Minireview

Is Inducible Nitric Oxide Synthase a Target for Chemoprevention?

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Abstract

The molecular messenger nitric oxide (NO) is synthesized endogenously from L-arginine by three isoforms of the enzyme NO synthase. The isoform most consistently associated with neoplasia is the inducible form, inducible nitric oxide synthase (iNOS). However, the role played by the NO/iNOS system in tumor development is complex, and both promoting and inhibitory effects on neoplasia have been reported. This review attempts to clarify the role of iNOS in carcinogenesis, with particular emphasis on the early stages of tumor development, offers possible explanations for the confused picture presented in the literature regarding the association of the NO/iNOS pathway with neoplasia, and identifies selective iNOS inhibitors that may have chemopreventive potential.

Introduction

The free radical NO[2] is a ubiquitous signaling molecule that affects numerous physiological and pathological processes. Homeostatic actions include immune function, blood flow, platelet aggregation, neurotransmission, and memory and are generally associated with low NO levels. Excess production of NO is involved in inflammatory and immunological disorders, pain, neurological diseases, atherosclerosis, and cancer (1). Unlike other chemical messengers, NO's rapid diffusibility, membrane permeability, and chemical instability abrogate the need for both specific extracellular receptors and degradative pathways (2). NO interacts with numerous biological targets and modulates gene expression via effects on transcription factors [*e.g.,* NF-κB, activator protein 1, specificity protein 1, early growth response (gene)-1, vitamin D receptor/retinoid X receptor, and hypoxia-inducible factor 1], elements of signal transduction pathways (*e.g.,* G proteins, Janus-activated kinase, mitogen-activated protein kinases, caspases, and protein phosphatases), mRNA stability and translation, and DNA methyltransferases. Effects on gene expression can be either activating or inhibitory, even on the same pathway. The plethora of NO targets, as well as the biphasic effects of NO on gene expression, underscore the complexity of NO's biological actions. Despite the vast array of data that has been generated, NO-mediated signaling pathways *in vivo* remain largely unknown (3, 4).

The constitutive isoforms of NOS, eNOS and nNOS, are calcium dependent and generate low levels of NO. eNOS is expressed in endothelial cells, cardiac myocytes, and hippocampal pyramidal cells and is involved in maintaining vascular tone, inhibiting adhesion of platelets and white cells, suppressing smooth muscle cell proliferation, and promoting angiogenesis. The isoform nNOS, which is expressed in neurons, skeletal muscle, and lung epithelium, participates in relaxation of vascular and nonvascular smooth muscle and acts as a neurotransmitter. Up-regulation of nNOS is associated with neurotoxicity and stroke damage; this isoform can also generate superoxide under some circumstances (1, 5).

In contrast to the constitutive NOS isoforms that generate low levels of NO, iNOS produces high NO levels; the activity of iNOS is calcium independent and largely regulated at the level of synthesis and stability of mRNA and protein. A number of agents up-regulate iNOS, most notably lipopolysaccharides and inflammatory cytokines. Appropriate stimulation leads to expression in many human cells including macrophages, hepatocytes, smooth muscle, chondrocytes, cardiac myocytes, and a variety of cancer cells. iNOS is also found in normal bronchial and gastrointestinal epithelium, apparently due to basal levels of appropriate stimuli (3–7). When produced in immune cells, iNOS is instrumental for pathogen defense, cytokine production, and T-helper-type cell expansion. Apart from these activities, up-regulation of iNOS has traditionally been thought to act solely as a pathological mediator. However, it is becoming increasingly clear that iNOS has physiological roles including osteoclastic bone resorption, prevention of gut inflammation, healing of the skin and intestinal mucosa, and ischemic preconditioning in the heart. The effects of iNOS in the inflammatory response are particularly complex, and the enzyme appears to be involved in both promotion and resolution of inflammation (5).

The expression of iNOS is regulated by transcription factors including NF-κB, activator protein 1, signal transducer and activator of transcription 1α, interferon-regulatory pro-

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[2] The abbreviations used are: NO, nitric oxide; ACF, aberrant crypt foci; AG, aminoguanidine; COX, cyclooxygenase; DCIS, ductal carcinoma *in situ*; NF-κB, nuclear factor κB; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NOS, nitric oxide synthase; PBIT, *S,S′*-1,4-phenylene-bis(1,2-ethanediyl)bis-isothiourea; PIN, prostatic intraepithelial neoplasia.

Table 1 Changes in iNOS expression/activity during tumor development in animals

Target species (carcinogen)	NOS expression/activity	iNOS effect on neoplasia (↑/↓)[a]	Ref. no.
Colon			
Rat (AOM)[b]	↑ Activity colonic mucosa	↑	9
Rat (AOM)	↑ Activity colonic mucosa	↑	8
Rat (AOM)	↑ Expression adenocarcinoma	↑	87
Rat (AOM)	↑ Expression dysplastic ACF, adenoma, adenocarcinoma	↑	11
Oral cavity			
Hamster (DMBA)	↑ Expression hyperplasia, dysplasia, squamous cell carcinoma	↑	49 and 50
Esophagus			
Rat (NMBA)	↑ Expression preneoplastic tissue, papilloma	↑	54
Rat (esophageal-duodenal astomosis, iron)	↑ Expression esophagus	↑	55
Lung			
Rat (cigarette smoke)	↑ Expression terminal bronchiole lesions	↑	88
Mouse (urethane)	↑ Expression tumors	↑	89

[a] iNOS expression has promoting (↑) or inhibitory (↓) effect on neoplasia.
[b] AOM, azoxymethane; DMBA, 7,12-dimethylbenz (*a*) anthracene; NMBA, *N*-nitroso-*N*-methylbenzylamine.

Table 2 Effect of iNOS inhibitors in animal cancer studies

Target species (carcinogen/tumor)	Agent[a]	Results	iNOS effect on neoplasia (↑/↓)[b]	Ref. no.
Intestine				
Rat (AOM)[c]	SC-51	↑ Colonic ACF formation	↑	8
Rat (AOM)	PBIT	↑ Colonic ACF formation	↑	9
Rat (AOM)	AG	↑ Colonic ACF formation	↑	8
Mouse ($Apc^{min+/-}$)	AG	NE Small intestine adenoma formation	NE	16
Mouse ($Apc^{min+/-}$; high-fat diet)	PBIT	↑ Colonic adenocarcinoma formation; slightly but significantly ↑ intestinal tumors	↑	18
Mouse (iNOS-expressing human colon tumor xenografts)	1400W	↑ Growth	↑	10
Mouse (colon adenocarcinomas with major iNOS source intratumoral macrophages)	1400W	NE Growth	NE	10
Mammary				
Mouse (iNOS-expressing adenocarcinoma)	1400W	↑ Growth	↑	10

[a] See text for iNOS selectivity.
[b] iNOS expression has promoting (↑) or inhibitory (↓) effect on neoplasia.
[c] AOM, azoxymethane; NE, no effect.

tein 1, nuclear factor interleukin-6, and high-motility group I (Y) protein. Various upstream signaling pathways are used to enhance or inhibit expression, depending on stimulus and cell type. NO itself affects iNOS transcription; low levels activate NF-κB and up-regulate iNOS expression, whereas high levels decrease transcription (3).

Association of iNOS with Tumorigenesis

iNOS has been associated with the development of human and animal cancers *in vivo*. Cancers with evidence of iNOS deregulation during the early stages of tumorigenesis are likely targets for chemoprevention. Tables 1–4 show changes in iNOS regulation during cancer development in humans and animals, emphasizing the early stages of tumorigenesis, which are likely targets for chemoprevention. These tables also show the probable effect of iNOS on tumor development (↑, promoting; ↓, inhibitory) based on results of the individual studies.

Colon. The majority of studies in both carcinogen-induced and genetic models support a role for iNOS in the promotion of colon carcinogenesis. iNOS activity is increased in the colonic mucosa of carcinogen-treated rats (Table 1). The partially selective iNOS inhibitors SC-51 [L-N^6-(1-iminoethyl)lysine tetrazoleamide] and PBIT decrease the formation of preneoplastic colonic ACF (Refs. 8 and 9; Table 2), and the selective iNOS inhibitor 1400W [*N*-3-((aminomethyl)benzyl)acetamidine] diminishes the growth of established human colon cancer xenografts in nude mice (10). In addition to increased activity, iNOS expression is also up-regulated in carcinogen-induced rat dysplastic ACF, adenomas, and adenocarcinomas, but not in hyperplastic ACF (11). The tumor-enhancing effects of iNOS in the colon may be associated with the ability of NO to increase the expression/activity of the enzyme COX-2 (12), which is significantly involved in colon cancer promotion (13). In this regard, SC-51 inhibits COX-2 activity in the colonic mucosa of carcinogen-treated rats, likely via cross-talk between the iNOS and COX-2 signaling pathways (8). NO-releasing nonsteroidal anti-inflammatory drugs, which prevent the development of ACF in rats independently of COX-1 or COX-2

Table 3 Tumor development in iNOS knockout mice

Target	Result	iNOS effect on neoplasia (↑/↓)[a]	Ref. no.
Intestine			
Apc$^{min/+}$ iNOS$^{-/-}$	↓ Adenoma formation	↑	16
Apc$^{min/+}$ iNOS$^{-/+}$			
Apc$^{min/+}$ iNOS$^{-/-}$	↑ Adenoma formation	↓	19
Lung			
iNOS$^{-/-}$ mice treated with urethane	↓ Tumor formation	↑	89

[a] iNOS expression has promoting (↑) or inhibitory (↓) effect on neoplasia.

inhibition (14), strongly inhibit the induction and expression of iNOS in colon cancer cell lines (15).

In support of a role for iNOS in colon tumor promotion, loss of iNOS expression decreases intestinal tumor formation in Min mice (Ref. 16; Table 3). These mice carry a nonsense mutation in the *Apc* tumor suppressor gene, which is highly associated with colorectal cancer risk in humans (17). Furthermore, oral PBIT significantly (but slightly) inhibits intestinal tumorigenesis in Min mice and significantly inhibits the development of colonic adenocarcinomas. Lower dose combinations with a COX-2 inhibitor are also efficacious (18). On the contrary, Scott *et al.* (19) reported that iNOS knockout Min mice develop slightly, but significantly, more intestinal adenomas than iNOS-replete littermates, suggesting that iNOS may have an inhibitory effect on tumor development. Additional studies are needed to clarify the role of iNOS in tumor formation in the context of *Apc* loss.

In humans, the association of iNOS with colorectal tumor development appears to be even more complex (Table 4). Studies have shown that iNOS expression is up-regulated in carcinomas compared with patients' normal-appearing colonic mucosa (20, 21), and iNOS activity is higher in adenomas than in normal-appearing tissue and lowest in metastases (22). However, other studies suggest that iNOS plays a protective role against cancer development. Moochhala *et al.* (23) reported that iNOS is strongly expressed in normal-appearing colonic mucosa; both expression and activity are down-regulated during progression to carcinoma. Hao *et al.* (24) also found strong iNOS expression in normal-appearing colonic mucosa that significantly decreases in both ACF and carcinomas. Furthermore, iNOS expression is not associated with the severity of dysplasia in ACF and is similar in multiple lesions from the same patient, leading the authors to conclude that iNOS down-regulation may be a very early event in the progression of colorectal neoplasia.

Breast. Previous studies largely support a role for iNOS in the promotion of breast neoplasia, at least during the early stages of tumor development. Vakkala *et al.* (25) reported that iNOS expression increases with DCIS grade and further increases in invasive lesions; increased expression also correlates with increased tumor vascularization and apoptotic index. Others found that the intensity of expression increases from benign disease to invasive ductal carcinoma grade II but decreases in grade III lesions, suggesting that the enzyme may stimulate growth of early lesions but inhibit later stages (26). De Paepe *et al.* (27) reported very low iNOS expression in normal breast tissue that increases in hyperplastic lesions but decreases in DCIS and invasive cancers, although the expression level is still higher than that in normal tissue. Still others reported that iNOS expression correlates positively with metastatic breast cancer, but not with tumor grade (28). In two studies comparing cancers with benign tumors, total NOS activity increased; however, higher activity was associated with both low (29) and high (30) tumor grade.

Prostate. Expression of iNOS has consistently been reported to be up-regulated in cancerous prostatic tissue compared with normal and adjacent normal-appearing tissue (31–34); however, the enzyme has been reported to be both expressed in (35, 36) and absent from (31, 34) benign lesions. Expression in precancerous high-grade PIN and cancer is also more intense than that in low-grade PIN and benign lesions, suggesting that up-regulation is associated with progression to malignancy (36).

Bladder. Significantly increased iNOS expression has also been consistently found in bladder cancers (37–40). In one study, all 94 transitional cell carcinomas examined exhibited some immunostaining for iNOS. All dysplastic lesions adjacent to carcinomas exhibited staining patterns similar to malignant tissue, suggesting that up-regulation of iNOS is an early event during bladder carcinogenesis (38). A consistent lack of correlation between increased iNOS expression in bladder cancers and clinicopathological factors (37–39) also implies that iNOS deregulation occurs early during bladder neoplasia.

Skin. iNOS is also up-regulated during progression of malignant melanoma. Although iNOS is absent from benign melanocytic nevi, its expression increases during progression from cutaneous melanoma *in situ*, to invasive melanomas, to s.c. metastases. In primary cutaneous melanomas, expression does not correlate with histopathological parameters or disease-specific survival (41). However, others have reported a correlation between increased expression and poor survival (42); still others have found expression to be inversely related to metastatic potential (43). In mice, iNOS deficiency decreases pulmonary metastases, impairs angiogenesis, and suppresses pleural effusion of injected murine melanoma cells (44). iNOS is up-regulated in human skin squamous cell carcinoma (45) but is down-regulated in skin basal cell carcinomas, which may contribute to the lack of aggressiveness of the latter (46).

Head and Neck. iNOS is not expressed in normal human oral mucosa but is up-regulated in epithelial dysplasia; expression correlates with severity of dysplasia and is not related to tumor grade in squamous cell carcinomas (47, 48). Consistent with human studies, iNOS is up-regulated in hamster oral hyperplastic, dysplastic, and cancerous tissue (49, 50). iNOS activity is also increased in human head and neck cancers compared with unaffected mucosa. Increased activity correlates with tumor vascularization and is higher at the invasive tumor edge than in the tumor core (51).

Esophagus. iNOS is frequently overexpressed in premalignant Barrett's esophagus and associated adenocarcinomas (52), as well as early-stage mucosal squamous cell cancers (53). In rat models of both of these types of esophageal cancer, the enzyme is overexpressed in precancerous lesions (54, 55).

Table 4 Changes in iNOS expression/activity during tumor development in humans

Target	iNOS expression/activity[a]	iNOS effect on neoplasia (↑/↓)[b]	Ref. no.
Colon	↓ Expression ACF; not associated degree of dysplasia in ACF	↓	24
	↓ Expression carcinoma		
	↓ Expression/activity adenoma, carcinoma	↓	23
	↑ Activity adenoma; ↓ activity correlates with ↑ Dukes stage, lowest metastases	↑ (early)/↓ (late)	22 and 90
	↑ Expression carcinoma	↑	20
	↓ Expression correlates with ↑ Dukes stage	↓ (late)	91
	↑ Expression cancer; no correlation with clinicopathological findings except vascular invasion	↑	21
Breast	↑ Expression hyperplasia	↑/↓ (?)	27
	↓ DCIS, invasive cancer compared with hyperplasia (↑ compared normal tissue)		
	↑ Expression correlates with ↑ DCIS grade, further ↑ in cancer	↑	25
	↑ Expression metastases; no correlation with tumor grade	↑	28
	↑ Expression benign disease to grade II carcinoma, ↓ grade III carcinoma	↑/↓ (late)	26
	↑ Total NOS activity cancer;	↑/↓	29
	↓ Total NOS activity correlates with ↑ proliferation, high tumor grade		
	↑ Total NOS activity cancer; activity correlates with tumor grade	↑	30
Prostate	↑ Expression high-grade PIN, cancer compared with low-grade PIN, BPH[c]	↑	36
	↑ Expression cancer	↑	31
	↑ Expression cancer	↑	32
	↑ Expression cancer	↑	33
	↑ Expression cancer	↑	34
Bladder	↑ Expression dysplasia, cancer	↑	38
	↑ Expression cancer	↑	37
	↑ Expression cancer	↑	39
	↑ Expression cancer	↑	40
Skin (melanoma)	↑ Expression during progression	↑	41
	↓ Expression correlates with metastases	↓ (late)	43
	↓ Expression correlates with poor survival	↑ (late)	42
Skin (basal cell carcinoma)	↓ Expression cancer	↓ (associated with lack of aggressiveness?)	46
Skin (squamous cell carcinoma)	↑ Expression in cancer	↑	45
Oral cavity	↑ Expression correlates with severity of dysplasia	↑	47 and 48
Head and neck	↑ Activity cancer; activity correlates with angiogenesis and metastases	↑	51
Esophagus	↑ Expression Barrett's esophagus, adenocarcinoma	↑	52
	↑ Expression esopageal squamous cell carcinomas, early-stage mucosal squamous cell cancer	↑	53
Lung	↑ Expression in NSCLC correlates with VEGF, angiogenesis	↑	92
Gastric	↑ Expression cancer, no correlation with inflammation, clinicopathological features	↑	93
	↑ Expression cancer	↑	94
	↑ Expression cancer	↑	95
	↑ Nitrotyrosine cancer		
	↓ Expression cancer	↓	96

[a] Compared with normal/normal-appearing tissue unless indicated otherwise.
[b] iNOS expression has promoting (↑) or inhibitory (↓) effect on neoplasia.
[c] BPH, benign prostatic hyperplasia; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor.

Dual Effects of iNOS/NO on Tumorigenesis

It is clear that a great deal of evidence supports a role for iNOS in promoting tumor development in both humans and animals. A number of activities may contribute to the tumor-enhancing effects of NO, including induction of DNA damage (56), increased angiogenesis and blood flow (57), prevention of apoptotic cell death (58), and suppression of the immune system (59).

On the other hand, numerous reports also indicate that NO can inhibit neoplasia. NO is cytotoxic to tumor cells (60) and can decrease tumor growth and metastasis *in vivo* (61–65). The inhibitory effects of NO on tumorigenesis have been

associated with antioxidant actions (66), inhibition of angiogenesis (67) and platelet aggregation (68), and enhancement of vasodilation (69), differentiation (70), and apoptosis (58, 63, 71).

This apparent paradox, in which NO both enhances and inhibits tumorigenesis, has been attributed to several factors including local NO concentrations, cell type, cellular genetics, and redox status (72–76). Low levels of NO appear to increase the tumor-promoting effects of NO, whereas high levels are cytostatic/cytotoxic. iNOS activity in genetically engineered human colon cells with increased growth and angiogenic potential is at least 1–2 orders of magnitude lower than that associated with antitumor actions such as necrotic and apoptotic cell death (72). Importantly, NOS activity in these cells is similar to that observed in human breast cancers (30).

It should be noted that most studies that demonstrate an inhibitory effect of NO/iNOS on tumorigenesis used biological systems that produce high levels of NO or used exogenous NO donors. Thus, continuous high levels of NO synthesized by macrophages or endothelial cells are cytotoxic/cytostatic toward tumor cells (60, 71), and high NO levels produced by tumor cells themselves, via stimulation of iNOS expression or transfection with iNOS genes, are associated with decreased tumor growth and metastasis *in vivo* (62–65). Moreover, exogenous NO donors enhance differentiation (70) and apoptosis (58) and inhibit angiogenesis (67).

It is possible that the high levels of NO produced experimentally in studies that show that NO inhibits neoplasia may not be relevant in the context of the natural course of tumor development *in vivo*. This would explain the overwhelming *in vivo* evidence (notwithstanding the complex situation in the colon) that iNOS enhances carcinogenesis (Tables 1 and 4). In this regard, it is instructive to examine the analysis of Kolb (58), who concluded that in human leukemia, apoptosis is largely associated with delivery of NO by chemical donors to tumor cell lines, whereas antiapoptotic effects appear to be related to the endogenous production of NO by NOS. It has also recently been pointed out that although it is clear that NOS-expressing cells produce NO, it remains unknown whether NOS directly synthesizes NO. Either NO or other reactive nitrogen species such as NO^- or peroxynitrite may be the immediate products of NOS (6). This observation may also explain some of the paradoxical effects that have been observed between activation of NOS and effects of exogenous NO donors in biological systems (*e.g.,* that NOS activity is associated with inflammation and cell damage, whereas exogenous NO donors have anti-inflammatory and cell protective activity).

The question thus becomes not whether iNOS promotes or prevents cancer—it appears to have the capability to do both—but rather, what role does iNOS play during the natural course of carcinogenesis *in vivo*. Most available data suggest that iNOS is present at levels that promote tumor development.

iNOS Inhibitors

Deciphering the role of iNOS in tumorigenesis has been limited by the lack of selective enzyme inhibitors (Fig. 1). Many studies have used inhibitors that lack any selectivity (*e.g., N*ω-monomethyl-L-arginine and *N*ω-nitro-L-arginine methyl ester). Still others have drawn conclusions regarding the actions of iNOS based on studies using inhibitors mischaracterized as "selective." The most notable example is probably AG. Although it is about 10-fold selective for iNOS over eNOS, it is minimally selective over nNOS. Moreover, it has numerous other biological activities, including inhibition of polyamine metabolism and catalase, as well as administering production of advanced glycosylation products. Interestingly, in various studies AG has been reported to be selective for all of these activities (6).

It has been proposed that inhibitors with less than 10-fold selectivity for iNOS be regarded as nonselective, those with 10–50-fold selectivity be regarded as partially selective (provided the necessary controls and condition/doses are used), and those with 50-fold or higher selectivity be regarded as selective iNOS inhibitors (5). In addition to nonselectivity, poor cellular and tissue penetration and significant toxicities have also been problems in developing clinically useful iNOS inhibitors (6). Selectivity is important for therapeutic utility in light of the important homeostatic functions of NO (1). However, total, chronic inhibition of iNOS may also be detrimental, given the increasingly apparent physiological functions of this isoform. Tissue or cell-specific inhibitors may prove to be the most clinically useful (5).

Partially selective inhibitors include SC-51 and PBIT. The former has minimal inhibitory activity toward iNOS *in vitro* but is rapidly converted to the partially selective iNOS inhibitor L-N^6-(1-iminoethyl)lysine *in vivo* (77). PBIT is competitive with L-arginine and demonstrates 190- and 5-fold selectivity for iNOS compared with eNOS and nNOS, respectively (78). However, the clinical utility of PBIT is hampered by poor cellular penetration and marked acute toxicity (79).

The acetamidine 1400W is a very potent, irreversible (or slowly reversible) iNOS inhibitor ($K_d \leq 0.007$ μM) with greater than 5000- and 200-fold selectivity for iNOS compared with eNOS and nNOS, respectively (79). Toxicity may limit human use of 1400W; nevertheless, it is one of the most potent and selective iNOS inhibitors available for experimentation (6). GW273629 (*S*-[2-[(1-iminoethyl)-amino]ethyl]-4,4-dioxo-L-cysteine) and GW274150 (*S*-[2-[(1-iminoethyl)amino]ethyl-L-homocysteine) are sulfur-substituted acetamidine amino acids that are competitive with L-arginine and are highly selective for iNOS over both eNOS and nNOS. Inhibition of iNOS is NADPH dependent and develops relatively slowly, whereas inhibition of the other isoforms is rapidly reversible (80). GW273629 has a short duration of action *in vivo* and is poorly bioavailable p.o. GW274150 shows higher bioavailability and has a longer half-life (81). Unlike 1400W, acute toxicity has not been observed with the sulfur-substituted compounds (6).

N-[(1,3-Benzodioxol-5-yl)methyl)-1-[2-(1*H*-imidazol-1-yl)pyrimidin-4-yl]-4-(methoxycarbonyl)piperazine-2-acetamide represents a new class of potent iNOS inhibitors that act by blocking dimerization of iNOS monomers rather than inhibiting the enzyme's catalytic activity. This compound

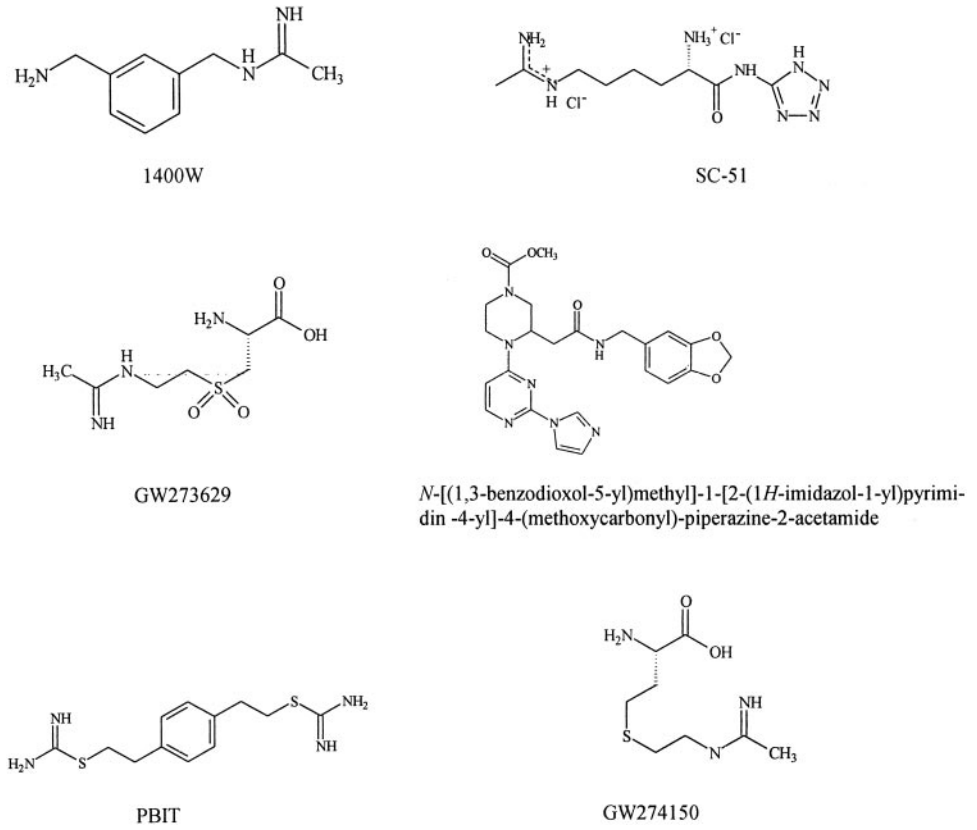

Fig. 1. Selective iNOS inhibitors.

decreases NO production in cells at 1000-fold lower concentrations than 1400W and shows 1000- and 5-fold selectivity for inhibition of iNOS dimerization compared with eNOS and nNOS, respectively. Diminished NO production has also been demonstrated *in vivo* (82, 83). Additionally, compounds that scavenge peroxynitrite, which has been implicated in NO pathology (1), may have clinical utility. One such compound, guanidinoethyldisulfide, although only 4-fold selective for iNOS over eNOS, is a potent peroxynitrite scavenger (84).

iNOS inhibitors with varying degrees of selectivity have demonstrated anticancer activity in experimental models (Table 2). The largest number of studies have been done in the colon. As noted above, the partially selective iNOS inhibitors SC-51 and PBIT decrease the formation of preneoplastic colonic ACF (8, 9), and the selective iNOS inhibitor 1400W diminishes the growth of established human colon cancer xenografts in nude mice (10). 1400W also inhibits the growth of iNOS-expressing murine mammary adenocarcinomas, although it failed to affect the growth of murine colon adenocarcinomas in which intratumoral macrophages were the major iNOS source (10).

Conclusions

In the past few years, data regarding the promoting effects of iNOS on tumor development *in vivo* have been mounting. A consistent association between up-regulation of iNOS and cancers of the bladder, prostate, oral cavity, and esophagus has been observed. Moreover, deregulation appears to occur during early neoplastic progression in these organs, suggesting that intervention with iNOS inhibitors may be a viable chemopreventive strategy. Most animal studies also support a role for iNOS in the promotion of colon carcinogenesis, although the association of iNOS with human colon cancer is significantly more complex.

Chemopreventive effects of iNOS inhibitors have been demonstrated in preclinical colon cancer models. Additionally, iNOS inhibitors with varying degrees of selectivity (thioureas, AG, 2-amino-4-methyl pyridine, L-N^6-(1-iminoethyl)-lysine, and $N\omega$-nitro-L-arginine methyl ester) inhibit transformation of rat tracheal epithelial cells *in vitro*. This assay has demonstrated good predictive value for lung cancer prevention in rodents (85). However, a recent study also suggests that up-regulation of iNOS contributes to the apoptosis-inducing activity of the established chemopreventive agent *N*-(4-hydroxyphenyl)retinamide *in vitro* (86). Additional studies are clearly needed to determine the role of the NO/iNOS pathway in tumorigenesis *per se* and to establish the utility of iNOS inhibitors as chemoprevention agents. The complex biological actions of this ubiquitous signaling molecule will necessitate careful experimentation to adequately assess risk/benefit and to identify the most appropriate cohorts for preventive intervention.

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Molecular Cancer Therapeutics

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