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Review

Dichotomy of Nitric Oxide in Cancer Biology and its Therapeutic Implications

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Abstract

The role of Nitric oxide, which is an important signalling molecule, has become an active and controversial area of research in cancer. Nitric oxide has been designated as "Double-edged sword" in Cancer as it has exhibited both pro-apoptotic and anti-apoptotic effects. Nitric oxide synthase (NOS) levels have been associated with contrasting effects of tumor suppression & tumor progression. The discovery of generation of NO in mammalian tissues and its biological role in cancer has thrown light into tumor biology research. Through various studies conducted, it has become clear that concentration and time dependent regulation of Nitric oxide lead to tumor growth, cytostasis and cell death .The regulation of tumor growth by Nitric oxide represents an important new dimension in cancer research and understanding of mechanisms involved behind this process at molecular and cellular level is a major area of concern which will open up new avenues in Cancer Research and may help to provide new and better therapeutic interventions in diagnosis, treatment and cure of Cancer.

Keywords: Nitric oxide; Cancer; Angiogenic; Genotoxic; Tumor biology

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Introduction

In the year 1987, Nitric oxide was identified as Endothelium derived Relaxing factor (EDRF) by Ignarro et al [1] & Montana et al [2]. Nitric oxide is a highly reactive free radical due to the molecular arrangement which leaves an unpaired electron. The small size and lipophilicity are two properties due to which nitric oxide diffuses easily through the cell membrane (Kroncke 2001). This universal signalling molecule is involved in many physiological as well as pathological processes. Low or moderate NO levels were associated with immune function, blood flow, platelet aggregation, neurotransmission and memory. Increase production of Nitric oxide was found in inflammatory and immunological disorders, pain, neurological diseases, atherosclerosis and cancer [3].In the year 1992, Nitric oxide was awarded "the molecule of the year" and year 1998 became remarkable when Noble prize was awarded together to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad in Physiology or Medicine for their discoveries regarding nitric oxide as a signalling molecule in the cardiovascular system.

Hibbs and co-workers demonstrated that Nitric oxide is the active factor responsible for the macrophage mediated killing of tumor cells in model systems [4].No being a pleiotropic molecule is capable of altering many cellular processes depending upon its rate of generation due to which its role in cancer development has become a contentious area of research despite the fact that the role of Nitric oxide has been established in human cancers as well as experimental models. Several studies were conducted and it was evident that, at very high production of NO, killing of cancerous cell occurs and at very low levels, is found to be responsible for tumor growth. However, at intermediate levels, Nitric oxide is found to protect cancer cells from apoptosis. Thus, Nitric Oxide is shown to be both pro-apoptotic and anti-apoptotic depending upon factors like flux, dose, specific cells involved as well as the redox state of those cells. While, Nitric oxide is being labelled as a causative agent in cancer, many experimental studies both in vitro and in vivo shows this molecule to be protective against many chemical species which is known to induce cancer. Thus, the multitude effects of Nitric oxide related to other aspects of tumor biology have been studied. Thus, evaluation of the mechanism of Nitric oxide at molecular level is required in order to exploit its potential as cancer therapy taking into account the tumor biology (processes involved like Angiogenesis, blood flow, metastasis, proliferation, apoptosis, immune system surveillance) which could help to initiate further studies in this direction.

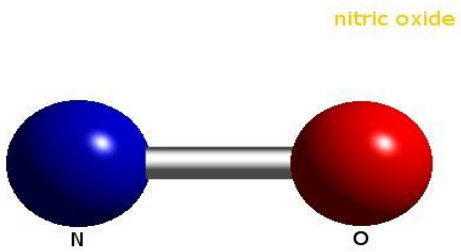

Figure 1 showing molecule of Nitric oxide.

Nitric oxide synthase (NOS) enzymes

Nitric oxide synthase is a haem protein which is similar to cytochrome P450. L-arginine is used as a substrate used during synthesis of nitric oxide which is converted to L-citrulline and this process is catalysed by an enzyme nitric oxide synthases. NO synthases are responsible for the synthesis of Nitric oxide from the substrate L-arginine.

Figure 2 Biosynthesis of Nitric oxide

Isoforms of Nitric oxide synthase (NOS)-

Nitric oxide synthase enzyme has three isoforms:

NOS 1 or neuronal nitric oxide synthase (nNos).

NOS 2 or inducible nitric oxide synthase (iNOS).

NOS 3 or endothelial nitric oxide synthase (eNOS).

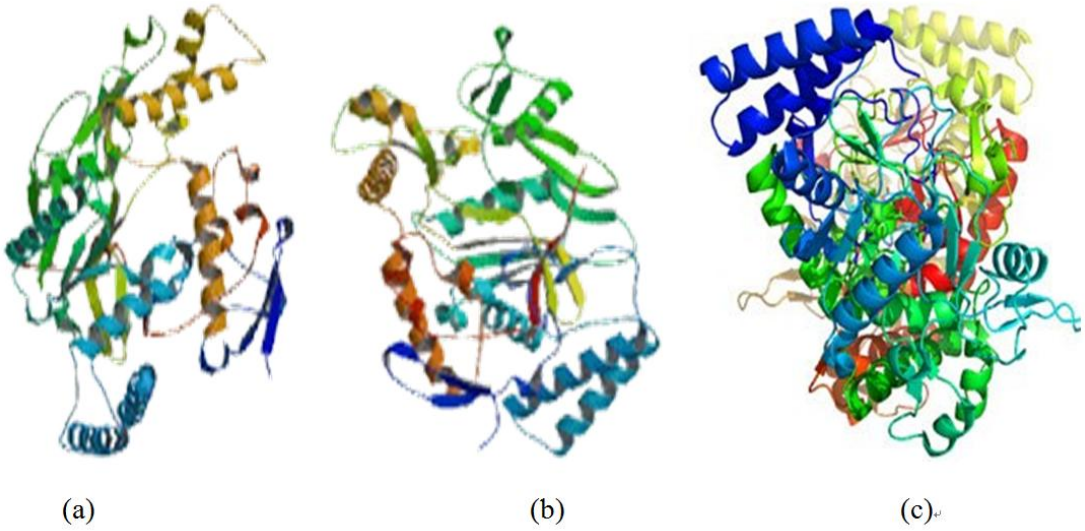

Figure 3 showing (a) Inducible NOS (iNOS); (b) Endothelial NOS (eNOS); (c) Neuronal NOS (nNOS)

These isoforms have been categorized as constitutive (both eNOS and nNOS) and inducible nitric oxide synthase (iNOS).Constitutive NOS(c NOS) is calcium dependent and produces low NO levels where as iNOS is calcium independent and generates high levels of NO. It is found that when cells are exposed to cytokines, high levels of NO are produced [5].

Inducible NOS (iNOS) - Inducible NOS is expressed mainly in macrophages, neutrophils and epithelial cells. This is governed by factors like lipopolysaccharides or cytokines. It has been found that the synthesis of iNOS can also be induced in glial cells, liver and cardiac muscle.

Endothelial NOS (eNOS) – This is a constitutive isoform dependent and expressed in endothelial lining of blood vessels. This isoform of NOS elicit CGMP dependent smooth muscle

relaxation in smooth muscle cells of blood vessel which increases blood flow.

Neuronal NOS (nNOS) – This is expressed constitutively in post synaptic terminals of neurons and is calcium dependent. Opening of voltage gated calcium channels results in membrane depolarization and which in turn activates nNOS [6].

All the three isoforms have been detected in tumors (Tozer and Everett 1997). NO produced endogenously had profound effects on tumour blood flow, angiogenesis and metastatic potential (Wink et al 1998) and was identified as an excellent target for cancer therapy. Still, the area of concern remains whether there is more therapeutic benefit by inhibiting NO production in tumors or by enhancing the production of Nitric oxide.

Chemical biology of Nitric oxide

The chemical reaction of Nitric oxide is based on its chemistry which occurs at different flux and concentration [7, 8].At low levels, NO was found to protect cell but in contrast, at higher levels, it is a known cytotoxin which has been implicated in tumor angiogenesis and progression [9].

The biological reactions of NO are divided into three main pathways [10].

1) Diffusion- NO diffuses the cell membrane by simple diffusion and reacts with cellular components. Once inside the cell, it reacts with non-heme iron or quench tyrosyl radical of ribonucleotide reductase leading to the inhibition of DNA synthesis [11, 12].
2) N_2O_3 (nitrous anhydride) is formed by Auto-oxidation

$$NO + NO_2 \longleftrightarrow N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+$$

Nitrous anhydride is formed by combination of Nitric oxide and nitrogen dioxide.

3) Peroxynitrite is formed by reaction with superoxide

Peroxynitrite formed is not a free radical and is a potent oxidant which reacts with almost all biological molecules [13]. Combination of carbon dioxide with the peroxynitrite anion forms nitroso peroxycarbonate adducts which on decomposition forms NO_3^- and CO_2.

$$ONOO^- + CO_2 \rightarrow \{ONO_2 \cdot CO_2^-\} \rightarrow NO_3^- + CO_2$$

Nitric oxide reacting with molecular oxygen, which is present in higher concentration than nitric oxide, to form peroxynitrite [14] takes place in aqueous or gaseous phase. NO_2 is a stable product of NO oxidation in gaseous phase where as NO_2 give rise to NO, NO_3^- [15] in aqueous solutions.

$$2NO + O_2 \rightarrow 2NO_2 \rightarrow NO^- + NO_3^- \rightarrow ONOO^-$$

The nitroxyl anion (NO^-) is found to be endothelium derived relaxing factor which is short lived and very reactive [16].

The chemical biology of Nitric oxide is divided into two types of effect.

1) Direct effect of NO
2) Indirect effect of NO

Direct effect of NO- This involves those chemical reactions in which NO is reacting directly with a biological target. For instance, low levels of NO can react directly with haem-containing proteins such as guanylate cyclase, oxyhemoglobin, and cytochrome p450 which is responsible for the neuromodulatory effect of nNOS and vasodilatory effect of eNOS.

At low concentration of NO, direct effects will predominate, while at higher concentration indirect effects mediated by NO/O_2^- [17]. NO protects tissue from peroxide mediated damage by scavenging metal oxo species [18].This free radical reacts with non-heme iron at the active site and inhibit lipid oxygenase activity [19].

The toxic effects of NO involve its oxidation

products where as NO alone is not capable of DNA damage [20]. It was shown that on exposure to NO generating agents, p53 is induced in both RAW 264.7 macrophages and RINm5F cells [21]. P53 is a protein responsible for maintaining genome stability. On exposure to DNA damaging agent, rapid increase in p53 level occurs. Generally, p53 has a short half life but DNA damage results in its accumulation in cells [22] which leads to DNA fragmentation and finally apoptosis.

NO mediates DNA damage by 3 mechanisms:

i. Formation of nitrosamines.
ii. Inhibition of DNA lesion repair system which is a genotoxic mechanism.
iii. Modification of DNA not directly by NO but by its oxidation products [23].

Indirect effects of NO- This involves those chemical reactions which are mediated by RNOS formed through reaction of NO either with O2 or with superoxide. These reactions require high local concentrations of NO, of which, NOS may be the sole biological source.

Indirect effect of NO is further divided into:

a) Oxidation
b) Nitrosation

Oxidation- Oxidation reaction is those where removal of electrons or hydroxylation reaction occurs, similar to those for (ROS) reactive oxygen species, leading to oxidative stress [24].

Nitrosation reaction- Nitrosation reaction is those in which RNOS donate NO to nucleophilic groups such as thiols and amines. Nitrosonium adducts formed in the biological systems are termed as nitrosative stress.

Thus, a diverse range of chemical reaction producing uncontrollable result is undoubtedly balanced by both nitrosative and oxidative stress [25].

Genotoxic mechanisms of Nitric oxide

The mechanism by which NO participate in genotoxic events involves the indirect chemistry of NO. For such event or reaction to occur, in vivo requires high local concentration of NO which is generated by iNOS. This would be reasonable to expect that sites of potential carcinogenic risk are those which inhibit prolonged expression of iNOS such as during chronic inflammation .Along with the formation of carcinogenic nitrosamines, NO increases the susceptibility of cells to other genotoxic agents which exhibit the indirect role of NO in genotoxicity.

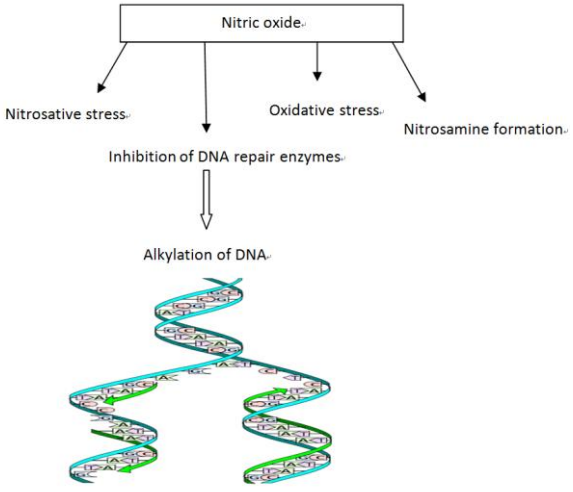

Figure 4 Potential genotoxic mechanisms of Nitric oxide

Figure 5 Factors regulating cancer biology

Role of Nitric oxide in cancer biology

Carcinogenesis is defined as a malignant transformation of a cell or group of cells. This process is divided into three stages:

i. Initiation
ii. Promotion
iii. Progression

Initiation phase involves modification of the genetic material of the cell due to single exposure to any carcinogenic agent which is irreversible. Promotion stage which is also irreversible involves multiple exposure to the promoter, alter gene expression and produces tumour.

The complex role of NO in cancer biology is based on earliest studies on NO.

(a) NO from macrophages were found to inhibit respiration in tumor cells [26, 27] while other studies indicated that through nitrosative process of NO, carcinogenic nitrosamines were derived from NOS [28].

(b) Later, it was found that RNOS reactive nitrogen oxide species derived from NO are carcinogenic. It may be due to alteration of DNA chemically as well as increasing the susceptibility to other genotoxic agent such as alkylating agents[29] and metals such as cadmium[30]

(c) Some studies suggested that expression of NOS reduce metastasis while other studies suggested that such tumors which express NOS are more aggressive in vivo.

Thus, parameters like Angiogenesis, blood flow, apoptosis, Metastasis, Immune surveillance are evaluated to explain the complex nature of NO in cancer.

Anti-carcinogenic effect or Tumor suppressing effect of NO

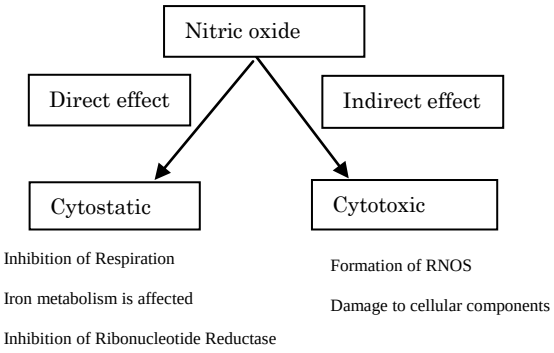

Figure 6 Cytostatic and cytotoxic mediated actions of NO

In earlier studies, Seminal experiments from macrophages inhibit cellular respiration in target cell [31] but later reports demonstrated that NO derived from macrophages, Kupffer cells, natural killer cells and endothelial cells exhibited tumoricidal activity against many

tumors [32].Thus, suggesting that NO tumor has a cytostatic and cytotoxic effect on tumor cells.

Aconitase and ribonucleotide reductase are the two molecular targets which has been utilised in the cytostasis or cytotoxicity mediated by NO. Mitochondrial aconitase [33] is the first NO target associated with tumoricidal activity of macrophages. Mitochondrial aconitase and iron-responsive binding protein (IRB) found in the cytosol are two enzymes possessing aconitase activity .Studies implied that aconitase activity is modified by oxidation. This oxidation process is mediated by superoxide and peroxynitrite and to lesser extent by Hydrogen peroxide and oxygen, but not by NO [34].These findings suggest that indirect effects are responsible for aconitase inhibition and direct effect of NO was studied through anaerobic solution of NO inactivated aconitase reversibly [35].

IRB protein regulates the transcription of iron responsive elements (IRE) which in turn regulates the transferrin receptor or ferritin.

The IRB exists in two forms:

1) Holoprotein- This has aconitase activity and cannot bind to the IRE.
2) Apoprotein- This has no aconitase activity but can bind to the IRE.

iNOS activity increases the cellular uptake of iron while nNOS activity enables the binding of IRB to IRE .In case of mitochondrial aconitase; superoxide and peroxynitrite inhibit the aconitase activity of IRB while NO does not [36].

Unlike superoxide or peroxynitrite, NO stimulates the binding of IRB to the IRE. Superoxide and peroxynitrite modify the IRB such that it cannot bind to the IRE effectively, by the oxidation of thiol group. These reactive species inhibit the protein irreversibly abolishing both aconitase activity and IRE binding. Thus, direct effects of NO would result in increased iron uptake where as indirect effect of NO or ROS result in decreased iron uptake. These direct and indirect effects are crucial in tumor growth as well as cytotoxic /cytostasis mechanisms mediated by the immune system against tumor cells.

NO also affects iron metabolism protein which is affected by NO including reaction of NO with ferritin to form Fe-NO complexes. Ferrochelatase involved in the synthesis of heme protein is also inhibited by NO. Therefore, NO suppresses cellular respiration and shifts iron metabolism which is contributing to the cytostatic properties of NO. The cytostatic effect of NO is implicated by inhibition of the enzyme Ribonucleotide Reductase which leads to suppression of DNA synthesis. This is due to reaction between NO and tyrosyl radical species formed in ribonucleotide reductase [37].

The viability of tumor lines were reduced on administration of Nitric oxide donors [38] by deleting intracellular stores of GSH and making other cells susceptible to other toxic mechanisms[39].When cells were treated with NO donors with short half –lives and exposed to high fluxes of NO for short periods of time, cells exhibited increased sensitivity to NO.

Differences in proliferation were shown in presence or absence of intracellular GSH, when longer acting NO donors were administered[38].Thus, high concentration of NO may result in the formation of RNOS which mediates cell death, while lower fluxes mediate cytostasis by interacting with metal/tyrosyl radicals. Macrophages in direct contact with tumor cells is expected to generate 4-5 μM NO [40] and thus mediate indirect effects ,whereas tumor cells farther away would experience lower fluxes of NO associated with direct effects.

The tumoricidal role of NO has been derived invitro; evidence in tumor bearing animals suggests that NO derived from leucocytes may have an antitumor role. Melanoma cells transfected with or stimulated to express iNOS show reduced cell growth invitro and limited

tumorigenesis and metastasis in vivo[41-44].These observations indicate that NO donors inhibit angiogenesis, tumor growth, and metastasis [45].

Expression of iNOS is suppressed in some tumors. Macrophages harvested from tumor bearing animals exhibit a reduced ability to produce NO and diminished tumoricidal activity[46-48].Several studies exhibited suppressed expression of iNOS in macrophages from tumor bearing mice, which is due to systemic formation of tumor derived suppressor agents such as IL-10, TGF-β1,PGE2 [49-51].This study suggests a relationship between NO production and tumoricidal activity.

Another consequence of NO production is apoptosis. Reduced expression of iNOS involves apoptotic events within growing tumor [52]. Mastocytoma cells [53], Sarcoma cells [42, 54], L929 cells [54, 55] and melanoma cells [43] are these cells which undergo extensive apoptosis upon exposure to NO, while other tumor cell lines such as A549 undergo limited apoptosis when exposed to chemical NO donors [56].The lymphocyte undergoing apoptosis was found to present phosphotidyl serine on their plasma membrane and macrophages are then stimulated to phagocyte these lymphocytes [57]. Phosphatidic acid suppresses the activity of iNOS both invitro[58,59] and in peritoneal macrophages from tumor bearing animals at transcriptional level[59].Thus, this leads to the further possibility that apoptosis and subsequent presentation of phosphotidyl serine may reduce NO generated from macrophages and results in reduction of antitumor activity within a given tumor.

NO was found to have potent influence on the metastatic potential of cells including motility, adhesion and invasion (reviewed by Williams and Djamgoz 2005). Treatment of metastatic disease is a major clinical problem and reports suggest that Nitric oxide produced endogenously by tumor cells may reduce their metastatic potential. Wei et al (2003) showed that iNOS expression in stroma supplying tumors slowed down their growth dramatically and reduce metastasis. The adhesion of tumor cells which is blocked to the venular side of microcirculation is another metastatic mechanism of NO.NO was shown to inhibit tumor cells adhesion [60] in a way similar to inhibition of leukocyte adhesion for ischemia reperfusion injury [61-63]. Thus, data suggested that low levels of NO produced by the endothelium will reduce metastasis to tissues such as lung. Inhaled Nitric oxide did not prevent the metastasis of melanoma cells to the lung [64].Other reports suggested that NO produced by endothelium of liver prevents metastasis of lymphoma cells [65] while NO produced in the vasculature of brain limits the spread of colon cancer to that tissue [66].In addition, NO secreted by microglial cells also suppress the spread of cancer to the brain [66].

Tumor promoting effect or Pro-carcinogenic effect of NO

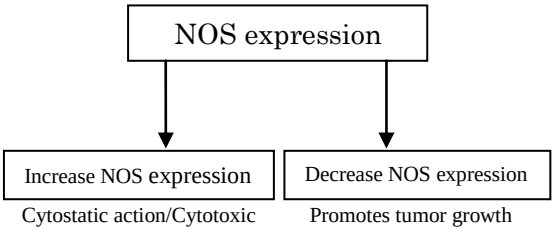

Figure 7 Conflicting effects of NOS expression

In contrast to the antitumor role of NO, NO was found to be an important mediator of tumor growth. The multistage carcinogenesis model was evaluated. NO was reported to act in other stages of cancer growth in addition to initiation.

The examples are

1) NO formed endogenously caused the neoplastic transformation of C3H 10T1/2 mouse fibroblasts cells [67].
2) Another example is NO mediated secretion of mucin by colonic adenocarcinoma cells, which was

consider to protect tumor has a role in promoting tumor [68].

3) Human adenocarcinoma (DLD-1) and murine mammary carcinoma (EMT-6) expressing iNOS showed inhibited growth in vitro.

Contrary to melanoma which expresses NOS, these cell lines are more aggressive when transplanted into mice [69, 70].This suggests that NO produced by these cells promote tumor growth. Other studies suggest that 5-FUDR activity in colon cancer may be due to reduction in iNOS expression and may account for the activity of chemotherapeutic drug [71].

Both constitutive and inducible forms of NOS were found to be present in tumors. Both the isoforms of NOS have been detected in human breast tumor [72],cervical tumors [73], tumors associated with CNS [74],colon [75] and Head and neck cancer [76]. Cytokine stimulation showed expression of iNOS in mammary carcinoma, melanoma and human colon adenocarcinoma as well as in breast cancer patients. All these data support the evidence that NO may play a critical role in growth and spread of tumors.

Another important mechanism of NO involved in tumor progression is regulation of Angiogenesis. Angiogenesis is defined as a process where tumor cells excrete certain protein that stimulates blood vessel growth into and around tumors. The tumor Keeps on growing, and eventually reaches a size where additional vasculature is required in order to maintain continued growth. Tumor expansion was found to be impossible without vascular proliferation (Folkman 1990).NO shown to be an important mediator of angiogenesis in various in vivo and invitro model systems. (Guoetal 1995, Murohara et al 1998, Jadeski & Lala 1999, Ziche & Morbidelli 2000; Kashiwagi et al 2005). Higher concentration of NO was found to be anti-angiogenic in various reports (Pipeli-synetors 1994; Lare & Ma 1996; Ray chaudhary et al 1996; Rowell et al 2000). It is well established that growth factors such as VEGF (Vascular Endothelial Growth Factor), Fibroblast growth factor (FGF), and Platelet derived growth factor are stimulators of angiogenesis. Angiogenesis process requires three processes which are initiated when VEGF binds to specific receptors on vascular endothelium.

1) Increased vascular permeability leads to formation of fibrin matrix which acts as a scaffolding for endothelial cells migration.
2) Endothelial proliferation and migration into the matrix is guided by cytokine stimulation. This process involves other co factors such as TNFα, TNFβ, bFGF and angiogenic activity of some of these factors are regulated by NO [77].
3) Hyperpermeability of vascular endothelium that is stimulated by VEGF occurs via stimulation of NO synthesis [78].

The following observations were noted which support the evidence for pro-angiogenic action of NO.

1) When glioblastoma and hepatocellular carcinoma cell lines were exposed to NO donor compounds (SNAP and NOR3).There was increase in VEGF production by stabilising m RNA levels [79].
2) When angiogenesis process is stimulated by substance P, use of NO donors leads to increase angiogenesis in the cornea pocket assay [80].
3) The in vitro proliferation coronary post capillary endothelial cells are stimulated by use of NO donors [81].
4) DLD1, the human colon tumor line which was incorporated with Nitric oxide synthase gene, grew more quickly and was better vascularised than the parent cell line [69].

There are enough of data to indicate that NO may actually down regulate angiogenesis.

1) Arterial smooth cells produce VEGF which is down regulated by NO. The

inhibition occurs by inhibition of AP1 binding to the VEGF promoter [82].

2) When exposure to exogenous NO took place, production of VEGF and its receptors were down regulated in exvivo perfused lungs and angiogenesis is inhibited in the chick corioallantoic membrane. Receptors were up regulated when NO synthase inhibitors were used [83, 84].
3) When animals are administered NO donor drugs, primarily tumor growth and metastatic frequency are lowered in the Lewis lung tumor model [45].
4) When NO donor drugs are administered proliferation and migration of endothelial cells is inhibited in vitro [85, 86].

Results from the tumor models suggest that NO stimulates angiogenesis. Though the data obtained from Lewis lung tumor model are difficult to interpret as the angiogenic process is not measured directly and due to administration of NO donor, hypotension was induced which made the tumor hypoxic [87].This slows down the growth of primary tumors.The discrepancies of the data in other model systems depends on factors like:

1) Environment in which cell were exposed to NO.
2) Types of cells exposed to NO
3) Presence or absence of other co factors involved in angiogenic process.

Another important mechanism by which NO exhibits pro-carcinogenic effect is by modulating the production of Prostaglandins. PGE2 production was shown to increase by NO which increases the blood supply of the tumor [46, 88]. NO enhances the Prostaglandin synthase activity [89] and studies have been conducted which suggest that PG synthase production has been favoured by shifting the balance in arachidonic acid metabolism and simultaneously limiting the lipoxygenase products. PGE2 was shown to suppress the NO-dependent macrophage tumor vasculature which promotes tumor growth by facilitating angiogenesis [90, 91].The hypothesis that enhanced permeability take up higher level nutrients which promotes tumor growth [92] failed to provide an explanation as studies have shown that nutrients like (glucose, oxygen) are not dependent on vascular permeability to a great extent [93] and the mechanism was found to be indirect one. The most potential mechanism is that NO which causes PGE2 activation in turn suppresses the NO production and tumoricidal activity of macrophages while facilitating angiogenesis.

Systemic effect of NO also involves suppression of proliferation and infiltration of leucocytes which is relevant in cancer biology. Studies indicates that T cell proliferation is suppressed by NO compromising the antitumor response of the host [47, 94].When NOS inhibitors were administered ,there was increased activity of lymphocytes activated killer cells, thus limiting tumorignesis [91].Thus, this study indicates that NO is essential in controlling the proliferation of tumor-infiltrating T-cells as well as at more distant sites.

Several studies done have reported that tumor cells producing NO may prevent infiltration of leucocytes .One of the study indicated that inhibitor effect causes greater infiltration of leucocytes in tumor [47].Another study was done where systemic LPS administration causes leukocyte adhesion in normal vasculature but not in tumor vascular suggesting that NO was released from tumor cells to prevent adhesion [78]. During Ischemia perfusion injury in which in which leukocyte infiltration is stimulated was suppressed by NO donors [95]. All these studies indicated that NO along with increasing the blood supply also down regulates expression of adhesion molecules such as VCAM which is important for inflammatory and immune cell adhesion to vascular endothelium [96]. In this, radiation balances the process in favour of the immune system.

Hence, the tumor suppressing and tumor promoting roles of NO can be described as both

direct and indirect effects. Low concentration of NO prevents binding of tumor cells to the endothelium mediated by direct effect. Other direct effects of NO also include prevention of leukocyte infiltration, suppression of T-cell proliferation, increase vascular permeability and angiogenesis. Whereas, the genotoxic effect of NO are mediated by indirect effects but the concentration of NO, where pro- to anti-malignant activity occurs, and which varies from one tumor type to another has not been defined. Thus, the amount and flux dictate both direct and indirect effects of NO, out of which amount is the important determinant which judges whether NO promotes or inhibits tumor growth.

Therapeutic potential of NO

The therapeutic potential of NO is explored by two opposite NO targeted strategies which will be difficult to interpret as different NO level will have variable consequences for different tumors as well as normal cells.

1) Suppression of endogenous production of NO.
2) Overproduction or over expression of NO.

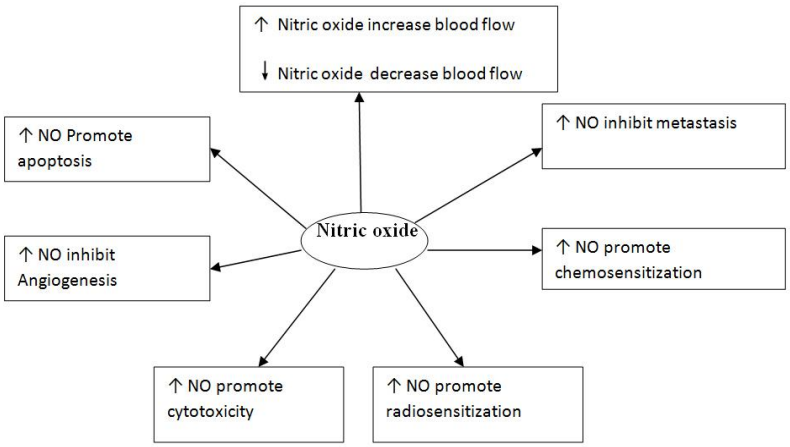

Figure 8 Potential of Nitric oxide as an anticancer agent

Suppression of endogenous production of NO

Kennovin et al 1994 showed that chronic administration of a non-isoform-specific L-arginine analogue causes inhibition of NOS in-vivo and resulted in regression of tumor growth in mice and rats. When specific iNOS inhibitors were administered to mice bearing different tumor types, tumor growth inhibition was found to be dependent on constitutive level of iNOS expression. Tumors which were expressing iNOS or genetically engineered to express iNOS showed growth inhibition where as growth of the parental, NOS-iNOS-expressing cell line was not affected by drug (Thomsen et al 1997). Thus, these studies confirmed that the dominant role of endogenous NO production in tumors, regardless of the iNOS isoform involved, is to promote growth, rather than to strengthen host

defence mechanisms that inhibit growth.

Kennovin et al 1994 demonstrated that in order to suppress the tumor growth, long term administration of NOS inhibitors are required but the withdrawal of NOS inhibitors results in rapid resumption of normal growth rate. Babal et al 1997 and pechanova et al 2004 showed that L-NAME causes a variety of undesirable cardiovascular changes including myocardial fibrosis and serious hypertension(Kanagy 1997) 210 mm Hg systolic after 21 days compared with normal value of ~ 140 mm Hg in a rat model at a dose lower than required for tumor growth inhibition. Atherosclerosis developed when L-NAME was administered which caused leukocyte to get attached with an arterial endothelium (Nabah et al 2005). Hence, chronic administration of non-specific NOS inhibitor does not seem to be a viable option for treatment in elderly cancer patients. The use of iNOS specific inhibitor can be used in such case because it avoids cardiovascular effects as iNOS does not affect NO generation in normal vascular architecture but it was found that this treatment option is viable only against tumors that express high levels of iNOS (Thomsen et al 1997) and Franchi et al 2005 demonstrated that even after using iNOS specific inhibitor against those tumors expressing high levels of iNOS but suppression of growth was not there in all regions within the tumor.

Overproduction of NO

Nitric oxide over expression can be evaluated as a therapeutic strategy through pro-apoptotic, antimetastatic, radio sensitizing and chemo sensitizing activities.

Activity as a single agent- The cytotoxic effect of NO to cancer cells is mediated by the generation of pro-apoptotic intermediates such as peroxynitrite and N2O3(Lechner et al 2005) and may inhibit DNA repair enzyme including poly(ADP-ribose) polymerase (Sidorkina et al 2003). The ideal way to generate high concentration of NO in cancer cells is iNOS gene transfer techniques. Juang et al 1997, 1998 used a murine melanoma cell line in vitro to transfer iNOS, then these cells were implanted in mice tumors, it was found that tumors grew more slowly and were less likely to metastasize than uninfected cells. Solar et al 2000 did in vivo transfection study where naked iNOS DNA injection in mouse thyroid cancer model where significant growth inhibition was found. One of the studies where liposomal vector was used to deliver a plasmid containing iNOS determined by constitutive or inducible promoters and inhibition of tumor growth was seen in syngenic mouse tumor and xenograft model (Worthington et al 2002, 2004, 2005).Application of NO over expression as a single modality fails to exploit its radio and chemo sensitizing potential. There has been evidence that pro-apoptotic activity of NO can be enhanced by using other classes of anticancer agents. Recent study was conducted using a breast cancer cell line which demonstrated NO induced apoptosis, when inhibitor of farnesyltransferase was added, but no effect was seen in breast epithelial cells (Pervin et al 2001).

Radio sensitizing activity- NO was demonstrated to be a potent radio sensitizer in bacteria and mammalian cells (Howards-Flanders 1957; Gray et al 1958; Dewey 1960) soon after the demonstration of oxygen effect in radiation biology (Gray et al 1953).The importance of NO as a radio sensitizer was rediscovered (Mitchell et al 1993,1996,1998; Janssens et al 1999).NO was produced by several mechanisms and it was shown that higher concentration in the micro molar range enhanced the in vitro radio sensitizing ratio of 2.1-2.5 as effective as oxygen and much more effective as any radio sensitizing drugs which has been tested in vivo. Griffin et al 1996 obtained similar results using NO donors. Recently gene therapy strategies have been used to radiosensitize tumor cells both in vivo and in vitro. Matsumoto et al 2001 demonstrated radio sensitizing effect of NO at

high concentration but at much lower levels leads to a radio protective bystander effect. Thus, a great therapeutic potential lies in the fact when high concentration is achieved in tumors while maintaining the lower concentration in normal tissue.

NO plays a major role in radiation-induced bystander mechanisms in addition to radio sensitization. Shao et al 2003;Sokolov et al 2005 indicated radiation induced NO generation which is contributing to the bystander effect by showing Nitric oxide specific scavenger present in culture medium, reduced cellular damage in the surrounding cell population.

Chemo sensitizing activity- Kroncke 2001; Kroncke et al 2002 demonstrated that NO is capable of nitrosating or oxidizing Zn finger-containing proteins leading to the denaturation. Earlier studies have shown that Zn finger-containing DNA repair proteins including Fpg (Wink & laval 1994), DNA ligase (Graziewicz et al 1996) and 06-methylguanine-DNA-methyl transferase (Laval and wink 1997) can be inhibited by NO donor compounds in vitro and in vivo.

There has been evidence which proves that levels of NO directly mediate the effects of some of the cytotoxic agents like cisplatin (Son & Hall 2000) and 5-Fluorouracil (Oshima et al 2001).High concentration of NO was found to have chemo sensitizing activity. Wink et al 1997 showed that when V79 lung fibroblasts were treated with either NO saturated medium for 30 minutes or NO donor drugs for 60 minutes, resulted in sensitization with subsequent cisplatin exposure. Another study was carried out by Azizzadeh et al 201 where similar results were obtained in Head and neck squamous carcinoma cells using different NO donors, but in this case only long acting donors were effective as chemo sensitizer. Liu et al 2004 showed that class of NO releasing agent (diazeniumdiolates) enhanced cisplatin cytotoxicity in rat liver epithelial cells line by increasing intracellular concentration of cisplatin via activation of MAP kinase pathways. When MCF-7 human breast cancer cells were exposed in vitro to NO gas, NO donors (when given before doxorubicin) or iNOS gene transfer along with doxorubicin, NO was found to chemo sensitize. Jea et al 2003 showed that NO donor (nitrosocaptopril) enhanced the transmembrane uptake of taxol and was found to increase the cytotoxic effect in two prostate cancer cell lines in vitro but none of the effect was seen in neuroblastoma cell lines. Role of P-glycoprotein mediated drug transport was suggested.

Konovalova et al 2003 studied the in vitro chemo sensitization of NO. The group used combination of NO donor 3, 3-Bis (nitroxymethyl) oxetane with cyclophosphamide and doxorubicin in mouse models of lung cancer, melanoma and leukemia. This showed impressive results by prolonging survival of leukemia bearing animals as compared to cytotoxic drugs alone. NO donors in combination with cyclophosphamide enhanced inhibition of metastasis from subcutaneously implanted melanomas compared with cyclophosphamide alone. NO therapy inhibited the development of resistance to cyclophosphamide in leukemic cells. This group reiterated the potential of NO therapy and further studies have been instigated in this area. Though, NO acts as a chemo sensitizer in combination with cytotoxic agents, but there are exceptions. iNOS derived NO was found to confer resistance in a rat glioma cell line against chloroethylnitrosourea (Yen et al 2001) via mechanism involving S-nitrosoglutathione (Yen et al 2004), a potent antioxidant obtained due to interaction between NO and glutathione. Further, full understanding of mechanisms involving the chemo sensitizing activity of NO needs to be evaluated.

Discussion

NO level has a fundamental aspect in cancer biology .The multidimensional roles of NO in

cancer are based on timing, location and concentration. When a tissue is exposed to high level of NO for prolonged period either during chronic inflammation or environmental exposure, accumulate mutations due to NO or mediated by genotoxic agents. As the tumor progresses, NO derived from iNOS kill tumor cells. Although, NO can mediate capillary leakiness, stimulate angiogenesis and limit infiltration of leucocytes but it has been found that NO could also limit metastasis and could cause apoptosis of tumor cells. This dichotomy of Nitric oxide has been a great challenge for scientists working in cancer therapy. Thus, the timing, and location of NO is important determinant in cancer cell biology. This is also important to evaluate the use of systemic NOS inhibitors and NO donors which involves a combined properties (chemical, biochemical, toxicological & physiological) properties of NO.

Hence, NO has a tremendous potential as an anticancer agent if targeted to tumor at high concentration. NO donors have shown many anticancer effects invitro but the dose which required in vivo resulted in unacceptable systemic effects such as hypotension which made it unsuitable for clinical use.NOS activation confined to tumor volume and gene therapy combined with NO generating capability proves to be a therapeutic gain. More specific targeting, gene activation combined with radiotherapy and chemotherapy will result in effective tumor control but we need to overcome problem or search a remedy for problems associated with gene therapy such as delivery, tumor targeting and toxicity of viral vectors which will allow further exploitation of NO. Nitric oxide being a mediator of cancer has led investigators to develop strategies by manipulating in vivo production and exogenous delivery of this molecule as therapeutic gain. Attempts to develop NO-based cancer therapy are still in budding stages, and an extended understanding of the levels of NOS expression, timing, and the concentrations of NO produced in the tumor vasculature which is key to the development of novel strategies for diagnosis, prevention, treatment and cure of cancer. Understanding tumor biology at molecular and cellular level which has been affected by NO will allow researchers to exploit the potential anticancer properties of drugs interfering with NO metabolism.

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