International Journal of Natural Medicine and Health Sciences
ISSN (Online):2790-2471 ISSN (Print): 2790-2463
Volume 1(4) September 2022
Journal homepage: https://journals.iub.edu.pk/index.php/ijnms

Review

A Review on anticancer potential of Nitric Oxide

Faheem Hadi[a], Tahir Maqbool[a], Tahir Muhammad[a], Shabana Akhtar[a], Muhammad Rafiq[a], Sana Javaid Awan[b], Tania Ahmad Shakoori[c], Asima Tayyeb[d*]

[a] Centre of Research in Molecular Medicine, Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan.
[b] Kinnaird College for Women University, Lahore, Pakistan.
[c] Department of Physiology and Cell Biology, University of Health Sciences, Lahore, Pakistan.
[d*] School of Biological Sciences, The University of Punjab, Lahore, Pakistan.

Article Info.

Received: 01-07-2022
Revised: 22-08-2022
Accepted: 09-09-2022
Online: 30-09-2022

Correspondence:
asima.sbs@pu.edu.pk

Abstract

Nitrous oxide (N2O; laughing gas) is clinically used as a safe anesthetic (dentistry, ambulance, childbirth) and appreciated for its anti-anxiety effect. Nitrous oxide (N2O) is a free radical gas which performs various physiological and pathological processes in body. NO is produced by different enzymatic pathways and plays role in homeostasis. Over past years, NO has emerged as a molecule of interest in many ailments including cancer. But its role in cancer is still controversy. It can display dose-dependent anticancer therapy on one hand and induce pro-cancer properties on the other hand. But as compared to conventional treatments, NO proved better tumor cell resistance. This review mentions dichotomous nature of NO that may encourage future research assessing the role of NO in cancer prevention and treatment either as a single agent or in combination with other antineoplastic compounds.

Citation: Hadi F, Maqbool T, Muhammad T, Akhtar S, Rafiq M, Awan SJ, Shakoori TA, Tayyeb A. A Review on Anticancer Potential of Nitric Oxide. IJNMS. 2022; 1(4): 31-35.

Introduction

Nitric oxide (NO) is a small molecule with a short life span of 1-5 sec *in-vivo* and is highly unstable[1] that became more prominent in 1992[2]**.** NO is called as a biological messenger[3]. It readily diffuses into the cell membrane to react with molecular targets and act as a signaling molecule in the body[4]. NO is among the simplest molecules and chemists are studying its structure and function for many years. Recent two decades have revealed many new and unexpected roles of NO as a key regulator at physiological level. Karlin and coworkers mentioned the relationship of NO compounds with biological denitrification[5]. Nagano and Yoshimura (2002) reviewed imaging techniques to detect biological NO in the body using spectroscopy and fluorometry[6].

Formation of Nitric Oxide

NO is produced in several tissues of the body[7]. Four forms of NO synthetase (NOS) synthesize NO, namely as endothelial NOS (in vascular endothelium), inducible NOS (in macrophages), neuronal NOS (in sensory nerve endings) and mitochondrial NOS (in mitochondria)[3,8]. NO synthases reacts with its substrate amino acid arginine and co-substrates O_2 and NADPH to form citrulline and NO^2 as shown in figure 1 (A).

Functions of Nitric Oxide

Among plants and animals, many redox molecules (H_2S, ROS, CO, NO etc.,) have shown their role in various physiological processes. In plants, NO regulates processes related to cellular signal transduction networks. A major source of NO formation in plants is nitrate reductase, which also regulates its homeostasis. NO regulates many physiological processes in plants such as stress regulation (freezing, hypoxic, & osmotic stress tolerance), development (abscisic acid-induced stomatal closure, floral transition & development of root hairs & leaves) and act as a gaseous biological signaling molecule. In animals, NO deals with immunomodulatory responses and oncogenesis[9]. NO also plays a key role in many physio pathological reactions related to hypoxia and cellular immunity[4]. It controls many functions in the body such as smooth muscle relaxation, inhibition of platelet aggregation, neurotransmission, leukocyte adhesion and vasodilation[2,3]. At cellular level, NO mediate functions via activating guanylyl cyclase (sGC) in the cytosol, that converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). cGMP acts as a secondary messenger and ultimately activates cGMP-dependent protein kinases pathways[7] as shown in figure 1 (B). Lack of NO in the body can induce diabetes mellitus, chronic kidney disease, heart failure, arteriosclerosis and hypertension[3].

Properties of Nitric Oxide

Cytotoxic and Anti-Atherogenic Properties

NO and its metabolites show cytoprotective/cytotoxic effects by the inhibition of the mitochondrial respiration, DNA and protein damage and apoptosis and necrosis[1]. NO involves in a reaction to form peroxynitrite, which can cause apoptotic death in the lymphocytes and tumor cells, but not in hepatocytes. Apoptotic death is controlled by the activation of caspases by NO and triggering mitochondrial permeability[10]. In-vitro, NO obtained from endothelial cells, natural killer cells, Kupffer cells and macrophages has showed cytotoxic effects[2]. NO has also showed anti-atherogenic properties in the macrophages[3].

Effect on Gastrointestinal Tract

NO donors help in healing of ulcers in Gastrointestinal tract (GIT), especially damage by ethanol and inhibition of NOS delays healing process in gastric mucosa (Figure 2). NO also dilates blood vessels of gastric mucosa which improves blood flow and protects from damage of strong irritants in food[3].

Role in Regulation of Heavy Metals Toxicity

In plants, the role of NO has established as a multifunctional messenger in the regulation of multiple biological processes during the last decade. NO is capable of removing heavy metals such as aluminum, copper and cadmium and prevent the plants from the toxic effects. This protective efficacy of NO is assumed with its regulation of antioxidant enzymes involved in ROS scavenging. For example, antioxidant enzyme, Superoxide dismutase (SOD) is involved in removal of cadmium. This type of pattern can be found in rice leaves and other parts of wheat. In contrast, the involvement of NO was also found in amplification of metal toxicity. For example, NO decrease cadmium detoxification by mediating S-nitrosylation of phytochelatins. Furthermore, NO promote accumulation of cadmium in roots of Arabidopsis by upregulating iron transporters such as IRT1. However, further research is needed to elucidate the role of NO in regulating toxicity of heavy metals[11].

Role in Repairing Liver Fibrosis

NO has also been investigated as hepatoprotective against liver fibrosis when administered with mesenchymal stem cells in an in-vivo study. Similarly, many studies mentioned that NO has negative regulatory properties specifically on activated hepatic stellate cells migration, contraction and proliferation in fibrotic liver[11].

Role as Pulmonary Vasodilator

In a study conducted at birth of lambs, during the fetal to neonatal transition for the respiratory onset, NO acts as main vasodilator which prevents from pulmonary vascular resistance. After production, NO diffuses to pulmonary artery smooth muscle cells, where activation of cGMP-protein kinase G signaling pathway takes place along with influx and sensitivity of contractile machinery of Ca^{2+}. These events lead to migration and proliferation of cells. Regarding the role of NO signaling in the adaptation of pulmonary circulation to extrauterine life, most of the studies have been conducted at near sea level, which can vary at high altitude. Studies conducted on sheep, yak or llama suggested that NO-dependent mechanisms contribute to chronic hypoxia adaptation to different degrees at high altitude. Lowland sheep displayed less production of NO as compared to high altitude newborn sheep. Inhalation of NO reduced vasoconstriction in rats suffered from pulmonary hypertension[12].

Role in Cancer

Pro-Cancer Activity

NO is a free radical and involves in the formation of cancer. NO has also showed pro-neoplastic properties such as promoting genomic instability, stimulation of angiogenesis, inhibition of apoptosis in cancer cells and cancer cell proliferation[2]. Some NO donors like sodium nitroprusside (SNP) also stimulated angiogenesis in a study[1]. In another study of lung cancer, NO inhibits p53

pathway in tumor cells, leading to 90% carcinogenesis due to defects in p53 pathway[4]. Many pro-tumorigenic compounds like estrogen have been proved to activate eNOS expression in tumor cells[8]. Progression of oral cancer due to presence of NO activity needs further evaluation[4]. NO promote apoptosis by many cellular pathways[1]. **S**tudies have indicated that NO may be involved in tumor growth and progression as it limits cell proliferation of leukocytes which can lead to antitumor response. NO and its derivatives support cancer progression of pancreas, skin, breast, colon and lung by enhancement of metastasis, angiogenesis, cell proliferation, post-translational modification of proteins, suppression of DNA repair enzymes, inhibition of antitumor immunity and apoptosis[1]. Excessive synthesis of NO in the body can contribute pathophysiological conditions such as in cancer of neck, CNS, larynx and head. NO promotes angiogenesis and hence, metastasis. Similar studies have been conducted by different scientists, as gastric cancer is initiated and progressed by NO, which can lead to malignancy. Incidence of oral cancer by smoking and chewing tobacco is much assumed due to many factors including formation of NO products as an inflammatory response in tobacco users[4].

Anti-Cancer Activity

NO donors have also been used as a preventive agent against several cancers[13]. NO can regulate apoptotic pathways hence, apoptosis of tumor cells is possible via NO activity. NO can damage tumor membrane or inhibit many cellular pathways that can induce apoptosis[1]. NO cytotoxic effect through salvage pathway has been documented in a study and high concentrations of according to choudhari et al (2013) NO have anti-metastasis property[4]. Many studies have also mentioned that high levels of NO can induce apoptosis in many cell types primarily by the effect of peroxynitrite that increases mitochondrial permeability[11]. In a study, inducible nitric oxide synthase (iNOS) was proved to suppress cancer formation[8]. Proliferation of lung tumor cells were noted in mice containing zero NO activity as compared to other group with proper NO activity in a study[1]. Many studies reported NO anticancer activity in cells via p53 upregulation, BCL-2 expression changes, cytochrome c emission and caspases activation. Defective mitochondrial and DNA functions were also induced by NO to kill cancer cells[13]. Diazeniumdiolates, class of NO donors, have much attractive properties such as target tissue specific delivery, long half-lives and effective radio and chemo-sensitizing agents. NO enhanced radio sensitivity in colorectal cancer patients in the presence of oxygen, whereas normal cells were unaffected in the study[1]. Most of the studies conducted to check antitumor capability of NO were in-vitro but little results have been found in cancer patients regarding death of cancer cells such as in oral squamous cell carcinoma and other solid tumors. Some factors lead the variation in cancer therapy such as duration, dose administered, redox state and type of cell lines used for treatment. Another NO donor molecule, fluorescent nanocrystals, have been found as effective antitumor agents. Neuroblastoma cells have been killed by combination of chemotherapy and nano-delivery. Other photolabile NO-releasing prodrugs also confirmed enhanced radio sensitization of A549 lung carcinoma cell line[4].

III Anti-Cancer Activity on different Cell Lines

NO didn't affect normal cells such as vascular smooth cells, cardiac myocytes, ovarian follicles, lymphoma cells, endothelial cells and liver cell line[1], however several cases have been reported as its tumorigenic effect on different cell lines. In pulmonary artery endothelial cells and cultured hepatocytes, NO showed anti-apoptotic activity[10]. NO inhibited proliferation of several cancers and cell lines such as human prostate metastatic cells[4], oral carcinoma, MCF-7 and Saos-2 cells, liver cancerous cell line (BEL-7402), gastric cancer cells, breast cancer cells (MDA-MB-231), human bladder carcinoma cell lines. Low doses of NO also stimulated the growth of cancer cells such as choriocarcinoma cells (JEG-3), ovarian carcinoma cells (HOC-7) and human melanoma cell line (C32TG). When NOS2-transfected pancreatic cancer cells were injected into mice, lower rate of tumorigenesis was observed[1]. Another NO donor like SNAP (S-nitroso-*N*-acetylpenicillamine) and GSNO (S-nitrosoglutathione) have been proved as antitumor agents against many cell lines. SNAP showed results against murine mammary adenocarcinoma cells (EMT-6), Chinese hamster lung cells (V79) and cervical carcinoma cell line (HeLa). GSNO induced cell cycle arrest and apoptosis in colon cancer cells. A typical compound that has been studied is 3-morpholinosydnonimine (SIN-1). SIN-1 caused neurotoxicity in rodent cortical cells and showed antineoplastic properties against glioma C-6 cells and induced apoptosis in lymphoblastoid cells (WTK). Diazeniumdiolates (NONOates), with a large spectrum of half-lives (2 s to 20 h), have been extensively studied in cancer therapeutics in-vitro and in-vivo. They include a wide range of compounds which exhibited anti-proliferative properties against many cell lines such as colon cancer cells (HT29), murine mammary adenocarcinoma cells, neuroblastoma cells (NB69), breast cancer cells (MDA-MB-231), mouse melanoma cells (B16F10), Chinese hamster lung fibroblasts (V79), prostate cancer cells (PC-3), head and neck squamous cell carcinoma cells. JS-K NO donors have been recognized as a strongest antitumor agent which showed activity against leukemia cells (HL-60), liver cancer in rats, murine prostate cancer xenografts and leukemia xenografts in mice. Sodium Nitroprusside (SNP) is related to a class of NO donors named as metal-NO complexes[2]. SNP has been clinically approved as antihypertensive agent, but recent trials also noticed its antitumor effect in different cancer cell lines *in vitro*, such as LN-Z308 and U251. In spite of this, SNP also influenced prostate, gastric and cervical cancer cell lines as well as radio sensitization was observed in pancreatic and glioma cancer cells[11]. For example, SNP induced apoptosis in T-cell lymphoma cells. SNP showed similar results as other NO donors, high concentrations of NO (10-100 µg/mL of SNP) induced cellular apoptosis, while low levels of NO (less than 2 µg/mL of SNP) inhibited apoptosis as also mentioned in work of Yang et al. SNP sensitized human colorectal cancer cells (CX-1)[10] and four gastric cancer cells[13] to TRAIL-induced apoptosis through the activation of caspases as compared to SNP alone. SNP also inhibited angiogenesis in-vivo[1].

IV Dual Nature of NO

NO donors showed dual nature. On one side, they showed pro-neoplastic properties and on other side, antineoplastic properties have been observed (Figure 3). This feature depends upon sensitivity of cancer cells, duration of exposure and concentration used[1]. If dose of NO is less than required for cytotoxicity, then NO will have supporting role in cancer progression[4]. For instance, in-vitro, NO has anticancer properties at high concentrations (>200 nM) and pro-neoplastic function was observed below this concentration. But concentration of NO always produced misconception in human trials or *in vivo* models due to limitation of half-life of NO in body and other factors[2]. In another study, 0.01-0.25 mM concentration of NO donors supported proliferation in cancer cells, whereas, >0.5 mM produced condition of cytostatic in cells). Another study depicted the same results. At low doses (1-10 Amol/L), NO stimulates growth of myoblast cells, whereas reverse phenomena was observed at high doses (50 Amol/L)[8]. Similar result has been found with SNAP concentrations. Low SNAP concentrations (0.1–0.3 mM) caused an increase in angiogenesis in microvascular endothelial cells, whereas high SNAP concentration (0.5–4 mM) inhibited angiogenesis[1]. In another previous work, study indicated that small dose NO releasing-aspirin (at a concentration of 1 mmol/l) alone had no obvious cytotoxic effect on the human hepatoma cell line SMMC-7721, but at high doses, NO-aspirin (at concentrations of 3 mmol/l and 10 mmol/l) had induced apoptosis[13].

V Nitric Oxide-Drug Hybrids

NO can be used alone or in combination with some other cytotoxic agent in cancer therapy[4]. The effects of NO have led to the development of hybrid drugs where each compound can act in synergism with NO to provide antitumor effects. This concept leads to the development of NO linked to a COX-2 inhibitor (NO-NSAIDs). A wide range of NO-NSAIDs are currently being investigated for their role in cancer treatment. These compounds have exhibited anticancer properties *in vitro* and *in vivo* even in COX-2 negative cells. NO-NSAIDs exert their anticancer property through inhibition of proliferation and initiation of cell cycle arrest, induction of apoptosis and modulation of Wnt and NF-κB signaling pathways. NO-NSAIDs inhibited colon adenocarcinoma cells (HT-29) as compared to NSAID alone[1]. In esophageal cancer cells, SIN-1 showed destruction of cells, in combination with the carcinogen myosimine[2].

Conclusion

NO is a free radical that has been utilized in the treatments of many ailments related to liver, lungs and gastrointestinal tract. It also helps removal of heavy metals from the body, indirectly aiding liver in detoxification. Role of NO in cancer treatment is still in controversy due to certain factors. There is dire need to scrutinize more about efficacy of NO in management of cancer diseases. In future, NO may prove much fruitful in treating cancer and other fatal diseases.

Conflict of Interests

Authors have no conflict of interests.

References

1. Burke AJ, Sullivan FJ, Giles FJ, Glynn SA. The yin and yang of nitric oxide in cancer progression. *Carcinogenesis.* 2013;34(3):503-512.
2. Huerta S. Nitric oxide for cancer therapy. *Future science OA.* 2015;1(1).
3. Magierowski M, Magierowska K, Kwiecien S, Brzozowski T. Gaseous mediators nitric oxide and hydrogen sulfide in the mechanism of gastrointestinal integrity, protection and ulcer healing. *Molecules.* 2015;20(5):9099-9123.
4. Korde Choudhari S, Chaudhary M, Bagde S, Gadbail AR, Joshi V. Nitric oxide and cancer: a review. *World journal of surgical oncology.* 2013;11(1):1-11.
5. Wasser IM, De Vries S, Moënne-Loccoz P, Schröder I, Karlin KD. Nitric oxide in biological denitrification: Fe/Cu metalloenzyme and metal complex NO x redox chemistry. *Chemical Reviews.* 2002;102(4):1201-1234.
6. Nagano T, Yoshimura T. Bioimaging of nitric oxide. *Chemical reviews.* 2002;102(4):1235-1270.
7. Yao X, Wu Y, Zhu M, Qian H, Chen Y. Nitric oxide/cyclic guanosine monophosphate inducers sodium nitroprusside and L-arginine inhibit the proliferation of gastric cancer cells via the activation of type II cyclic guanosine monophosphate-dependent protein kinase. *Oncology Letters.* 2015;10(1):479-484.
8. Ying L, Hofseth LJ. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer research.* 2007;67(4):1407-1410.
9. Pan Q-N, Geng C-C, Li D-D, et al. Nitrate reductase-mediated nitric oxide regulates the leaf shape in Arabidopsis by mediating the homeostasis of reactive oxygen species. *International Journal of Molecular Sciences.* 2019;20(9):2235.
10. Lee YJ, Lee KH, Kim H-RC, et al. Sodium nitroprusside enhances TRAIL-induced apoptosis via a mitochondria-dependent pathway in human colorectal carcinoma CX-1 cells. *Oncogene.* 2001;20(12):1476-1485.
11. Blackburn RV, Galoforo SS, Berns CM, Motwani NM, Corry PM, Lee YJ. Differential induction of cell death in human glioma cell lines by sodium nitroprusside. *Cancer: Interdisciplinary International Journal of the American Cancer Society.* 1998;82(6):1137-1145.
12. Herrera EA, Ebensperger G, Hernández I, Sanhueza EM, Llanos AJ, Reyes RV. The role of nitric oxide signaling in pulmonary circulation of high-and low-altitude newborn sheep under basal and acute hypoxic conditions. *Nitric Oxide.* 2019;89:71-80.
13. Yang L, Lan C, Fang Y, et al. Sodium nitroprusside (SNP) sensitizes human gastric cancer cells to TRAIL-induced apoptosis. *International immunopharmacology.* 2013;17(2):383-389.

.

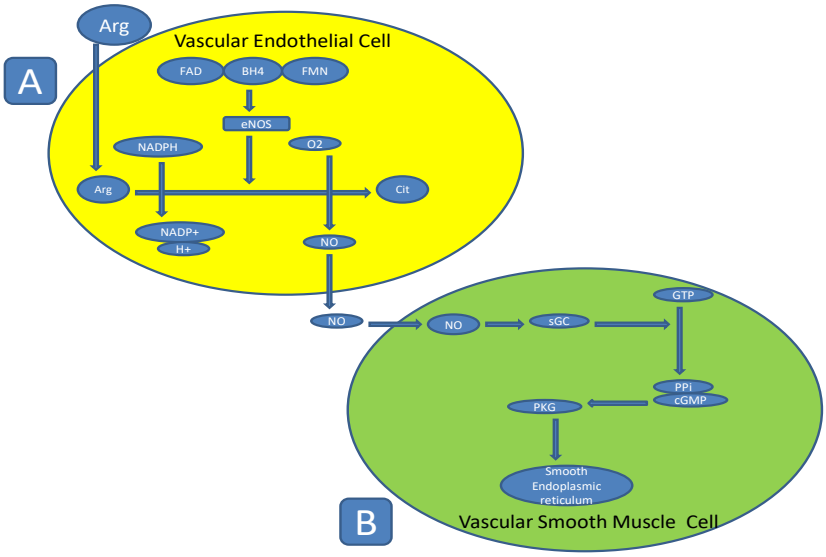

Figure 1. (A) Formation of NO via NO synthase which reacts with its substrate amino acid arginine and co-substrates O_2 and NADPH to form citrulline. **(B)** Conversion of cGMP from GP via sGC.

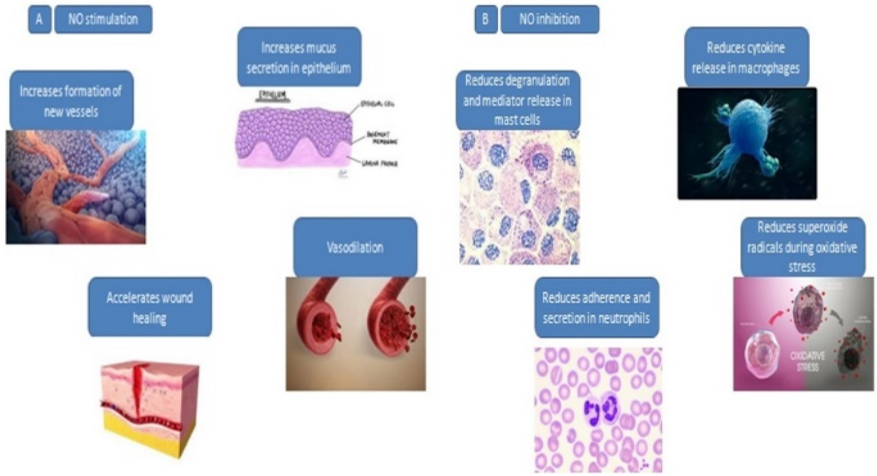

Figure 2. Beneficial actions of nitric oxide (NO) in the mechanism of gastrointestinal mucosal defense (A) stimulation (B) inhibition.

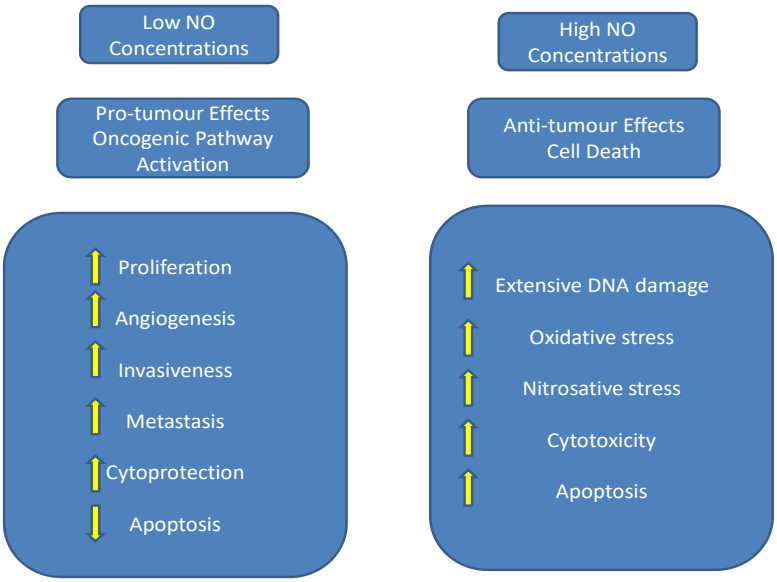

Figure 3. Concentration-dependent effects of NO in cancer. Low levels of NO (<100 nM) promote increased proliferation and angiogenesis. Medium levels of NO (100–500 nM) promote increased invasiveness, metastasis, cytoprotection and repress apoptosis. High levels of NO (>500 nM) promote DNA damage, oxidative/nitrosative stress, cytotoxicity and apoptosis.