Nitrosative stress in cancer therapy

David G. Hirst, Tracy Robson

School of Pharmacy, Medical Biology Centre, Queens University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, U.K..

TABLE OF CONTENTS

1. ABSTRACT

Reactive nitrogen species play important roles in cell signalling, but when present at high concentrations they can subject cells to nitrosative stress, which may lead to cell death. Nitric oxide (NO˙) is now recognized as playing important roles in cancer aetiology and progression and it can influence the outcome of cancer treatment. It is synthesised by the action of nitric oxide synthases (NOSs) on the amino acid arginine. Although NO˙ is not highly reactive with biological molecules, it reacts readily with other oxygen radicals to generate highly damaging reactive nitrogen species such as peroxynitrite, nitrogen dioxide and dinitrogen trioxide.   These are potent inducers of apoptosis and necrosis. They may also inhibit DNA repair mechanisms, leading to mutation and carcinogenesis.   Both inhibition and over-production of NO˙ have been investigated as strategies for cancer therapy. There is clear evidence that administration of competitive inhibitors of NOS can significantly slow the growth of solid tumors in rodent models, probably by reducing blood flow, and this creates a hypoxic environment that is conducive to the activation of bioreductive anticancer agents. Alternatively, generation of NO˙ concentrations in the high micromolar range by NO˙ donor drugs or gene therapy with inducible NOS is directly cytotoxic to cells and has been shown to inhibit tumor growth. At these high concentrations NO˙ is also an excellent sensitizer to radiation and to some chemotherapeutic agents, particularly cisplatin. Thus, manipulation of NO˙ levels in tumors offers exciting opportunities to improve the effectiveness of cancer treatment.

2. INTRODUCTION

2.1. Free radicals in cancer biology

The role of free radicals as key players in the biology and therapy of cancer is well-established (1). By far the most biologically important of these are reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can be generated both by endogenous metal-catalysed reactions and by exposure of cells to certain anticancer agents such as bleomycin (2) and tirapazamine (3) or to ionizing or UV radiation (4). ROS are known to activate numerous signalling cascades. The most significant of these involve nuclear transcription factors and protein kinases, which in turn control cell cycle checkpoints, stress responses and differentiation and are upstream regulators of numerous cytokines (1). Reactions involving ROS are also seen as potential targets for cancer therapy (5) and have been widely studied.

The most important source of nitrogen for the generation of reactive nitrogen species (RNS) *in vivo* is nitric oxide (NO˙). It is well known to be a product of the catalytic action of the nitric oxide synthase enzyme family on L-arginine (6). However, recent evidence suggests that it can also be formed by reduction of nitrite, which can arise in the body by ingestion or from bacterial metabolism (7). Low concentrations of NO˙ exert subtle effects in cells, some of which are the key homeostatic regulators in several tissues. Nitrosative stress on the other hand arises when the rate of generation of RNS exceeds the capacity of a cell to neutralise them and damaging interactions with macromolecules (lipids, proteins and nucleic acids) compromise cellular function (1). It can originate from the

endogenous generation or exogenous addition of nitric oxide (NO⋅), which in turn interacts with ROS to generate numerous nitrogen oxides of widely differing reactivity (8).

The past five years have seen an explosion of interest in the impact of NO⋅ on the aetiology, progression, treatment and prognosis of cancer. In 2003 Wink and Mitchell, two major contributors to the field, summarised the available data on the subject in a concise review (9), concluding that "In tumor biology, NO⋅ continues to confuse and confound us". Since then over 3000 articles have been published containing evidence of relevance to this field; some of these have helped clarify the role of NO⋅, while others have added additional complexity to the picture. This review will attempt to summarise our current understanding of the importance of RNS in cancer biology and their potential roles in cancer therapy.

2.2. Synthesis of (NO⋅) in biological systems

The cellular generation of NO⋅ occurs by an oxidation reaction involving L-arginine as the substrate and tetrahydrobiopterin (10) nicotine adenine dinucleotide, flavin adenine dinucleotide, flavin mononucleotide, and protoporphyrin IX (a source of haem) as cofactors (11,12). This reaction, is catalysed by three main isoforms of the nitric oxide synthase (NOS) enzyme family (13), although NOS activity has also been demonstrated in mitochondria and this can probably be attributed to a fourth distinct isoform (14). Endothelial NOS (eNOS) and neuronal NOS (nNOS) are expressed constitutively and regulated post translationally by association with other proteins including caveolins (15). Their expression is largely, but not exclusively, restricted to the vasculature (16) and nervous system respectively (17), where they are known to generate NO⋅ as a signalling molecule at picomolar to nanomolar concentrations, mainly as a homeostatic regulator of blood pressure and central nervous activity. They also play a role in gut secretion and motility (18). eNOS and nNOS are dependent on Ca^{2+}/calmodulin for their activation, probably because of the presence of specific sequences in the reductase domains of the enzymes (19).

The inducible isoform of NOS (iNOS) is not constitutively expressed in normal tissues and its concentration is regulated mainly at the transcriptional and translational levels (20), though some post translational regulation is now recognised (21). Once generated in the cell, iNOS is capable of generating very high concentrations (high micromolar) of NO⋅ over a prolonged period (20). An increasing number of cytokines and other signalling molecules are now known to mediate iNOS expression (22); in addition, iNOS expression is sensitive to environmental stress factors such as hypoxia (23). While all three isoforms of NOS have been detected in tumors (24), given the conditions prevailing in the tumor microenviroment, such as hypoxia and increased superoxide production, it is not surprising that iNOS is the isoform most usually associated preferentially with malignant tissue and that it is rarely detected in normal tissues in the absence of pathology (22). Thus, the conditions existing in the microenvironment of tumors are conducive to the generation of high concentrations of NO⋅

and there is compelling evidence that this influences their biology, progression and response to therapy. There is also strong evidence that NO⋅ generation plays a key role in the aetiology of many cancers (25) and may be a major mediator of carcinogenesis associated with inflammation (22, 26, 27) particularly in the colon (28) and gastro-oesophageal junction (29).

2.3. Biological reactions involving NO⋅ and generation of RNS

A broad spectrum of biologically reactive molecules is now known to be generated by the interaction of NO⋅ with O_2 and reactive oxygen species under physiological conditions (8). These in turn react with thiols, proteins and lipids with profound consequences for the integrity of the cell. However, the most significant reaction of NO⋅ in the absence of pathology is its binding to the prosthetic haem group of soluble guanylate cyclase, which massively increases the activity of the enzyme (30). This leads to rapid synthesis of cyclic GMP, which in turn mediates relaxation of vascular smooth muscle (31). This is the most important mechanism maintaining steady state control of vascular tone.

Two key reactions of NO⋅ with either O_2 or the O_2^- radical initiate the generation of a wide spectrum of RNS (8). Some of these species are highly reactive with cellular macromolecules, causing, for example, lipid peroxidation and nitrating tyrosine-containing proteins (32), impairing or modifying their function. Perhaps the best characterised reaction of NO⋅ with reactive oxygen species is its very rapid interaction with superoxide (O_2^-). This leads to generation of peroxynitrite ($OONO^-$), a potent oxidising agent (33, 34) and mediator of cellular damage that triggers apoptosis in many cell types via a variety of mechanisms (35-38). Common features are lipid peroxidation (39, 40) and the activation of caspase-3 (41). There is also compelling evidence that NOSs can generate O_2^- in addition to NO⋅ (42-45) creating the ideal circumstances for $OONO^-$ formation. Clearly, however, this does not happen with high efficiency in healthy tissue, largely because O_2^- generation from NOS occurs only under conditions where its L-arginine substrate is depleted (46) and/or tetrahydrobiopterin is absent (43, 44) leading to uncoupling of the enzyme. In addition, superoxide dismutases, an enzyme family present as three isoforms in mammalian cells, act as highly efficient antioxidants, rapidly catalysing the conversion of O_2^- to much less reactive species such as H_2O_2 and molecular oxygen (47, 1). There is also compelling evidence that one isoform in particular, Mn-SOD, affects tumor growth and progression (48, 1). While detailed consideration of these mechanisms lies beyond the scope if this review, the presence of O_2^- appears to be a crucial determinant of whether or not exposure to NO⋅ will lead to apoptosis in a given cell type; furthermore, this may form the basis of high levels of apoptosis induction by NO⋅ in transformed cells (49-50) particularly compared to normal cells (51). Generally, then, peroxynitrite formation from NO⋅ and O_2^- is a key reaction in determining if apoptosis will be triggered. In the absence of O_2^-, lipid peroxidation will not occur and indeed NO⋅ has been shown

to be protective against peroxidation and other damaging radical reactions (52).

The final fate of peroxynitrite is dependent on the conditions. In most tissues, where CO_2 is present at significant concentrations, $CO_3^{\cdot-}$ and $NO_2^{\cdot}$ are generated and these species are capable of undergoing damaging reactions with other cellular constituents (53). In the absence of CO_2, peroxynitrite undergoes slow decomposition to $NO_2^{\cdot}$ (see below) and $OH^{\cdot}$. While these are also potentially very damaging radical species their rate of generation by this reaction is very slow and in any event is unlikely to occur in metabolising tissues with mM concentrations of CO_2. An additional basis for tumor specificity may be the dependence of one mechanism of $NO^{\cdot}$-induced apoptosis on hypoxia, though this appears to be independent of peroxynitrite (54).

If the cellular concentration of $NO^{\cdot}$ is high enough (55) it can be oxidised by physiological concentrations of O_2 to generate, via several intermediates including $NO_2^{\cdot}$, the potent nitrosating agent dinitrogen trioxide (N_2O_3) (8). This is the principal nitrosating agent derived from $NO^{\cdot}$ and is capable of interacting with many biologically significant molecules (53). Of particular interest is the interaction with thiols to generate S-nitrosothiols, which in turn can react with other intracellular thiols or thiol-containing proteins (56). In some systems this may convey regulatory signals (57-58) and in the case of cancer cells there is evidence that apoptosis is regulated by S-nitrosoglutathione (via bax signalling) (59) and S-nitrosylation of procaspase-9 (53). Additionally, the nitrosation of glutathione by N_2O_3, and possibly also $NO_2^{\cdot}$, occurs most efficiently at an oxygen tension of 3% (60) typical of the level likely to exist in many viable tumor cells *in vivo* (61).

The nitrogen dioxide radical ($NO_2^{\cdot}$) can be formed in several different reactions though some are too slow to be of any consequence *in vivo* (8). The most biologically significant are the reaction of $NO^{\cdot}$ at high concentrations with molecular oxygen (which also yields N_2O_3) and the decomposition of peroxynitrite in the presence of CO_2 to yield $NO_2^{\cdot}$ as well as $CO_3^{\cdot-}$ (62). The latter reaction is of particular interest because the cogeneration of $NO_2^{\cdot}$ and $CO_3^{\cdot-}$ shows selectivity for important biological molecules and, acting together, they are highly effective and selective in nitrating proteins (63,8), which can contribute to numerous disease processes (64) including cancer (65).

3. NO$^{\cdot}$ AND CANCER THERAPY

We have recently reviewed the importance of $NO^{\cdot}$ in cancer therapy (25) and there is now an extensive literature available (22, 66-69). As a general rule, malignant tumors express significant levels of NOS enzymes and often at higher concentrations than their normal tissue counterpart. This has been clearly demonstrated in a wide range of tumors including those of the bladder (70), brain (71, 72), breast (73-75), cervix (76), colon (77), endometrium (78), lung (79, 80), ovary (81), pancreas (82), prostate (83), and in melanoma (84). This

implies that tumors develop within an environment with high levels of nitrosative stress and are exposed to a range of RNS (see section 2.3). Potential therapies could therefore be designed to reverse this condition, which as we shall see, is responsible for maintaining the malignant phenotype of many cancers; alternatively, the damaging effects of RNS could be specifically enhanced. Strategies employing these two approaches will now be reviewed.

3.1. Inhibition of RNS generation.

We will focus mainly on methods of intervention to modify the generation of RNS in a manner that could be used to treat established solid tumors. However, it is worthy of note that over-expression of iNOS has been associated with generation of excessive levels of RNS during chronic inflammation and may play an important role in carcinogenesis by causing DNA damage (26, 85-89); there may, therefore, be a role for RNS inhibition in the chemoprevention of cancer. The generation of RNS in tissues including tumors occurs almost exclusively via the catalytic action of the NOS enzyme family; consequently, this offers a discrete target through which generation of $NO^{\cdot}$ can be inhibited. Furthermore the availability of a wide variety of inhibitors with selectivity for the NOS family of enzymes, or its specific isoforms, has allowed this concept to be tested both *in vitro* and *in vivo* (90-92).

Regardless of the underlying mechanism, the evidence from preclinical studies using NOS inhibitors in rodent tumor models *in vivo* clearly demonstrates inhibition of tumor growth. This was first shown over ten years ago when administration of N^G-nitro-L-arginine methyl ester (L-NAME) at concentrations of 1 -6 mg/ml in the drinking water was shown to reduce tumor growth rates by a factor of ~2, in a fully and rapidly reversible manner, such that tumor growth rates returned to normal within 24 h of drug withdrawal (93). Several other investigators have since demonstrated very similar results using L-NAME (94) or a similar arginine analogue N^G-nitro-L-arginine (NNLA) (95). A more recent study in a human pancreatic cancer model in nude mice showed that the addition of 1 mg/ml NNLA to the drinking water again reduced tumor growth rate by a factor of 2 (96); furthermore, these authors investigated several additional tumor parameters to elucidate the mechanisms behind the growth inhibition. Blood vessel diameters were reduced by 50% as was vessel perfusion (measured by Hoechst 33342) in the NNLA-treated tumors; there was also a small increase in apoptosis. A reduction in tumor perfusion in response to NOS inhibitors has previously been reported by others in a variety of experimental systems. Window chamber models have been used to demonstrate reductions in diameter and perfusion of rat tumor isografts (97) and human tumor xenografts (98). Similar results have also been obtained in orthoptic glioma (99, 100) and tissue isolated rat tumor models (101,102). Because the NOS inhibitors used in all of the above studies will inhibit all NOS isoforms, and all isoforms are known to be expressed in tumors (24) these data do not allow a more detailed analysis of the isoform/s responsible for enhancing tumor perfusion. This information would be important for planning a clinical strategy because the inhibition of nNOS and particularly

eNOS has been shown to induce significant cardiovascular pathology (103-106), specifically after chronic administration of L-NAME, as would be required to maintain tumor growth inhibition (93). One study, however, clearly demonstrates the importance of iNOS in the tumor growth response to NOS inhibitors (107). Treatment of mouse tumor isografts and human tumor xenografts with the specific iNOS inhibitor 1400W by continuous infusion, reduced tumor growth rates to the same degree (50%) as that seen in most studies with the non-specific NOS inhibitors. Tumors that did not express iNOS showed no response to 1400W. This suggests that iNOS is the main contributor to NO˙ generation in tumors, at least in the models tested and that its inhibition could be the basis for a clinical strategy to inhibit tumor growth. Few human studies with 1400W have been carried out, but there is evidence that it can be used safely in man (108). In summary, it is reasonable to conclude that tumors are exposed to high levels of nitrosative stress, predominantly as a consequence of iNOS expression; this in turn leads to a highly dilated vasculature that shows marked constriction in response to inhibition of the source of NO˙ production.

NOS inhibitors have also been shown to enhance the effectiveness of other therapies that depend on reduced blood flow or hypoxic conditions for their effectiveness. The toxicity of the bioreductive cytotoxin, RB6145, was significantly enhanced by administration of the non-specific NOS inhibitor L-nitro arginine in mouse tumors (KHT and SCCVII), while the inhibitor had no effect on RB6145 toxicity in bone marrow (109). Inhibition of NO˙ synthesis with L-NAME was found to increase heat-induced growth delay of FSaII tumors in mice, probably as a result of a reduction in the cooling effect of blood flow (110).

Another well established action of NO˙ at constitutive levels is to stimulate angiogenesis, both in tumors and normal tissues. Numerous investigators have studied the role of NO˙ (mainly with the use of non-isoform specific NOS inhibitors) on different aspects of angiogenesis (111-115) and have observed that inhibition of NO˙ synthesis results in potent inhibition *in vitro* and *in vivo*. A potent anti-angiogenic effect was also demonstrated for aminoguanidine, an inhibitor with specificity for iNOS (116). One *in vivo* study also showed that NO˙ specifically activated angiogenesis by longitudinal splitting of capillaries and had no effect on sprout formation (117). Several studies have attempted to identify downstream mediators of the angiogenic action of NO˙. One likely candidate is vascular endothelial growth factor (VEGF); it has been known for some time that NO˙ can upregulate VEGF in liver and brain tumor cells via a guanylate cyclase-dependent mechanism that is dependent on de novo protein synthesis and leads to stabilisation of VEGF RNA (118). Increased expression of VEGF in response to administration of the NO˙ donor S-nitroso-glutathione was demonstrated in normal cells (119, 120). The association between NO˙ and VEGF has also been established in human primary astrocytomas and their expression correlated directly with grade of disease (71).

One particularly valuable study directly demonstrated the bipolar character of NO˙ in angiogenesis (121). Exposure of endothelial cells to 50-200 μM concentrations of the NO donor *S*-nitro-*N*-acetyl penicillamine (SNAP) caused a dose-dependent increase in several angiogenic end points and in the phosphorylation of PKC, ERK and c-Jun, and AP-1 activation. At higher concentrations (up to 4 mM) these endpoints were markedly inhibited by SNAP in a dose-dependent manner.

IL-8 is another cytokine with a well-established role as a mediator of angiogenesis (122, 123) and there is now considerable evidence that NO˙ upregulates IL-8 (together with VEGF) expression (probably at the transcriptional level) in colon cancer (124) and melanoma (125) cells.

In addition to upregulating pro-angiogenic cytokines, NO˙ has been shown to down-regulate inhibitors of angiogenesis (such as angiostatin) in heart muscle (126). To what extent, then, does abrogation of this process by NOS inhibition contribute to the reduced growth rate of tumors *in vivo*? Few data are available, but one recent study (96) showed that vessel density determined histologically in a pancreatic tumor model was reduced modestly (~20%) when the host animals were given the NOS inhibitor NNLA, but the most dramatic effect was a 40% reduction in the cross sectional area of the tumor vasculature. Given that the resistance of a blood vessel to flow is inversely proportional to the 4th power of the radius, blood flow reduction is likely to be the predominant mechanism contributing to the 50% reduction in tumor growth rate seen in that study.

3.2. Enhancing nitrosative stress for therapeutic benefit

As we have seen (2.3), NO˙ is not highly reactive with cellular macromolecules, but is capable of undergoing interactions with molecular oxygen to generate dinitrogen trioxide and with O_2^- to yield $ONOO^-$ which, on decomposition in the presence of CO_2 yields NO_2^-. All of these RNS are capable of damaging reactions with lipids, proteins and nucleic acids. A common consequence is the induction of apoptosis following recognition of the damage by the cell, though death by necrosis also occurs (127). However, there is also clear evidence that NO˙ can inhibit apoptosis in some cells, while promoting it in others (128) and that this probably occurs via inhibition of mitochondrial respiration and inhibition of caspases by *S*-nitrosylation (129). In attempting to generalise the cellular response to NO˙, it is clear that, concentration is important. This is a notoriously difficult parameter to control *in vitro* let alone *in vivo,* which may account for enormous variation in responses between studies. One study, however, used a membrane delivery system to maintain tight control of NO˙ concentration in lymphoblastoid cells and was able to demonstrate that thresholds for steady state concentration (~0.5 μM) and total cumulative dose (~150 μM.min) must both be exceeded if cell death was to occur (130). Interestingly, a lymphoblastoid line lacking wild type p53 required a higher cumulative dose (~300 μM) to kill the cells (131). Variations in NO˙ production are not the only confounding factors determining response in a given cell system: interaction with thiols, metal ions,

Table 1. Enhancement of the effects of chemotherapeutic drugs by NO·

Cisplatin	Experimental model	Sensitizer enhancement ratio	Cell/animal survival ratio	Source of NO·	Reference
Cisplatin	Fibroblasts *in vitro*	NA	~60	NO· gas in solution	155
Cisplatin	..	NA	800-3000	NO· donor drugs	155
Cisplatin	Hamster lung fibroblasts *in vitro*	2.8 at 50% survival	NA	NO· donor drug	156
Cisplatin	Mouse leukemia *in vivo*	NA	2.6 (animal survival ratio at 60 d)	NO· donor drug	159
Cisplatin	Rat liver epithelial cells *in vitro*	3.7-5.8 at 50% survival	NA	NO· donor drugs	157
Cisplatin	Human ovarian cancer cells *in vitro*	NA	~3	NO· donor drug	158
Oxaliplatin	Colon cancer *in vitro*	NA	3.7-4.0	NO· donor drug	146
Oxaliplatin	Colon cancer *in vivo*	NA	2.7 (apoptotic ratio; 60% reduction in growth rate)	NO· donor drug	146
Doxorubicin	Human breast cancer *in vitro*	NA	8	NO· gas in solution	163
Doxorubicin	Human breast cancer *in vitro*	NA	~1.6	NO· donor drug	160
Taxol	Prostate cancer *in vitro*	5-12	NA	NO· donor drug	164
Taxol	Neuroblastoma *in vitro*	1-3	NA	NO· donor drug	164
Cyclophosphamide	Mouse melanoma *in vitro*	NA	1.6 (inhibition of metastasis)	NO· donor drug	159
Cyclophosphamide	Mouse leukemia	NA	100% versus 0% animal survival	NO· donor drug	159

NA= not available

protein tyrosine and interaction with ROS will all differ (128).

Many investigators have studied the effect of delivering high concentrations of NO· and its reactive products. Delivery methods include nitric oxide donor drugs and iNOS gene transfer that can achieve concentrations of NO· in the micromolar range. This lead to apoptosis in a wide variety of human cancer cells including bladder (132-134) breast (135), colon (136, 137), pancreas (138, 68) and prostate (139). There is also some evidence that NO· mediated apoptosis may show some selectivity for transformed compared with normal cells (fibroblasts) and that this can be attributed to increased superoxide production in transformed cells leading, on interaction with NO·, to peroxynitrite production (51). Further evidence suggests that solid tumors containing hypoxic regions may be specifically vulnerable to peroxynitrite-induced apoptosis because of enhanced generation of ROS (including $O_2^{·-}$) under hypoxic conditions (140). Is there any *in vivo* evidence? The first study used direct injection of a plasmid containing the iNOS gene into an experimental thyroid cancer model in rats and showed significant inhibition of tumor growth even though only 1% of the cells in the tumors had been transfected (141). Several studies have since investigated apoptosis induction by NO· using iNOS DNA constructs and have seen extensive apoptosis within 24 hours of transfection in rodent tumor (142, 143) or human tumor xenograft (144,145) models. Nitric oxide donor drugs have since been shown to induce similar effects in colon cancer xenografts (146). Thus, expression of NO· in tumors at high concentrations has therapeutic potential, but none of the above studies suggests that NO· could be effective as a stand alone treatment; it is likely to be combined with other conventional or novel therapies.

3.3. Enhancing conventional therapies
NO· or its reaction products have been shown to interact with and damage proteins; in particular, it can nitrosate zinc finger containing enzymes leading to their denaturation (147, 148). This includes many of the DNA repair proteins such as Fpg (149), DNA ligase (150), O^6–methylguanine-DNA-methyltransferase (151), poly (ABP-ribose) polymerase (152), and enzymes involved in nucleotide excision repair (153). Compromised repair capacity induced by high concentrations of NO· should, therefore, increase the toxicity of most DNA damaging agents; there is now a considerable body of evidence to support this, particularly for cisplatin (154).

Chemosensitization by RNS has been studied most extensively in combination with cisplatin. A variety of techniques have been used to deliver high NO· concentrations (high μM) to cells in culture including NO· gas saturation, NO· donor drugs or iNOS gene transfer. The first study (155) used NO·-saturated medium or the NO· donors DEA/NO or PAPA/NO to sensitize fibroblasts to cisplatin. While no enhancement ratio can be calculated from their data, surviving fraction after cisplatin was reduced by over 1000 fold by the addition of NO· donors. Since then, chemosensitization has also been demonstrated in head and neck carcinoma cells (156), in liver cells (157) and in ovarian cancer cells (158). The latter study also included evidence for increased intracellular retention of cisplatin by a mechanism involving mitogen activated kinases, with obvious implications for multidrug resistance. Whatever the mechanism, enhancement of the antitumor effect of platinum-based drugs can also be achieved *in vivo*. Significantly prolonged survival was seen in leukaemia bearing mice treated with a combination of cisplatin and an NO· donor compared with animals treated with cisplatin alone (159). A recent study using oxaliplatin in combination with the NO· donor NCX 4040 achieved a 60% reduction in tumor growth rate compared with oxaliplatin alone and a 3 fold increase in the number of apoptotic cells (146).

Recently, a study in breast cancer cells grown as spheroids demonstrated that the NO· donor DETA/NO· was

able to reverse acquired resistance to doxorubicin (160). There is also evidence that inhibition of drug efflux by high concentrations of NO⁻ (induced by cytokines) may be another mechanism of enhancement of the cytotoxicity of this drug (161). These authors have also shown that the ability of statins to revert the resistance of colon cancer cells to this agent may be mediated by NO⁻ (162). An important insight into the mechanisms of NO⁻ induced enhancement of doxorubicin is provided by a recent study (163), which demonstrated the importance of the sequencing of NO⁻ delivery in relation to cytotoxic drug exposure. NO⁻ delivered 30 min before doxorubicin enhanced its cytotoxicity by eight fold (in terms of cell survival), but when doxorubicin was given 30 min before or simultaneously there was little or no enhancement. These authors proposed that time might be required for NO⁻ to modify critical signalling pathways regulating apoptosis. We suggest that inhibition of DNA repair enzymes by NO⁻ (153) may also occur during that time. Finally, using AdiNOS gene transfer this group were able to show enhanced doxorubicin toxicity to breast cancer cells, but not to normal cardiac myoblasts under the same conditions. This provides a basis for selectivity of NO⁻ therapy.

Limited data exist for other drug combinations. NO⁻ delivery using a donor drug increased the cytotoxicity of taxol in prostate cancer cell lines, but had no effect in neuroblastoma cell lines (164). An NO⁻ donor has also been used in combination with 5-fluorouracil, but in this case toxicity was only additive (146). Enhancement of the cytotoxicity of cyclophosphamide by an NO⁻ donor has been demonstrated *in vivo*, leading to an impressive delay in tumor regrowth (158).

NO⁻ is also a potent radiation sensitizer. The radiosensitization of hypoxic cells by NO⁻ was first described at about the same time as the oxygen effect in radiation biology was characterised in mammalian systems (165-167). While the evidence existed then that NO⁻ has a sensitizing efficiency similar to that of O_2, it did not generate the same level of interest as in the "oxygen effect". This may have been because NO⁻ was considered to be an environmental pollutant and not the fundamental regulator of cellular signalling pathways that we now recognise (168, 169). Within the last ten years, however, extensive studies have been carried out to quantify the radiosensitizing effect of NO⁻ using a variety of strategies. While it is possible to deliver authentic NO⁻ gas in radiosensitization experiments *in vitro* (170) most investigators have found it more practical to use a variety of drugs that donate NO⁻ in the cellular environment (171-172). Radiosensitization by NO⁻ generated via a redox reaction with Agneli's salt was also demonstrated (173). In these studies NO⁻ gave sensitizer enhancement ratios of 1.6 -2.0 when the donors were added at 0.1 – 1.0 mM concentrations; this is similar to what would be expected for oxygen.

Rather than introducing NO⁻ donor drugs, an alternative approach is to exploit the action of iNOS as a potent and long lasting generator of NO⁻ from L-arginine.

The first study to do this (174) achieved an enhancement ratio of 2.5 in hypoxic EMT-6 tumor cells in culture by stimulating them with interferon gamma, even though the peak NO⁻ levels generated by this method were lower than those achieved with an NO⁻ donor. This suggests that the duration of NO⁻ exposure rather than just the concentration at the time of irradiation is important to the cellular response and in this respect differs from the effect of oxygen (175).

Developments in gene transfer have opened up the possibility of introducing iNOS into cells as a suicide gene therapy to be combined with other modalities, including radiation. In our own studies we have focussed on gene transfer using liposomal delivery of vectors expressing high levels of iNOS in rodent and human tumor cells *in vitro* and as solid tumors *in vivo*. Constitutive (CMV) and radiation- inducible (WAF-1) promoters were used to drive expression of the iNOS gene in RIF-1 tumor cells in culture and achieved enhancement of radiosensitivity similar to that seen with oxygen (176). This strategy can also be applied successfully *in vivo*: we used direct injection of WAFiNOS and CMViNOS constructs in a liposomal vector to transfect RIF1 (mouse) and HT29 (human) tumors and achieved enhancement ratios of 1.6 -2.0 in combination with radiation (142, 144). Adenoviral delivery can also be used successfully with iNOS gene therapy of tumors (177). Very similar enhancement ratios were obtained in human colon cancer xenografts in combination with single dose and fractionated (2 Gy) irradiation. It is perhaps surprising that enhancement ratios close to the full oxygen effect can be achieved *in vivo* with a technique that expresses iNOS in a very small proportion of the tumors cells (transfection efficiencies ~1%). Therefore, in considering the role of NO⁻ in the sensitivity of tumors *in vivo* it is important to differentiate the direct effects of NO⁻ from physiological changes that could alter oxygenation. Two explanations are likely: a) NO⁻ generation could lead to enhanced tumor blood flow and oxygen delivery, so sensitizing previously hypoxic cells. b) NO⁻ is a highly diffusible molecule with very short half life in the presence of oxygen or haem proteins (178), but in regions of tumors with few blood vessels and very low oxygen tensions its lifetime could be much longer (several seconds) and diffusion distance greater. There is evidence for the former mechanism: intra venous administration of the NO⁻ donor SIN-1 within a narrow dose range (0.5-2.0 mg/kg) resulted in increased oxygenation and radiosensitivity, which was attributed to improved blood flow (179), furthermore, iNOS gene therapy using adenoviral delivery (177) caused an increase in tumor vascularity, which could allow non-transfected bystander cells to be sensitized. However, it is reasonable to suppose that diffusion of NO⁻ must play an important part in tumor radiosensitization *in vivo* after iNOS activation or transfection because the catalytic action of NOS on arginine to generate NO⁻ requires O_2 as the source of an oxygen atom (13). Further studies are required to distinguish the relative importance of these two mechanisms, which will probably differ between tumors.

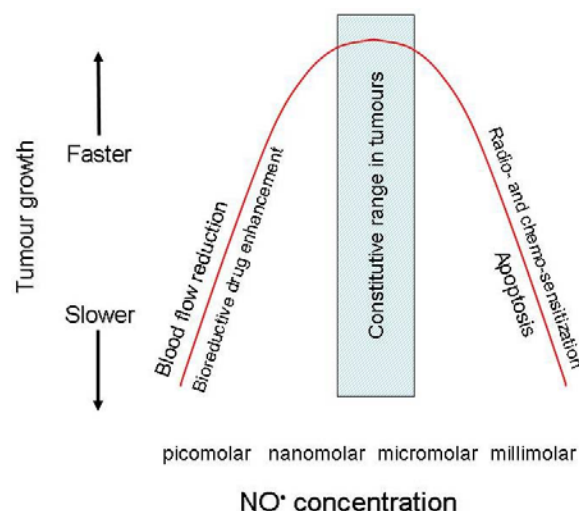While NO• may radiosensitize by fixation of DNA radicals in a manner similar to oxygen (although this

Figure 1. Diagramatic representation of the bell-shaped relationship between NO˙ concentration and tumor response. The abnormally high NO˙ concentration found in tumors compared with normal tissues is mainly generated by iNOS and maintains the tumor vasculature in a highly dilated condition which supports rapid growth. Inhibition of that constitutive expression leads to reduced blood flow and nutrient deprivation, which can sensitize tumors to bioreductive cytotoxins. Delivery of very high NO˙ concentrations leads to high levels of nitrosative stress, triggering apoptosis. It also sensitizes the tumor cells to other cytotoxic insults.

remains a matter of debate) it also exerts more subtle influences by activating a host of signalling pathways within the cell that trigger apoptosis (180). There is also evidence that these death signals can be exported to other cells: microbeam irradiation of individual cells, even if the nucleus was excluded, leads to extensive DNA damage as measured by micronucleus formation in neighbouring unirradiated cells and the effect could be eliminated by the use of NO˙-specific scavengers (181, 182).

3.4. Enhancing novel therapies

NO˙ may also play an important role in the mechanisms of action of and interaction with novel cancer therapies. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to be a selective cytotoxin in some, though not all cancer cells (183-185). There is now clear evidence that NO˙ can sensitize cancer cells to TRAIL-induced apoptosis; sensitization of prostate cancer cells to TRAIL was also achieved when NO˙ at high concentration was delivered by the donor DETANONOate. Inactivation of NF-κB and inhibition of Bcl-$_{xL}$ were implicated (186). In another study in human colorectal cancer cells the NO˙ donor sodium nitroprusside enhanced TRAIL-induced apoptosis via a mitochondria-dependent pathway (187). However, NO˙ at lower constitutive concentrations may even protect prostate cancer cells from TRAIL-induced apoptosis (188). This is consistent with the conclusion that NO˙ at low levels in tumors are protective of cell survival, whereas high levels induce death signalling.

4. PERSPECTIVE

The generation of NO˙ in tumors mainly, though not exclusively by iNOS- plays a key role in maintaining the malignant phenotype. Inhibition of this process consistently reduced the rate of tumor growth, but was unable to prevent growth entirely. In addition, NOS inhibition created a poorly perfused, hypoxic environment (Figure 1). This could be exploited to enhance the cytotoxicity of bioreductive drugs, but would be detrimental to the effectiveness of conventional chemotherapy and radiotherapy. The opposing strategy of targeting very high NO˙ levels to tumors looks more promising in that it can be used to enhance conventional therapies as well as directly causing cell death. While it has clearly been shown in several studies to delay tumor growth by inducing extensive apoptosis, it has also consistently enhanced radiotherapy and chemotherapy given in clinically relevant schedules. Thus, introduction of NO˙ augmentation as an adjuvant therapy should not be problematical. Furthermore, any undesirable systemic effects of NO˙ on blood pressure can be monitored easily and are readily reversible. These characteristics support the view that introduction of NO˙ augmentation therapy into clinical trials would be a low risk strategy with the potential for considerable patient benefit.

5. REFERENCES

1. Valko M, CJ Rhodes, J Moncol, M Izakovic, & M Mazur. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160, 1-40 (2006)
2. Yen HC, HM Chang, HJ Majima, FY Chen & SH Li. Levels of reactive oxygen species and primary antioxidant enzymes in WI38 versus transformed WI38 cells following bleomcyin treatment. *Free Radic Biol Med* 38, 950-959 (2005)
3. Wardman P. Electron transfer and oxidative stress as key factors in the design of drugs selectively active in hypoxia. *Curr Med Chem* 8, 739-761 (2001)
4. Feinendegen LE. Reactive oxygen species in cell responses to toxic agents. *Hum Exp Toxico.* 21, 85-90 (2002)
5. Pennington JD, TJ Wang, P Nguyen, L Sun, K Bisht, D Smart & D Gius. Redox-sensitive signalling factors as novel molecular targets for cancer therapy. *Drug Resist Updat.* 8, 322-330 (2005)
6. Li H & TL Poulos. Structure-function studies on nitric oxide synthases. *J Inorg Biochem* 99, 293-305 (2005)
7. Lundberg JO & E Weitzberg. NO generation from nitrite and its role in vascular control. *Arterioscler Thromb Vasc Biol.* 25, 915-922 (2005)
8. Pryor WA, KN Houk, CS Foote, JM Fukuto, LJ Ignarro, GL Squadrito & KJ Davies. *Free Radical Biology and Medicine*: It's a Gas, Man! *Am J Physiol Regul Integr Comp Physiol* 291, R491-511 (2006)
9. Wink DA & JB Mitchell. Nitric oxide and cancer: an introduction. *Free Radic Biol Med* 34, 951-954 (2003)
10. Wei CC, ZQ Wang, AL Meade, JF McDonald & DJ Stuehr. Why do nitric oxide synthases use tetrahydrobiopterin? *J Inorg Biochem* 91, 618-624 (2002)
11. Knowles RG & S Moncada. Nitric oxide synthases in mammals. *Biochem J* 249-258. (1994)

12. Stuehr DJ. Enzymes of the L-arginine to nitric oxide pathway. *J Nutr* 134, 2748S-2751S (2004)

13. Stuehr DJ. Mammalian nitric oxide synthases. *Biochim Biophys Acta* 1411:217-330 (1999)

14. Ghafourifar P & C Richter. Nitric oxide synthase activity in mitochondria. *FEBS Lett* 418, 291-6 (1997)

15. Feron O & JL Balligand. Caveolins and the regulation of endothelial nitric oxide synthase in the heart. *Cardiovasc Res* 69, 788-797 (2006)

16. van Haperen R, C Cheng, BM Mees, E van Deel, M de Waard, LC van Damme, T van Gent, T van Aken, R Krams, DJ Duncker & de R Crom. Functional expression of endothelial nitric oxide synthase fused to green fluorescent protein in transgenic mice. *Am J Pathol* 163, 1677-1686 (2003)

17. Wolf G. Nitric oxide and nitric oxide synthase: biology, pathology, localization. *Histol Histopathol* 12, 251-261 (1997)

18. Salzman AL. Nitric oxide in the gut. *New Horiz* 3, 352-64 (1995)

19. Chen PF & KK Wu. Structural elements contribute to the calcium/calmodulin dependence on enzyme activation in human endothelial nitric-oxide synthase. *J Biol Chem* 278, 52392-52400 (2003)

20. Hickey MJ, DN Granger & P Kubes. Inducible nitric oxide synthase (iNOS) and regulation of leucocyte/endothelial cell interactions: studies in iNOS-deficient mice. *Acta Physiol Scand* 173119-173126 (2001)

21. Kolodziejski PJ, JS Koo & NT Eissa. Regulation of inducible nitric oxide synthase by rapid cellular turnover and cotranslational down-regulation by dimerization inhibitors. *Proc Natl Acad Sci U S A*. 101, 18141-18146 (2004)

22. Lechner M, P Lirk & J Rieder. Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. *Semin Cancer Biol* Aug;15, 277-89 (2005)

23. Ferreiro CR, AC Chagas, MH Carvalho, AP Dantas, C Scavone, LC Souza, E Buffolo & PL da Luz. Expression of inducible nitric oxide synthase is increased in patients with heart failure due to ischemic disease. *Braz J Med Biol Res* 37, 1313-20 (2004)

24. Tozer, GM & S.A Everett. Nitric oxide in tumor biology and cancer therapy. Part 2: Therapeutic implications. *Clin. Oncol. (R Coll Radiol)* 9, 357-364 (1997)

25. Hirst, DG & T. Robson. Targeting nitric oxide for cancer therapy. *J Pharm Pharmacol (in press)*.

26. Kawanishi S, Y Hiraku, S Pinlaor & N Ma. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol Chem* 387, 365-372 (2006)

27. Sawa T & H Ohshima. Nitrative DNA damage in inflammation and its possible role in carcinogenesis. *Nitric Oxide* 14, 91-100 (2006).

28. Goodman JE, LJ Hofseth, SP Hussain & CC Harris. Nitric oxide and p53 in cancer-prone chronic inflammation and oxyradical overload disease. *Environ Mol Mutagen* 44, 3-9 (2004)

29. Iijima K, J Grant, K McElroy, V Fyfe, T Preston & KE McColl. Novel mechanism of nitrosative stress from dietary nitrate with relevance to gastro-oesophageal junction cancers. *Carcinogenesis* 24, 1951-1960 (2003)

30. Russwurm M & D Koesling. Guanylyl cyclase: NO hits its target. *Biochem Soc Symp* 71, 51-63 (2004)

31. Thippeswamy T, McKay JS, Quinn JP, Morris R. Nitric oxide, a biological double-faced janus--is this good or bad? *Histol Histopathol* 21, 445-458 (2006)

32. Radi R. Nitric oxide, oxidants, and protein tyrosine nitration. *Proc Natl Acad Sci U S A*. 101, 4003-4008 (2004)

33. Pryor WA & GL Squadrito. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 268, 699-722 (1995)

34. Squadrito GL & WA Pryor. The formation of peroxynitrite *in vivo* from nitric oxide and superoxide. *Chem Biol Interact* 96, 203-26 (1995)

35. Dickhout JG, GS Hossain, LM Pozza, J Zhou, S Lhotak & RC Austin. Peroxynitrite causes endoplasmic reticulum stress and apoptosis in human vascular endothelium: implications in atherogenesis. *Arterioscler Thromb Vasc Biol* 25, 2623-2629 (2005)

36. Vicente S, R Perez-Rodriguez, AM Olivan, A Martinez PalacianMP Gonzalez & MJ Oset-Gasque. Nitric oxide and peroxynitrite induce cellular death in bovine chromaffin cells: Evidence for a mixed necrotic and apoptotic mechanism with caspases activation. *J Neurosci Res* Apr 19, (2006) [Epub ahead of print]

37. Vinas JL, A Sola & G Hotter. Mitochondrial NOS upregulation during renal I/R causes apoptosis in a peroxynitrite-dependent manner. *Kidney Int* 69, 1403-1409 (2006)

38. Shacka JJ, MA Sahawneh, JD Gonzalez, YZ Ye, TL D'alessandro, & Estevez AG. Two distinct signaling pathways regulate peroxynitrite-induced apoptosis in PC12 cells. *Cell Death Differ* Jan 20, (2006) [Epub ahead of print]

39. Hogg N & B Kalyanaraman. Nitric oxide and low-density lipoprotein oxidation. *Free Radic Res* 28, 593-600 (1998)

40. Hall ED, MR Detloff, K Johnson & NC Kupina. Peroxynitrite-mediated protein nitration and lipid peroxidation in a mouse model of traumatic brain injury. *J Neurotrauma* 21, 9-20. (2004)

41. Lin KT, JY Xue, MC Lin, EG Spokas, FF Sun & PY Wong. Peroxynitrite induces apoptosis of HL-60 cells by activation of a caspase-3 family protease. *Am J Physiol* 274, C855-860 (1998)

42. Pou S, WS Pou, DS Bredt, SH Snyder & GM Rosen. Generation of superoxide by purified brain nitric oxide synthase, *J Biol Chem* 267, 24173–24176 (1992)

43. Xia Y & JL Zweier. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages, *Proc Natl Acad Sci USA*. 94, 6954–6958 (1997)

44. Vasquez-Vivar J, B Kalyanaraman, P Martasek, N Hogg, BS Masters, H Karoui, P Tordo & KAJ Pritchard. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors, *Proc Natl Acad Sci U. S. A.* 95, 9220–9225 (1998)

45. Weaver J, S Porasuphatana, P Tsai, S Pou, LJ Roman & GM Rosen. A comparative study of neuronal and inducible nitric oxide synthases: generation of nitric oxide, superoxide, and hydrogen peroxide. *Biochim Biophys Acta* 1726, 302-308 (2005)

46. Porasuphatana S, P Tsai & GM Rosen. The generation of free radicals by nitric oxide synthase. *Comp Biochem Physiol C Toxicol Pharmacol* 134, 281-9 (2003)

47. Mates JM & F Sanchez-Jimenez. Antioxidant enzymes and their implications in pathophysiologic processes. *Front Biosci* 4, D339-345 (1999)

48. Behrend L, G Henderson & RM Zwacka. Reactive oxygen species in oncogenic transformation. *Biochem Soc Trans* 31, 1441-1444 (2003)

49. Bauer G. Signaling and proapoptotic functions of transformed cell-derived reactive oxygen species. *Prostaglandins Leukot Essent Fatty Acids* 66, 41-56 (2002)

50. Qiao S, W Li, R Tsubouchi, M Haneda, K Murakami & M Yoshino. Involvement of peroxynitrite in capsaicin-induced apoptosis of C6 glioma cells. *Neurosci Res* 51, 175-83 (2005)

51. Heigold S, C Sers, W Bechtel, B Ivanovas, R Schafer & G Bauer. Nitric oxide mediates apoptosis induction selectively in transformed fibroblasts compared to nontransformed fibroblasts. *Carcinogenesis* 23, 929-941 (2002)

52. Nicolescu AC, JN Reynolds, LR Barclay & GR Thatcher. Organic nitrites and NO: inhibition of lipid peroxidation and radical reactions. *Chem Res Toxicol* 17, 185-196 (2004)

53. Dedon PC & SR Tannenbaum. Reactive nitrogen species in the chemical biology of inflammation. *Arch Biochem Biophys* 423, 12-22 (2004)

54. Lee VY, DS McClintock, MT Santore, GR Budinger & NS Chandel. Hypoxia sensitizes cells to nitric oxide-induced apoptosis. *J Biol Chem* 277, 16067-16074 (2002)

55. Liu X, MJ Miller, MS Joshi, DD Thomas & JR Lancaster Jr. Accelerated reaction of nitric oxide with O2 within the hydrophobic interior of biological membranes. *Proc Natl Acad Sci U S A*. 95, 2175-2179 (1998)

56. Stamler JS. S-nitrosothiols and the bioregulatory actions of nitrogen oxides through reactions with thiol groups. *Curr Top Microbiol Immunol* 196, 19-36 (1995)

57. Gaston B, D Singel, A Doctor & Stamler JS. S-Nitrosothiol Signaling in Respiratory Biology. *Am J Respir Crit Care Med* Mar 9, (2006) [Epub ahead of print]

58. Marshall HE, DT Hess & JS Stamler. S-nitrosylation: physiological regulation of NF-kappaB. *Proc Natl Acad Sci U S A*. 101, 8841-8842 (2004)

59. Ho YS, HL Liu, JS Duh, RJ Chen, WL Ho, JH Jeng, YJ Wang & JK Lin. Induction of apoptosis by S-nitrosoglutathione and Cu^{2+} or Ni^{2+} ion through modulation of bax, bad, and bcl-2 proteins in human colon adenocarcinoma cells. *Mol Carcinog* 26, 201-211 (1999)

60. Jourd'heuil D, FL Jourd'heuil & M Feelisch. Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide. Evidence for a free radical mechanism. *J Biol Chem*. 278, 15720-15726 (2003)

61. Kallinowski F, R Zander, M Hoeckel & P Vaupel. Tumor tissue oxygenation as evaluated by computerized-pO2-histography. *Int J Radiat Oncol Biol Phys* 19, 953-61 (1990)

62. Squadrito GL & WA Pryor. Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med* 25, 392-403 (1998)

63. Kikugawa K, Kato T, Okamoto Y. Damage of amino acids and proteins induced by nitrogen dioxide, a free radical toxin, in air. *Free Radic Biol Med* 16, 373-382 (1994)

64. Halliwell B, K Zhao & M Whiteman. Nitric oxide and peroxynitrite. The ugly, the uglier and the not so good: a personal view of recent controversies. *Free Radic Res* 31, 651-669 (1999)

65. Masri FA, SA Comhair, T Koeck, W Xu, A Janocha, S Ghosh, RA Dweik, J Golish, M Kinter, DJ Stuehr, SC Erzurum & KS Aulak. Abnormalities in nitric oxide and its derivatives in lung cancer. *Am J Respir Crit Care Med* 172, 597-605 (2005)

66. Williams EL & MB Djamgoz. Nitric oxide and metastatic cell behaviour *Bioessays*. 27, 1228-1238 (2005)

67. Thatcher GR. An introduction to NO-related therapeutic agents. *Curr Top Med Chem*. 5, 597-601 (2005)

68. Kashfi K & B Rigas. Molecular targets of nitric-oxide-donating aspirin in cancer. *Biochem Soc Trans* 33, 701-4 (2005)

69. Ekmekcioglu S, CH Tang & EA Grimm. NO news is not necessarily good news in cancer. *Curr Cancer Drug Targets* 5, 103-115 (2005)

70. Swana HS, SD Smith, PL Perrotta, N Saito, MA Wheeler & RM Weiss. Inducible nitric oxide synthase with transitional cell carcinoma of the bladder. *J Urol* 161, 630-634 (1999)

71. Pan JW, RY Zhan, Y Tong, YQ Zhou & M Zhang. Expression of endothelial nitric oxide synthase and vascular endothelial growth factor in association with neovascularization in human primary astrocytoma. *J Zhejiang Univ Sci B* 6, 693-698 (2005)

72. Hara A & I Okayasu Cyclooxygenase-2 and inducible nitric oxide synthase expression in human astrocytic gliomas: correlation with angiogenesis and prognostic significance. *Acta Neuropathol (Berl)* 108, 43-48 (2004)

73. Vakkala M, K Kahlos, E Lakari, P Paakko, V Kinnula & Y Soini. Inducible nitric oxide synthase expression, apoptosis, and angiogenesis in insitu and invasive breast carcinomas. *Clin Cancer Res* 6, 2408-2416 (2000)

74. Bulut AS, E Erden, SD Sak, H Doruk,, N Kursun & D Dincol. Significance of inducible nitric oxide synthase expression in benign and malignant breast epithelium: an immunohistochemical study of 151 cases. *Virchows Arch* 447, 24-30 (2005)

75. Loibl S, A Buck, C Strank, G von Minckwitz, M Roller, HP Sinn, V Schini-Kerth, C Solbach, K Strebhardt & M Kaufmann. The role of early expression of inducible nitric oxide synthase in human breast cancer. *Eur J Cancer* 41, 265-271 (2005)

76. Chen HH, WC Su, CY Chou, HR Guo, SY Ho, J Que & WY Lee. Increased expression of nitric oxide synthase and cyclooxygenase-2 is associated with poor survival in cervical cancer treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 63, 1093-1100 (2005)

77. Kojima M, T Morisaki, Y Tsukahara, A Uchiyama, Y Matsunari, R Mibu & M Tanaka. Nitric oxide synthase expression and nitric oxide production in human colon carcinoma tissue. *J Surg Oncol* 70, 222-229 (1999)

78. Li W, RJ Xu, LH Jiang, J Shi, X Long & B Fan. Expression of cyclooxygenase-2 and inducible nitric oxide synthase correlates with tumor angiogenesis in endometrial carcinoma. *Med Oncol* 22, 63-70 (2005)

79. Marrogi AJ, WD Travis, JA Welsh, MA Khan, H Rahim, H Tazelaar, P Pairolero, V Trastek, J Jett, NE Caporaso, LA Liotta & CC Harris. Nitric oxide synthase, cyclooxygenase 2, and vascular endothelial growth factor

in the angiogenesis of non-small cell lung carcinoma. *Clin Cancer Res* 6, 4739-4744 (2000)

80. Rubio L, FJ Vera-Sempere, JA Lopez-Guerrero, J Padilla & MJ Moreno-Baylach. A risk model for non-small cell lung cancer using clinicopathological variables, angiogenesis and oncoprotein expression. *Anticancer Res* 25, 497-504, (2005)

81. Raspollini MR, G Amunni, A Villanucci, V Boddi, G Baroni, A Taddei & Taddei GL. Expression of inducible nitric oxide synthase and cyclooxygenase-2 in ovarian cancer: correlation with clinical outcome. *Gynecol. Oncol.* 92, 806-812 (2004)

82. HU Kasper, H Wolf, U Drebber, HK Wolf & MA Kern. Expression of inducible nitric oxide synthase and cyclooxygenase-2 in pancreatic adenocarcinoma: correlation with microvessel density. *World J Gastroenterol* 10, 1918-1922 (2004)

83. Aaltomaa SH, PK Lipponen, J Viitanen, JP Kankkunen, MY Ala-Opas & VM Kosma. The prognostic value of inducible nitric oxide synthase in local prostate cancer. *BJU Int* 86, 234-239 (2000)

84. Massi D, A Franchi, I Sardi, L Magnelli, M Paglierani, L Borgognoni, U Maria Reali & M Santucci. Inducible nitric oxide synthase expression in benign and malignant cutaneous melanocytic lesions. *J Pathol* 194, 194-200 (2001)

85. Chaiyarit P, N Ma, Y Hiraku, S Pinlaor, P Yongvanit, D Jintakanon, M Murata, S Oikawa & S Kawanishi. Nitrative and oxidative DNA damage in oral lichen planus in relation to human oral carcinogenesis. *Cancer Sci* 96, 553-559 (2005)

86. Garcea G, AR Dennison, WP Steward & DP Berry. Role of inflammation in pancreatic carcinogenesis and the implications for future therapy. *Pancreatology* 5, 514-29 (2005)

87. Ohshima H, H Tazawa, BS Sylla & T Sawa. Prevention of human cancer by modulation of chronic inflammatory processes. *Mutat Res* 591,110-122 (2005)

88. Inano H & M Onoda. Nitric oxide produced by inducible nitric oxide synthase is associated with mammary tumorigenesis in irradiated rats. *Nitric Oxide*12, 15-20 (2005)

89. Ishimura N, SF Bronk & GJ Gores, Inducible nitric oxide synthase upregulates cyclooxygenase-2 in mouse cholangiocytes promoting cell growth. *Am J Physiol* 287, G88-G95 (2004)

90. Tinker AC & AV Wallace. Selective inhibitors of inducible nitric oxide synthase: potential agents for the treatment of inflammatory diseases? *Curr Top Med Chem* 6, 77-92 (2006)

91. Dudzinski DM, J Igarashi, D Greif & T Michel. The regulation and pharmacology of endothelial nitric oxide synthase. *Annu Rev Pharmacol Toxicol* 46, 235-276 (2006)

92. Erdal EP, EA Litzinger, J Seo, Y Zhu, H Ji & RB Silverman. Selective neuronal nitric oxide synthase inhibitors. *Curr Top Med Chem* 5, 603-24 (2005)

93. Kennovin GD, DG Hirst, MRL Stratford & FW Flitney. Inducible nitric oxide synthase is expressed in tumor-associated vasculature: inhibition retards tumor growth. In: The Biology of Nitric Oxide. Eds: Moncada S, Feelisch M, Busse R., Higgs EA. Portland Press, London, pp. 259-263 (1994)

94. de Wilt JH, ER Manusama, B van Etten, ST van Tiel, AS Jorna, AL Seynhaeve, TL ten Hagen & AM Eggermont. Nitric oxide synthase inhibition results in synergistic anti-tumor activity with melphalan and tumor necrosis factor alpha-based isolated limb perfusions. *Br J Cancer* 83, 1176-1182 (2000)

95. Orucevic A & PK Lala. Effects of N(G)-Nitro-L-arginine methyl ester, an inhibitor of nitric oxidesynthesis, on IL-2-induced LAK cell generation *in vivo* and *in vitro* in healthy and tumor-bearing mice. *Cell Immunol* 169, 125-132 (1996)

96. Camp ER, A Yang, W Liu, F Fan, R Somcio, DJ Hicklin & LM Ellis. Roles of nitric oxide synthase inhibition and vascular endothelial growth factor receptor-2 inhibition on vascular morphology and function in an *in vivo* model of pancreatic cancer. *Clin Cancer Res* 12, 2628-2633 (2006)

97. Meyer RE, S Shan, J DeAngelo, RK Dodge, J Bonaventura, ET Ong & MW Dewhirst. Nitric oxide synthase inhibition irreversibly decreases perfusion in the R3230Ac rat mammary adenocarcinoma. *Br J Cancer* 71, 1169-1174 (1995)

98. Fukumura D, F Yuan, M Endo & RK Jain. Role of nitric oxide in tumor microcirculation. Blood flow, vascular permeability, and leukocyte-endothelial interactions. *Am J Pathol* 150, 713-725 (1997)

99. Swaroop GR, GP Malcolm, PA Kelly, I Ritchie & IR Whittle. Effects of nitric oxide modulation on tumor blood flow and microvascular permeability in C6 glioma. *Neuroreport* 9, 2577-2581 (1998)

100. Swaroop GR, PA Kelly, HS Bell, J Shinoda, S Yamaguchi & IR Whittle. The effects of chronic nitric oxide synthase suppression on gliomapathophysiology. *Br J Neurosurg* 14, 543-548 (2000)

101. Tozer GM, VE Prise & DJ Chaplin. Inhibition of nitric oxide synthase induces a selective reduction in tumor blood flow that is reversible with L-arginine. *Cancer Res* 57, 948-955 (1997)

102. Tozer GM, VE Prise, R Motterlini, BA Poole, J Wilson & DJ Chaplin. The comparative effects of the NOS inhibitor, Nomega-nitro-L-arginine, and the haemoxygenase inhibitor, zinc protoporphyrin IX, on tumor blood flow. *Int J Radiat Oncol Biol Phys* 42, 849-853 (1998)

103. Babal P, O Pechanova, I Bernatova & S Stvrtina. Chronic inhibition of NO synthesis produces myocardial fibrosis and arterial media hyperplasia. *Histol Histopathol* 12, 623-629 (1997)

104. Kanagy NL. Increased vascular responsiveness to alpha 2-adrenergic stimulation during NOS inhibition-induced hypertension. *Am J Physiol* 273, H2756-2764 (1997)

105. Pechanova O, Z Dobesova, J Cejka, J Kunes & J Zicha. Vasoactive systems in L-NAME hypertension: the role of inducible nitric oxide synthase. *J Hypertens* 22, 167-173 (2004)

106. Nabah YN, T Mateo, M Cerda-Nicolas, A Alvarez, M Martinez, AC Issekutz & MJ Sanz. L-NAME induces direct arteriolar leukocyte adhesion, which is mainly mediated by angiotensin-II. *Microcirculation* 12, 443-53 (2005)

107. Thomsen LL, JM Scott, P Topley, RG Knowles, AJ Keerie & AJ Frend. Selective inhibition of inducible nitric

oxide synthase inhibits tumor growth *in vivo*: studies with 1400W, a novel inhibitor. *Cancer Res* 57, 3300-3304 (1997)

108. Ferguson JW, AR Dover, S Chia, NL Cruden, PC Hayes & DE Newby. Inducible nitric oxide synthase activity contributes to the regulation of peripheral vascular tone in patients with cirrhosis and ascites. *Gut* 55, 542-546 (2006)

109. Butler SA, PJ Wood, S Cole, C Williams, GE Adams & IJ Stratford. Enhancement of bioreductive drug toxicity in murine tumors by inhibition of the activity of nitric oxide synthase. *Br J Cancer* 76, 438-444 (1997)

110. Griffin RJ, A Ogawa, BW Williams & CW Song. Hyperthermic enhancement of tumor radiosensitization strategies. *Immunol Invest* 34, 343-359 (2005)

111. Guo J-P, MM Panday, PM Consigny & AM Lefer. Mechanisms of vascular preservation by a novel NO donor following carotid artery intimal injury. *Am J Physiol* 269, H1122–H1131 (1995)

112. Murohara T, T Asahara, M Silver, C Bauters, H Masuda, C Kalka, M Kearney, D Chen, JF Symes, MC Fishman, PL Huang & JM Isner. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest* 101, 2567-2578 (1998)

113. Jadeski LC & PK Lala. Nitric oxide synthase inhibition by N(G)-nitro-L-arginine methyl ester inhibits tumor-induced angiogenesis in mammary tumors. *Am J Pathol* 155, 1381-1390 (1999)

114. Ziche M & L Morbidelli. Nitric oxide and angiogenesis. *J Neurooncol* 50, 139-148 (2000)

115. Kashiwagi S, Y Izumi, T Gohongi, ZN Demou, L Xu, PL Huang, DG Buerk, LL Munn, RK Jain & D Fukumura. NO mediates mural cell recruitment and vessel morphogenesis in murine melanomas and tissue-engineered blood vessels. *J Clin Invest* 115, 1816-1827 (2005)

116. Wang GY, B Ji, X Wang & JH Gu. Anti-cancer effect of iNOS inhibitor and its correlation with angiogenesis in gastric cancer. *World J Gastroenterol* 11, 3830-3833 (2005)

117. Williams JL, D Cartland, A Hussain & S Egginton. A differential role for nitric oxide in two forms of physiological angiogenesis in mouse. *J Physiol* 570, 445-454 (2006)

118. Chin K, Y Kurashima, T Ogura, H Tajiri, S Yoshida & H Esumi. Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. *Oncogene* 15, 437-442 (1997)

119. Frank S, B Stallmeyer, H Kampfer, N Kolb & J Pfeilschifter. Nitric oxide triggers enhanced induction of vascular endothelial growth factor expression in cultured keratinocytes (HaCaT) and during cutaneous wound repair. *FASEB J* 13, 2002-2014 (1999)

120. Frank S, B Stallmeyer, H Kampfer, C Schaffner, & J Pfeilschifter. Differential regulation of vascular endothelial growth factor and its receptor fms-like-tyrosine kinase is mediated by nitric oxide in rat renal mesangial cells. *Biochem J* 338, 367-74 1 (1999)

121. Jones MK, K Tsugawa, AS Tarnawsk & D Baatar. Dual actions of nitric oxide on angiogenesis: possible roles of PKC, ERK, and AP-1. *Biochem Biophys Res Commun* 318, 520-528 (2004)

122. Wakabayashi Y, T Shono, S Hori, K Matsushima, M Ono & M Kuwano. Dual pathways of tubular morphogenesis of vascular endothelial cells by human glioma cells: VEGF/bFGF and IL-8. *Jpn J Cancer Res* 86, 1189–1197 (1995)

123. Charalambous C, LB Pen, YS Su, J Milan, TCChen & FM Hofman. Interleukin-8 differentially regulates migration of tumor-associated and normal human brain endothelial cells. *Cancer Res* 65, 10347-10354 (2005)

124. Hellmuth M, J Paulukat, R Ninic, J Pfeilschifter & Muhl H. Nitric oxide differentially regulates pro- and anti-angiogenic markers in DLD-1 colon carcinoma cells. *FEBS Lett* 563, 98-102 (2004)

125. Andrew PJ, H Harant & IJ Lindley. Nitric oxide regulates IL-8 expression in melanoma cells at the transcriptional level. *Biochem Biophys Res Commun* 214, 949-956 (1995)

126. Matsunaga T, DW Weihrauch, MC Moniz, J Tessmer, DC Warltier & WM Chilian. Angiostatin inhibits coronary angiogenesis during impaired production of nitricoxide. *Circulation* 105, 2185-2191 (2002)

127. Kim JE & SR Tannenbaum. S-Nitrosation regulates the activation of endogenous procaspase-9 in HT-29 human colon carcinoma cells. *J Biol Chem* 279, 9758-9764 (2004)

128. Choi BM, HO Pae, SI Jang, YM Kim & HT Chung. Nitric oxide as a pro-apoptotic as well as anti-apoptotic modulator. *J Biochem Mol Biol* 35, 116-126 (2002)

129. Kim PK, R Zamora, P Petrosko & TR Billiar. The regulatory role of nitric oxide in apoptosis. *Int Immunopharmacol* 1, 1421-1441 (2001)

130. Li CQ & GN Wogan. Nitric oxide as a modulator of apoptosis. *Cancer Lett* 226, 1-15 (2005)

131. Li CQ, B Pang, T Kiziltepe, LJ Trudel, BP Engelward, PC Dedon & GN Wogan. Threshold effects of nitric oxide-induced toxicity and cellular responses in wild-type and p53-null human lymphoblastoid cells. *Chem Res Toxicol* 19, 399-406 (2006)

132. Huguenin S, F Vacherot, J Fleury-Feith, JP Riffaud, DK Chopin, M Bolla & MC Jaurand. Evaluation of the antitumoral potential of different nitric oxide-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs) on human urological tumor cell lines. *Cancer Lett* 218, 163-170 (2005)

133. Huguenin S, F Vacherot, L Kheuang, J Fleury-Feith, MC Jaurand, M Bolla, JP Riffaud & DK Chopin. Antiproliferative effect of nitrosulindac (NCX 1102), a new nitric oxide-donating non-steroidal anti-inflammatory drug, on human bladder carcinoma cell lines. *Mol Cancer Ther* 3, 291-298 (2004)

134. Fabbri F, G Brigliadori, P Ulivi, A Tesei, I Vannini, M Rosetti, S Bravaccini, D Amadori, M Bolla & W Zoli. Pro-apoptotic effect of a nitric oxide-donating NSAID, NCX 4040, on bladder carcinoma cells. *Apoptosis* 10, 1095-1103 (2005)

135. Mortensen K, J Skouv, DM Hougaard & LI Larsson. Endogenous endothelial cell nitric-oxide synthase modulates apoptosis in cultured breast cancer cells and is transcriptionally regulated by p53. *J Biol Chem* 274, 37679-37684 (1999)

136. Chung P, T Cook, K Liu, Y Vodovotz, R Zamora, S Finkelstein, T Billiar & D Blumberg. Overexpression of the human inducible nitric oxide synthase gene enhancesradiation-induced apoptosis in colorectal cancer cells via a caspase dependent mechanism. *Nitric Oxide* 8, 119-126 (2003)

137. Gao J, X Liu & B Rigas. Nitric oxide-donating aspirin induces apoptosis in human colon cancer cellsthrough induction of oxidative stress. *Proc Natl Acad Sci U S A.* 102, 17207-17212 (2005)

138. Gansauge S, AK Nussler, HG Beger & F Gansauge. Nitric oxide-induced apoptosis in human pancreatic carcinoma cell lines is associated with a G1-arrest and an increase of the cyclin-dependent kinase inhibitor p21WAF1/CIP1. *Cell Growth Differ* 9, 611-617 (1998)

139. Royle JS, JA Ross, I Ansell, P Bollina, DN Tulloch & FK Habib. Nitric oxide donating nonsteroidal anti-inflammatory drugs induce apoptosis inhuman prostate cancer cell systems and human prostatic stroma via caspase-3. *J Urol* 172, 338-344 (2004)

140. Kim PK, R Vallabhaneni, BS Zuckerbraun, C McCloskey, Y Vodovotz & TR Billiar. Hypoxia renders hepatocytes susceptible to cell death by nitric oxide. *Cell Mol Biol (Noisy-le-grand)*. 51, 329-35 (2005)

141. Soler MN, P Bobe, K Benihoud, G Lemaire, BA Roos & S Lausson. Gene therapy of rat medullary thyroid cancer by naked nitric oxide synthase II DNA injection. *J Gene Med* 2, 344-52 (2000)

142. Worthington J, T Robson, M O'Keeffe & DG Hirst. Tumor cell radiosensitization using constitutive (CMV) and radiation inducible (WAF1) promoters to drive the iNOS gene: a novel suicide gene therapy. *Gene Ther* 9, 2263-269 (2002)

143. Worthington J, T Robson, S Scott & D Hirst. Evaluation of a synthetic CArG promoter for nitric oxide synthase gene therapy of cancer. *Gene Ther* 12, 1417-1423 (2005)

144. Worthington J, HO McCarthy, E Barrett, C Adams, T Robson & DG Hirst. Use of the radiation-inducible WAF1 promoter to drive iNOS gene therapy as a novel anti-cancer treatment. *J Gene Med.* 6, 673-680 (2004)

145. Cook T, Z Wang, S Alber, K Liu, SC Watkins, Y Vodovotz, TR Billiar & D Blumberg. Nitric oxide and ionizing radiation synergistically promote apoptosis and growth inhibition of cancer by activating p53. *Cancer Res* 64, 8015-8021 (2004)

146. Leonetti C, M Scarsella, G Zupi, W Zoli, D Amadori, L Medri, F Fabbri, M Rosetti, P Ulivi, L Cecconetto, M Bolla & A Tesei. Efficacy of a nitric oxide-releasing nonsteroidal anti-inflammatory drug and cytotoxic drugs in human colon cancer cell lines *in vitro* and xenografts. *Mol Cancer Ther* 5, 919-926 (2006)

147. Kroncke KD. Cysteine-Zn2+ complexes: unique molecular switches for inducible nitric oxide synthase-derived NO. *FASEB J* 15, 2503-2507 (2001)

148. Kroncke KD, LO Klotz, CV Suschek & H Sies. Comparing nitrosative versus oxidative stress toward zinc finger-dependent transcription. Unique role for NO. *J Biol. Chem* 277, 13294-13301 (2002)

149. Wink DA & J Laval. The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide *in vitro* and *in vivo*. *Carcinogenesis* 15, 2125–2129 (1994)

150. Graziewicz M, DA Wink & F Laval. Nitric oxide inhibits DNA ligase activity. Potential mechanisms for NO mediated DNA damage. *Carcinogenesis* 17, 2501–2505 (1996)

151. Laval F & DA Wink. Inhibition by nitric oxide of the repair protein O6–methylguanin-DNA-methyltransferase. *Carcinogenesis* 15, 443–447 (1997)

152. Sidorkina O, MG Espey, Miranda KM, DA Wink & J Laval. Inhibition of poly(ADP-RIBOSE) polymerase (PARP) by nitric oxide and reactive nitrogen oxide species. *Free Radic Biol Med* 35 1431-1438 (2003)

153. Chien YH, DT Bau & KY Jan. Nitric oxide inhibits DNA-adduct excision in nucleotide excision repair. *Free Radic Biol Med* 36, 1011-1017 (2004)

154. Turchi JJ. Nitric oxide and cisplatin resistance: NO easy answers. *Proc Natl Acad Sci USA* 103, 4337-8 (2006)

155. Wink DA, JA Cook, D Christodoulou, MC Krishna, R Pacelli, S Kim, W DeGraff, J Gamson, Y Vodovotz, A Russo & JB Mitchell. Nitric oxide and some nitric oxide donor compounds enhance the cytotoxicity of cisplatin. *Nitric Oxide* 1, 88-94 (1997)

156. Azizzadeh B, HT Yip, KE Blackwell, S Horvath, TC Calcaterra, GM Buga, LJ Ignarro & MB Wang. Nitric oxide improves cisplatin cytotoxicity in head and neck squamous cell carcinoma. *Laryngoscope* 111, 1896-1900 (2001)

157. Liu J, C Li, W Qu, E Leslie, CL Bonifant, GS Buzard, JE Saavedra, LK Keefer & MP Waalkes. Nitric oxide prodrugs and metallochemotherapeutics: JS-K and CB-3-100 enhance arsenic and cisplatin cytolethality by increasing cellular accumulation. *Mol Cancer Ther* 3, 709-714 (2004)

158. Bratasz A, NM Weir, NL Parinandi, JL Zweier, R Sridhar, LJ Ignarro & P Kuppusamy. Reversal to cisplatin sensitivity in recurrent human ovarian cancer cells by NCX-4016, a nitro derivative of aspirin. *Proc Natl Acad Sci USA* 103, 3914-3919 (2006)

159. Konovalova NP, SA Goncharova, LM Volkova, TA Rajewskaya, LT Eremenko & AM Korolev. Nitric oxide donor increases the efficiency of cytostatic therapy and retards the development of drug resistance. *Nitric Oxide* 8, 59-64 (2003)

160. Muir CP, MA Adams & CH Graham. Nitric oxide attenuates resistance to doxorubicin in three-dimensional aggregates of human breast carcinoma cells. *Breast Cancer Res Treat*. 96, 169-176 (2006)

161. Riganti C, E Miraglia, D Viarisio, C Costamagna, G Pescarmona, D Ghigo & A Bosia. Nitric oxide reverts the resistance to doxorubicin in human colon cancer cells by inhibiting the drug efflux. *Cancer Res* 65, 516-525 (2005)

162. Riganti C, S Orecchia, G Pescarmona, PG Betta, D Ghigo & A Bosia. Statins revert doxorubicin resistance via nitric oxide in malignant mesothelioma. *Int J Cancer* 119, 17-27. (2006)

163. Evig CB, Kelley EE, Weydert CJ, Chu Y, Buettner GR, Burns CP. Endogenous production and exogenous exposure to nitric oxide augment doxorubicin cytotoxicity for breast cancer cells but not cardiac myoblasts. Nitric Oxide. 2004 May;10(3):119-29.

164. Jia L, J Schweizer, Y Wang, C Cerna, H Wong & M Revilla. Effect of nitric oxide on cytotoxicity of Taxol: enhanced Taxol transcellular permeability. *Biochem. Pharmacol* 66, 2193-2199 (2003)

165. Howard-Flanders, P. Effect of nitric oxide on the radiosensitivity of bacteria. *Nature* 180, 1191-1192 (1957)

166. Gray LH, FO Green & Hawes CA. Effect of nitric oxide on the radiosensitivity of tumor cells. *Nature* 182, 952-953 (1958)

167. Dewey DL. Effect of oxygen and nitric oxide on the radio-sensitivity of human cells in tissue culture. *Nature* 186, 780-782 (1960)

168. Moncada S. Adventures in vascular biology: a tale of two mediators. *Philos Trans R Soc Lond B Biol Sci* 361, 735-759 (2006)
169. Brennan PA & S Moncada. From pollutant gas to biological messenger: the diverse actions of nitric oxide in cancer. *Ann R Coll Surg Engl* 84, 75-78. 2002
170. Mitchell JB, DA Wink, W DeGraff, J Gamson, LK Keefer & MC Krishna. Hypoxic mammalian cell radiosensitization by nitric oxide. *Cancer Res* 53, 5845-5848 (1993)
171. Mitchell JB, JA Cook, MC Krishna, W DeGraff, J Gamson, J Fisher, D Christodoulou & DA Wink. Radiation sensitization by nitric oxide releasing agents. *Br J Cancer* 27, S181-184 (1996)
172. Janssens MY, VN Verovski, DL Van den Berge, C Monsaert & GA Storme. Radiosensitization of hypoxic tumor cells by S-nitroso-N acetylpenicillamine implicates a bioreductive mechanism of nitric oxide generation. *Br J Cancer* 79, 1085-1089 (1999)
173. Mitchell JB, W DeGraff, S Kim, JA Cook, J Gamson, D Christodoulou, M Feelisch & DA Wink. Redox generation of nitric oxide to radiosensitize hypoxic cells. *Int J Radiat Oncol Biol Phys* 42, 795-798 (1998)
174. Janssens MY, DL Van den Berge, VN Verovski, C Monsaert & GA Storme. Activation of inducible nitric oxide synthase results in nitric oxide-mediated radiosensitization of hypoxic EMT-6 tumor cells. *Cancer Res* 58, 5646-5648 (1998)
175. Harrop HA, KD Held & BD Michael. The oxygen effect: variation of the K-value and lifetimes of O2-dependent damage in some glutathione deficient mutants of Escherichia coli. *Int J Radiat Biol* 59, 1237-1251 (1991)
176. Worthington J, T Robson, M Murray, M O'Rourke, G Keilty & DG Hirst. Modification of vascular tone using iNOS under the control of a radiation-inducible promoter. *Gene Ther*. 7, 1126-1131 (2000)
177. Wang Z, T Cook, S Alber, K Liu, I Kovesdi, SK Watkins, Y Vodovotz, TR Billiar & D Blumberg. Adenoviral gene transfer of the human inducible nitric oxide synthase gene enhances the radiation response of human colorectal cancer associated with alterations in tumor vascularity. *Cancer Res* 64, 1386-1395 (2004)
178. Thomas DD, X Liu, SP Kantrow & JR Lancaster Jr. The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O2. *Proc Natl Acad Sci USA* 98, 355-360 (2001)
179. Wood PJ, JM Sansom, IJ Stratford, GE Adams, C Szabo, C Thiemermann & JR Vane. Changes in energy metabolism and X-ray sensitivity in murine tumors by the nitric oxide donor SIN-1. *Br J Cancer* 27, S177-180 (1996)
180. Mikkelsen RB & P Wardman. Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms. *Oncogene* 22, 5734-5754 (2003)
181. Shao C, V Stewart, M Folkard, BD Michael & Prise KM. Nitric oxide-mediated signaling in the bystander response of individually targeted glioma cells. *Cancer Res* 63, 8437-8442 (2003)
182. Shao C, M Folkard, BD Michael & KM Prise. Targeted cytoplasmic irradiation induces bystander responses. *Proc Natl Acad Sci USA*. 101, 13495-134500 (2004)

183. Rieger J, U Naumann, T Glaser, A Ashkenazi & M Weller. APO2 ligand: a novel lethal weapon against malignant glioma? *FEBS Lett* 427, 124-128 (1998)
184. Walczak H, RE Miller, K Ariail, B Gliniak, TS Griffith, M Kubin, W Chin, J Jones, A Woodward, T Le, C Smith, P Smolak, RG Goodwin, CT Rauch, C Schuh J & DH Lynch. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. *Nat Med* 5, 157-163 (1999)
185. Kelley SK & A Ashkenazi. Targeting death receptors in cancer with Apo2L/TRAIL. *Curr Opin Pharmacol* 4, 333-339 (2004)
186. Huerta-Yepez S, M Vega, A Jazirehi, H Garban, F Hongo, G Cheng & B Bonavida. Nitric oxide sensitizes prostate carcinoma cell lines to TRAIL-mediated apoptosis via inactivation of NF-kappa B and inhibition of Bcl-xl expression. *Oncogene* 23, 4993-5003 (2004)
187. Lee YJ, KH Lee, HR Kim, JM Jessup, DW Seol, TH Kim, TR Billiar & YK Song. Sodium nitroprusside enhances TRAIL-induced apoptosis via a mitochondria-dependent pathway in human colorectal carcinoma CX-1 cells. *Oncogene* 20, 1476-1485 (2001)
188. Tong X & H Li. eNOS protects prostate cancer cells from TRAIL-induced apoptosis. *Cancer Lett* 210, 63-71 (2004)

Abbreviations: AdiNOS: adenovirus/inducible nitric oxide synthase; DETA/NO: diethylenetriamine nitric oxide adduct; DETANONOate: (Z)-1-[2-(2-aminoethyl)-N-(2-ammonio-ethyl) amino]diazen-1-ium-1,2-diolate; CMV: cytomegalovirus; L-NAME: N^G-nitro-L-arginine methyl ester); NNLA: N^G-nitro-L-arginine; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; SNAP: *S*-nitro-*N*-acetyl penicillamine; VEGF: vascular endothelial growth factor; WAF1: wild-type p53 activated fragment

Key words: Nitrosative Stress, Reactive Nitrogen Species, Cancer, Nitric Oxide, Inos, Enos, Chemotherapy, Radiotherapy, Apoptosis, Review

Send correspondence to: Professor David Hirst, School of Pharmacy, Medical Biology Centre, Queen's University, 97 Lisburn Road, Belfast, BT9 7BL, U.K., Tel: 44-0-2890972027, Fax: 44-0-2890247749, E-mail: d.hirst@qub.ac.uk