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Review

Site-directed delivery of nitric oxide to cancers

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ABSTRACT

Nitric oxide (NO) is a reactive gaseous free radical which mediates numerous biological processes. At elevated levels, NO is found to be toxic to cancers and hence, a number of strategies for site-directed delivery of NO to cancers are in development during the past two decades. More recently, the focus of research has been to, in conjunction with other cancer drugs deliver NO to cancers for its secondary effects including inhibition of cellular drug efflux pumps. Among the various approaches toward site-selective delivery of exogenous NO sources, enzyme activated nitric oxide donors belonging to the diazeniumdiolate category afford unique advantages including exquisite control of rates of NO generation and selectivity of NO production. For this prodrug approach, enzymes including esterase, glutathione/glutathione S-transferase, DT-diaphorase, and nitroreductase are utilized. Here, we review the design and development of various approaches to enzymatic site-directed delivery of NO to cancers and their potential.

Contents

1. Introduction

Nitric oxide (NO) has multifaceted roles in biological systems including neurotransmission, regulation of blood pressure, cardiac function, platelet aggregation, regulating multiple functions in reproductive systems, signal transduction, antimicrobial defense, maintenance of cellular redox status, and anti-proliferative activity [1–5]. NO is produced endogenously by nitric oxide synthase (NOS). The alternative NOS-independent pathway prevalent in hypoxic and acidic condition is catalyzed by various reductive enzymes like bacterial nitrate reductases and xanthine oxidase, to reduce nitrates and nitrites to NO [6–12]. The relationship between NO and cancer is complex and is dependent on its concentration, dosage, duration and its location of release [13,14]. At elevated concentrations, NO and associated reactive nitrogen species (RNS) can react spontaneously with metal ions and biomacromolecules possibly resulting in their inactivation. Some common RNS-induced modifications are nitration and nitrosylation of proteins, lipids and DNA, deamination of DNA leading to mutations possibly causing loss in functions triggering induction of necrosis and/or apoptosis; a favorable outcome in targeting cancers (Fig. 1) [10,13–16].

During episodes of oxidative stress, nitric oxide can react with superoxide, a reactive oxygen species (ROS), to produce peroxynitrite, which is highly toxic to cells [17,18]. Thus, a synergy between ROS and RNS often leads to enhanced cytotoxicity. A common phenotype associated with cancers is enhanced ROS in comparison with the paired normal tissue [19,20] and hence, introduction of NO might result in greater inhibition of proliferation. In support of this claim,

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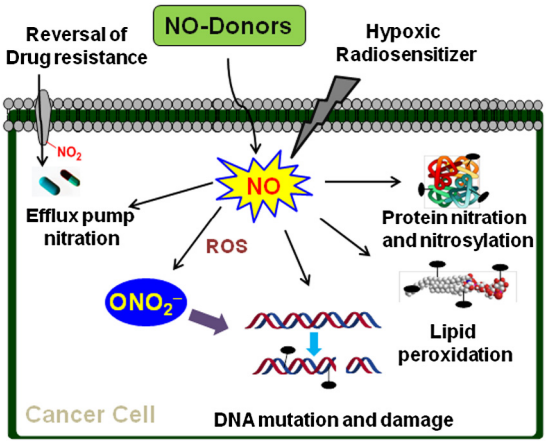

Fig. 1. Cellular effects of increased exposure to nitric oxide.

in a panel of lung cancer cells, the ones with higher basal levels of ROS were more sensitive to cytotoxic effect of NO, in part, suggesting the intermediacy of peroxynitrite [21].

NO is also known to reverse chemotherapy resistance in colon cancer cells [22,23]. Among the various mechanisms involved in the drug resistance, the important ones are the upregulation of efflux pumps in resistant cells that pump out the drugs out of the cell [24–26]. Due to diminished accumulation of cytotoxic drugs in cancer cells, desired anti-proliferative effects are not observed. The most common classes of efflux pumps include P-glycoprotein (P-gp) and multiple drug resistance-associated proteins (MRP) and are particularly relevant due to their broad spectrum of substrates. It has been found that NO inhibited the MRP-3 efflux pump by nitrating the tyrosine and thereby led to inversion of drug resistance [22,27,28]. Thus, it is likely that NO can be used in conjunction with traditional cancer drugs that have become ineffective due to such modes of efflux.

A number of solid tumors are associated with extensive regions of low oxygen concentrations (hypoxia) and these cells are found to be resistant to most anticancer drugs [15,29–31]. Hypoxic cells are distant from blood vessels and as a result, are not adequately exposed to some types of anticancer drugs. The vasculature of tumors is chaotic and hence blood supply is not uniform to all regions. Also, hypoxia selects for cells that have lost sensitivity to p53-mediated apoptosis, and genes involved in drug resistance are upregulated, including genes encoding p-glycoprotein which might reduce sensitivity to some anticancer agents [15]. Multidrug resistance has emerged as a major clinical problem and hypoxia contributes to drug resistance in solid cancers. Recent studies show that tumor hypoxia induces resistance to anticancer drugs by interfering with endogenous NO signaling and hence, introduction of low concentrations of NO might attenuate hypoxia-induced drug resistance in tumor cells [27,28,32–34]. Furthermore, NO can also act as a hypoxic radiosensitizer [35–37] and this aspect is particularly useful as low oxygen concentrations in tumors result in diminished sensitivity to radiation therapy and poor prognosis. NO can accentuate radiation-induced DNA damage thus causing increased cell death (Fig. 1). Another important adaption of hypoxic cells resulting in uncontrolled growth and recurrence of tumors post-surgery is the expression of the transcription factor hypoxia inducible factor (HIF-1) [38]. HIF-1 has been reported to promote angiogenesis and promote metabolic adaptation through increase in glycolytic enzymes [39,40]. Increased expression of HIF in human cancer cells has been shown to increase tumor growth, angiogenesis, and metastasis, whereas genetic manipulations that decrease HIF expression result in decreased tumor growth, angiogenesis, and metastasis in animal models. This and other similar data supports inhibiting HIF-1 is an important strategy toward developing new drugs targeting hypoxic tumors. Under hypoxic conditions, nitric oxide blocks activation of HIF-1 [41,42] by inhibition of an activation step of HIF-1α to a DNA-binding form. Since NO diffuses easily across the cell membranes, it can show its tumoristatic effects in neighboring tumor cells as well through the bystander effect. Thus, selectively enhancing NO levels in tumors might have enormous therapeutic value [16,43–45].

1.1. Delivery of nitric oxide

Due to the involvement of NO in various biological activities leading to systemic side-effects as well as due to its reactive and unstable nature, controlled and localized generation of NO to cancer cells is challenging. NO donors like organic nitrates, diazeniumdiolates [15,46–51], *S*-nitrosothiols [9,43,52,53], metal-NO complexes [54–56], furoxans [57–59] have been reported to show anti-cancer effects in certain types of cancer cells [45]. However, most of the aforementioned methodologies suffer from drawbacks associated with potential toxicity due to non-specific activation and inability to deliver NO to the desired site of action. For selective and effective delivery of cytotoxic NO to tumors site directed drug delivery using enzymatically activated "prodrug" strategy (Fig. 2) and macromolecular scaffold-based drug delivery are promising. These strategies exploit the abnormalities that exist in tumors such as upregulation of certain enzymes in tumors, existence of bioreductive environment in tumor cells, low pH condition and imperfect vasculature [29,60]. In the prodrug strategy, NO-donors are anchored to a triggering group to form a non-toxic molecule, which is then metabolized into toxic form by specific enzymes, over-expressed in cancer cells and releases cytotoxic NO (Fig. 2).

The NO-donor prodrugs are inactive in their native form and also presumably non-toxic. The inactive form is metabolized into an active NO-releasing form by specific enzymes over-expressed in cancer cells. In designing and developing this NO prodrug concept, diazeniumdiolates are frequently used as NO surrogates. Diazeniumdiolates are known to spontaneously release NO in physiological media [61] and the versatility of this NO donor is exploited by anchoring the diazeniumdiolate anion to a group which gives directionality toward cancer cells and gets activated to release NO in cancer cells (Scheme 1). Depending on the type of enzymes used for activation, different classes of protected diazeniumdiolates have been designed (Scheme 1). However, such methodologies do not preclude the possibility of activation of the prodrug in normal cells as well, albeit at low concentrations, to generate NO. Hence, an alternate strategy that might be advantageous is the introduction of an exogenous enzyme that is not normally expressed in mammalian cells either by transfection methodologies (for GDEPT) or by the use of tumor-specific antigens conjugated to the enzyme (for ADEPT) [62–65]. Upon exposure to the exogenous enzyme, the inactive prodrug, which is a substrate for the enzyme is metabolized to produce the cytotoxic species either intracellularly or in the proximity of tumors. As normal cells do not express this enzyme, potential deleterious side-effects can be minimized (Fig. 2).

As with other cancer drugs, macromolecular scaffold-based NO delivery to cancers aims to exploit enhanced permeability and retention effect (EPR) of tumors [66,67]. Due to the large size of these particles and defective tumor vasculature, the NO carrying particles are selectively accumulated in tumor cells and subsequently release NO in tumors. The macromolecular scaffold-based approach has been reviewed recently *Chem. Soc. Rev.*, **2012**, *41*, 3731–3741 and *J. Mater. Chem. B*, **2014**, *2*, 341–356 and in this review, we discuss various approaches to localized delivery of NO in cancers focusing on prodrug-based methodologies.

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