxy 1-2-pyrrolidine-1-carboxylic ester displays novel cytotoxicity through reactive oxygen species-mediated oxidative damage in MCF-7 and MDA-MB-231 cells. *Chem Biol Interact* 192: 287–297, 2011.

180. Zhelev Z, Bakalova R, Aoki I, Gadjeva V, and Kanno I. Imaging of cancer by redox-mediated mechanism: a radical diagnostic approach. *Mol Biosyst* 6: 2386–2388, 2010.
181. Zhelev Z, Bakalova R, Aoki I, Matsumoto K, Gadjeva V, Anzai K, and Kanno I. Nitroxyl radicals for labeling of conventional therapeutics and noninvasive magnetic resonance imaging of their permeability for blood-brain barrier: relationship between structure, blood clearance, and MRI signal dynamic in the brain. *Mol Pharm* 6: 504–512, 2009.
182. Zhelev Z, Matsumoto K, Gadjeva V, Bakalova R, Aoki I, Zheleva A, and Anzai K. EPR signal reduction kinetic of several nitroxyl derivatives in blood in vitro and in vivo. *Gen Physiol Biophys* 28: 356–362, 2009.
183. Zhou Y, Wu Z, Cao X, Ding L, Wen Z, and Bian JS. HNO suppresses LPS-induced inflammation in BV-2 microglial cells via inhibition of NF-kappaB and p38 MAPK pathways. *Pharmacol Res* 111: 885–895, 2016.
184. Zhu G, Groneberg D, Sikka G, Hori D, Ranek MJ, Nakamura T, Takimoto E, Paolucci N, Berkowitz DE, Friebe A, and Kass DA. Soluble guanylate cyclase is required for systemic vasodilation but not positive inotropy induced by nitroxyl in the mouse. *Hypertension* 65: 385–392, 2015.

Address correspondence to:

Dr. Jin-Song Bian
Department of Pharmacology
Yong Loo Lin School of Medicine
National University of Singapore
Singapore 117600
Singapore

E-mail: phcbjs@nus.edu.sg

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Abbreviations Used

3-MST = 3-mercaptopyruvate sulfurtransferase
AS = Angeli's salt
CBS = cystathionine β -synthase
cGMP = cyclic GMP

CGRP = calcitonin gene-related peptide
CO = carbon monoxide
CSE = cystathionine γ -lyase
DMBA = 7,12-dimethylbenz(a)anthracene
DOX = doxorubicin
DTT = dithiothreitol
DTX = docetaxel
EPRI = electron paramagnetic resonance imaging
EPRS = electron paramagnetic resonance spectroscopy
GAPDH = glyceraldehyde 3-phosphate dehydrogenase
GSH = glutathione
H₂O₂ = hydrogen peroxide
H₂S = hydrogen sulfide
HIF-1 α = hypoxia-inducible factor-1 α
HNO = nitroxyl
HO-1 = heme oxygenase-1
HSP70 = heat shock protein 70
IL = interleukin
IPA/NO = isopropylamine NONOate
L-NNP = tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-O-1H-imidazole-3-cationic-1-oxyl-2)-pyrrolidine-1-carboxylic ester
MnSOD = manganese superoxide dismutase
MRI = magnetic resonance imaging
MRRI = magnetic resonance redox imaging
N₂O₃ = dinitrogen trioxide
NCA = 1-nitrosocyclohexyl acetate
NH₂OH = hydroxylamine
NO = nitric oxide
NO₂ = nitrogen dioxide
NOS = nitric oxide synthase
Nrf2 = nuclear factor (erythroid-derived 2)-like 2
ONOO⁻ = peroxynitrite
PARP = poly (ADP-ribose) polymerase
PhSO₂NHOH = Piloty's acid
PNCs = platinum-HNO complexes
ROS = reactive oxygen species
RyR = ryanodine receptor
sGC = soluble guanylate cyclase
SOD = superoxide dismutase
SR = sarcoplasmic reticulum