Redox Biology 6 (2015) 1–8

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research Paper

NO to cancer: The complex and multifaceted role of nitric oxide and the epigenetic nitric oxide donor, RRx-001 ☆

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ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form
30 June 2015
Accepted 1 July 2015
Available online 2 July 2015

Keywords:
Nitric oxide
Radiosensitization
Radiotherapy
Hypoxia
Cancer

ABSTRACT

The endogenous mediator of vasodilation, nitric oxide (NO), has been shown to be a potent radiosensitizer. However, the underlying mode of action for its role as a radiosensitizer – while not entirely understood – is believed to arise from increased tumor blood flow, effects on cellular respiration, on cell signaling, and on the production of reactive oxygen and nitrogen species (RONS), that can act as radiosensitizers in their own right. NO activity is surprisingly long-lived and more potent in comparison to oxygen. Reports of the effects of NO with radiation have often been contradictory leading to confusion about the true radiosensitizing nature of NO. Whether increasing or decreasing tumor blood flow, acting as radiosensitizer or radioprotector, the effects of NO have been controversial. Key to understanding the role of NO as a radiosensitizer is to recognize the importance of biological context. With a very short half-life and potent activity, the local effects of NO need to be carefully considered and understood when using NO as a radiosensitizer. The systemic effects of NO donors can cause extensive side effects, and also affect the local tumor microenvironment, both directly and indirectly. To minimize systemic effects and maximize effects on tumors, agents that deliver NO on demand selectively to tumors using hypoxia as a trigger may be of greater interest as radiosensitizers. Herein we discuss the multiple effects of NO and focus on the clinical molecule RRx-001, a hypoxia-activated NO donor currently being investigated as a radiosensitizer in the clinic.

1. Introduction

Nitric oxide (NO) is a familiar molecule, having been studied extensively over the past 3 decades, whose seeming simplicity of structure has activity impacting nearly every major organ system [1–3]. NO is a gaseous diatomic radical that readily passes through biological membranes and is a well characterized vasodilator and anti thrombotic agent in a cardiovascular setting. Less well understood are its protean effects and activity, good and bad, therapeutic and harmful, in cancer, which is the subject of this review.

In mammals, under normoxic conditions, NO is generated endogenously 'on demand' by the oxidative conversion of Arg to L-citrulline, catalyzed by a variety of nitric oxide synthases (Fig. 1). These synthases can be divided into functional classes, constitutive and inducible NOS (cNOS and iNOS), based on calcium sensitivity [4]. Although the action of NO includes induction of signaling through nitration of proteins the chief activity is vasodilation through binding of soluble guanylate cyclase, the primary target of NO, leading to synthesis of cGMP, the key mediator for downstream NO-related signaling. Under hypoxic conditions, NOS activity is disabled and NO is synthesized independently through the hypoxia-specific nitrite reductase activity of deoxyhemoglobin and others that effectively catalyze the conversion of serum nitrite to NO [5]. NO is highly promiscuous, binding with a wide range of targets resulting in varied and sometimes diverse activity. Metabolism is rapid, with metabolites including species such as peroxynitrite and N_2O_3 that display anti-tumor mechanisms either alone or in concert with radiation [6,7]. With rapid synthesis, high permeation and a short half-life, NO is a highly effective molecule

☆This article belongs to a special issue on Nitric Oxide and Cancer, edited by Jordi Muntané and Benjamin Bonavida.

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http://dx.doi.org/10.1016/j.redox.2015.07.002
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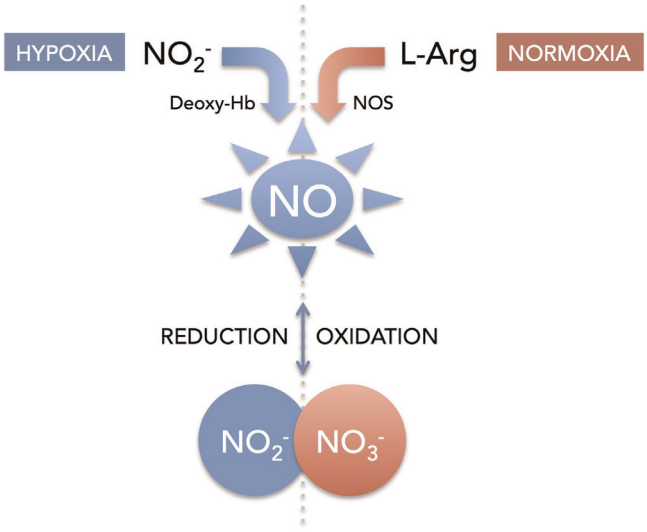

Fig. 1. The nitric oxide–nitrite–nitrate pathway. Under normal oxygen parameters (normoxia), nitric oxide (NO) is produced by NOS from L-Arg. In the absence of oxygen (hypoxia), nitrite is reduced by a variety reductases, including deoxyhemoglobin to produce NO. Further reduction/oxidation of NO can lead to production of metabolites.

for local and transient signaling, underlining the importance of its context-specific activity.

Resistance to radiotherapy and subsequent recurrence of disease is a significant problem in the treatment of cancer. A key factor contributing to the observed radioresistance is tumor hypoxia [8,9]. Hypoxic tumors can have an aggressive phenotype, strongly associated with progression, metastasis and chemo and radioresistance. The reversal of tumor hypoxia to transform the tumor to a phenotype that is more sensitive to radiation or chemotherapy has been a major are of research [10].

As a consequence of its local and context-dependent mechanism, the activity of NO as a radiosensitizer is complicated, and in some cases contradictory, depending very much on context of activation [11]. The context factors include the local concentration of NO, the site of action, the oxygenation and architecture of the local tissue and vessels, in essence, the tumor microenvironment. This context dependent, complex and highly varied activity of NO has led to a paradox with contradictory observations on the action of this species as a radiosenstizer with some articles substantiating and demonstrating radiosensitizing activity, with others reporting the opposite [2]. This paradox has been greatly debated, and NO with its biologically closely related partners, described as 'the good, the bad and the ugly' namely NO, superoxide and peroxynitrite respectively [12].

Like a two-edged sword, NO can function both positively and negatively in a variety of ways: for example NO can function as a pro and anti-apoptotic agent, promoting or protecting cells from oxidative stress, and acting both as a radioprotector and radiosensitizer.

As 'Il Brutto' or 'the bad', evidence points towards NO as a tumor promoter, with increased expression of iNOS in a number of human tumors such as pancreatic, breast, gynecologic and head and neck [13], and strongly suggests NO plays a role in metastasis as well as growth promotion possibly by enabling increased tumor blood flow *via* inducing vasodilation [12,14]. Furthermore, inhibition of NO synthase was found to decrease tumor blood flow and induce tumor shrinkage [15].

However, NO as 'Il Buono', acting as a hypoxic cell radiosensitizer [16] has been described extensively in the literature with radiosensitizing effects arising from endogenous induction [16] or through external application of NO-donors [17]. The NO activity observed was explained through effects on systemic and hypoxic vasodilation, RBC rheology and decreased oxygen utilization. These are discussed in greater detail below.

Since NO activity is heavily dependent on context and concentration, the paradoxical activity of this elusive molecule must be considered multidimensionally, by considering local concentration, interaction and reaction with immediate biological surroundings and metabolism to form other, and often more reactive, nitrogen oxides. The concentration specific activity of NO can be considered as having a series of thresholds: at very low levels, tumor growth is stunted [18], while relatively moderate concentrations promote tumor angiogenesis and cell survival [19]. Further increases in local NO concentration above this threshold switch NO activity to an anti-tumor role [20]. One rationalization or attribution that could account for these observations is through the effect of the formation of highly reactive NO metabolites such as peroxynitrite and N_2O_3, characterized as 'Il Cattivo' or 'The Ugly' by Beckman [12]. These reactive oxygen and nitrogen species (RONS) can go on to induce, directly or indirectly, cellular damage and changes in microenvironment that lead to apoptosis and can serve to promote radiosensitization. The concept of multiple thresholds for NO activity is critical to the understanding of its radiosensitizing and cytotoxic effects [18,19].

The delivery of nitric oxide to serve as a radiosensitizer needs to take into consideration local *versus* systemic effects. Often high doses of NO donors do not necessarily lead to high local tumor concentrations of NO, while resulting in systemic side effects such as hypotension. The local and context-specific activity of NO as a radiosensitizer suggests that a NO-mediated radiosensitizer should, ideally, deliver NO selectively to areas of tumor hypoxia. This review on NO as a radiosensitizer will therefore describe some aspects of nitric oxide physiology to place in context the radiosensitizing effects of this contradictory molecule and then will focus on the preclinical radiosensitizing effects of RRx-001, a novel hypoxia mediated and locally acting nitric oxide donor that is currently being explored in the clinic as a radiosensitizer.

2. Radiosensitization effects of oxygen and nitric oxide are closely linked

Oxygen is intricately linked with radiosensitization. The activity of radiation is multiplied two to three times in the presence of excess oxygen, while the most common radiation resistance mechanism is related to the low oxygen levels in solid tumors. Incident radiation ionizes water molecules leading to the formation of reactive oxygen species that produce DNA breaks with radical species at the end of the fragments [10]. Whether this damage leads to cell death or is repaired depends on whether oxygen is able to react with the DNA radicals to form peroxy radicals that are not as readily repaired. In the absence of oxygen, DNA radicals are quenched through thiol-mediated redox reactions and DNA repair mechanisms that enable the cell to survive. Consequently, reoxygenation of tumors has been extensively investigated as a radiosensitization strategy [10]. Though beyond the scope of this review, reoxygenation proved to provide only variable or poor results as a consequence of the difficulty of ensuring diffusion of oxygen to the hypoxic regions of the tumor [10]. Using an indirect approach, compounds that mimic the effect of oxygen by donating radicals that serve to 'fix' DNA damage have also been extensively explored. While many of these compounds did indeed possess radiosensitizing properties, clinical utility was limited either by toxicity or low activity [10,21].

In addition to the vasodilating effects of NO that can lead to greater tumor oxygenation and hence radiosensitization, discussed in more detail below, as a free radical and source of reactive

oxygen and nitrogen species (RONS), NO exerts more direct radiosensitizing effects. With comparable electron affinity to oxygen, nitric oxide would be expected to also fix DNA radiation damage in a similar way [22]. Indeed, radiation-generated guanine and adenine radicals reacting with NO in a combination experiment have been shown to result in nitrosation products such as 8-azaguanine, xanthine and 8-azaadenine [23,24]. However, because of its short half-life and rapid reaction with oxygen and reactive oxygen species, the radiosensitizing effects of NO are surprisingly long-lived. This may be due, in part, to the induction of vasodilation and associated improved diffusion of blood containing oxygen, NO and RONS deep into tumors to potentiate the effect of radiation [25,26]. Moreover, in the presence of low dose radiation, NO promotes p53 nuclear retention, sensitizing cells to apoptosis [27]. The formation of NO metabolites with oxygen and superoxide such as peroxynitrite [28] drive the enhanced radiosensitization effect of NO, enabling the parent molecule to essentially act with a pronounced bystander effect. Relatively small increases in intratumoral NO can lead to significant and prolonged effects. Peroxynitrite, in particular, can cause multiple cell insults such as lipid peroxidation, cysteine oxidation, and protein nitrosylation leading to apoptotic and necrotic cell death [29]. Thus the presence of peroxynitrite serves as an important anti-tumor mediator and radiosensitization mediator [30].

3. Indirect NO radiosensitization effects: increasing tumor oxygenation

While it is indisputable that NO has an effect on tumor blood flow, the activity observed in different studies has been contradictory. In some instances, observations supported increased tumor perfusion, with enhanced responses to radiation, while other studies have reported decreased blood flow that was associated with increased tumor proliferation [31]. For example, a study administering a low systemic dose of NO through application of transdermal nitroglycerin to patients with prostrate cancer reported a lowering of PSA, which was attributed to an increase in tumor blood flow [20]. Additionally, a promising response rate of 75% was found with concurrent chemoradiation in a Phase 2 study in locally advanced NSCLC patients treated with cisplatin and vinorelbine plus concurrent nitroglycerin with radiotherapy [32]. The differences in observations between studies could be explained by tumor microenvironment heterogeneity and differences between tumor types [33]. These contradictory observations could be rationalized by a redistribution of blood flow through the ‘steal effect’, a consequence on blood flow of a systemic vasodilatory response as a result of the administration of a systemic NO donor. Paradoxically, because tumor vasculature is fully dilated, NO donation results in an overall redistribution of blood to the systemic circulation, thus lowering tumor perfusion. Conversely, an anti-steal effect mediated through systemic vasoconstriction, for example, could increase blood flow to the tumor [34]. In addition to direct effects on the vasculature, NO has been shown to alter red blood cell elasticity and to reverse the surface crenulation that arises in RBCs after exposure to low oxygen in a similar way to the effect found in blood storage products [35]. NO donation would then be expected, therefore, to increase tumor blood perfusion by increasing red blood cell elasticity and reducing flow viscosity, thus enabling the delivery of oxygen into areas of the tumor that would not be readily oxygenated. These vascular changes would be expected to increase the efficacy of radiation.

4. Indirect NO radiosensitization effects: the effect of hypoxia

Tumor hypoxia can be described in two different ways: chronic hypoxia arises in tissue where blood perfusion is limited and is characterized by a decreasing oxygen tension gradient that is related to the distance from functioning blood vessels. Acute hypoxia however, arises from temporary blockages in blood flow to specific parts of the tumor by poor blood vessel patency. While delivering a blood-borne agent to chronically hypoxic regions is clearly challenging, it is anticipated that areas of acute hypoxia would be more sensitive to the vasodilatory and red blood cell viscosity modifying effects of NO to temporarily ease blockages and allow entry of blood flow and tissue reoxygenation, leading to an improved radiation response.

NO transport by red blood cells is complex and subject to contradictory theories. One hypothesized mechanism describes the transport of NO by hemoglobin with NO bound either to the heme iron or to a highly conserved residue on the beta chain, the cysteine 93. The beta Cys-93 residue of hemoglobin is highly conserved throughout mammalian species and is vital for controlling hemoglobin oxygen affinity and for serving as a key residue for NO binding and transport [36,37]. This residue is in close proximity to the His-92 that interacts directly with the heme iron and thereby closely controls hemoglobin oxygen affinity. Binding to this residue increases hemoglobin oxygen affinity, allowing for release of oxygen under greater hypoxia leading to, indirectly, increased radiosensitization. The transition of hemoglobin from its oxygenated (R) to deoxygenated (T) form, allows the release of NO to diffuse, either as the parent or as a S-nitrosothiol, to the vessel wall inducing the cascade leading to vasodilation, improved blood flow, oxygenation and, therefore, radiosensitization [38]. An alternative mechanism invokes the role of systemic nitrite that is reduced under hypoxic conditions by deoxyhemoglobin and other nitrite reductases to NO [5]. Regardless of transport and source of NO, both these competing hypotheses describe NO being delivered to areas of hypoxia to facilitate vasodilation and allow oxygen transport.

Under hypoxia, HIF-1 alpha [39], one of the primary responses to hypoxia, is stabilized and induces the expression of proteins important to the hypoxic response such as VEGF, GLUT2 and others, stabilizing the low oxygen phenotype and hence promoting resistance to radiation [10]. Acting directly on the mitochondrial respiratory chain through cytochrome c oxidase [1], NO and its metabolites (*e.g.* nitrate) inhibit mitochondrial respiration [40], leading to lower oxygen consumption [41]. This results in an increase in oxygen availability to targets that are not part of the respiratory chain [42] such as HIF-1 alpha. Thus NO mediated reoxygenation and lower oxygen use can lead to increased radiosensitivity [41] through the reversal of HIF-1 alpha stabilization and suppression of the hypoxic response [43].

5. Nitric oxide as a radioprotectant

Paradoxically, in addition to the extensive literature supporting the role of NO as a radiosensitizer, there have been a smaller number of reports suggesting a role for NO as a radioprotectant. Studies have demonstrated a greater survival of mice that were exposed to whole body radiation after treatment with the NO donor DEA/NO [44], while a number of papers have described the blocking of CD47 and TSP-1 interactions, thereby increasing NO levels, as a successful strategy to protect mice from the effects of total body irradiation [45,46]. Indeed CD47 deficient animals were observed to be less sensitive to the effects of radiation [47]. Interestingly, targeting of CD47 was reported by Maxhimer as resulting in both radiosensitization of tumors and radioprotection of normal tissue [45], an observation of potential relevance to the activity of RRx-001 described below. With thrombospondin-1 and CD47 closely linked to the activity of NO, a role for NO in

radioprotection could be anticipated. However, alternative hypotheses suggest that radioprotection arises from the induction of autophagy [47], by changes in local tumor blood flow or by a systemic 'steal' effect leading to lower oxygen delivery to bone marrow and, paradoxically, inducing a local hypoxia-mediated protective response [44]. In addition, the quenching of RONS by reaction with increased levels of NO could confer a direct antioxidant effect [48] as evidenced by increased nuclear translocation of Nrf2, a key member of the antioxidant response element, that results in expression of redox quenchers such as glutathione [49].

6. Radiosensitization strategies based on NO

As discussed above, systemic NO-donors have been shown to be radiosensitizers: while radiosensitization with NO gas itself has been demonstrated [50,51], the use of NO donors such as DEA/NO [52], the nitroxyl donor Angeli salt [53], DETANONOate [54], spermine nonoate [22] or isosorbide dinitrate [55] is more common. In addition to radiosensitization, in the studies of these NO donors, increases in tumor blood flow were also observed when the administration of NO synthase inhibitors led to radioresistance [55]. However, in other studies NO donors, in this case nitroprusside [56], resulted in a vascular steal effect, as described above, manifested by a drop in mean arterial blood pressure. Other studies have reported similar results: NO donors that have been reported elsewhere to be radiosensitizers that increased tumor blood flow, were also found to lower tumor oxygenation through a steal effect [57].

Although these reports highlight the inconsistent and sometime contradictory effects of NO on tumor blood flow, a conclusion that can be drawn from these publications is that the systemic effects of NO can greatly influence local intratumoral activity. One strategy that could circumvent the steal effect and lead to potentially better radiosensitization results would be to deliver NO in such a way that it would be released or produced in the tumor microenvironment, thereby affecting local vasodilation and RONS production without systemic effects.

A number of strategies to deliver or generate NO locally have been investigated. In addition to RRx-001, which delivers NO to the tumor under hypoxia (discussed below), approaches that induce the local production of NO by driving the iNOS gene have shown promise. In addition to exploiting the cytokine induced upregulation of iNOS, resulting in local NO synthesis [21], many of these approaches have explored using transfected viral vectors encoding human iNOS. For example, transfecting the iNOS gene under the control of a constitutive cytomegalovirus immediate early promoter resulted in significant production of NO *in vitro* under hypoxic, but not normoxic conditions [58]. Other strategies such as 'radiogenic therapy' [59] achieve iNOS gene transfer using WAF-1 [16] or pE9 [60] radiation-inducible promoters, essentially using the presence of incident radiation to activate NO production.

7. Local delivery of nitric oxide by RRx-001

RRx-001 is an aerospace industry-derived anti-cancer agent that acts as an ROS and RNS-mediated epigenetic modulator that has completed a Phase 1 first in human study [61] and is currently in Phase 2 clinical development. RRx-001 (Fig. 2) has also been reported to have radiosensitizing anti-tumor properties while acting as a radioprotectant in normal tissue. RRx-001 differs from other NO-donating compounds in that the molecule induces local, endogenous and biphasic production or release of NO, rather than fragmenting to release NO systemically. This activity is closely linked to the metabolism of RRx-001. Metabolic and disposition studies on RRx-001 have shown that, on infusion, the compound rapidly, irreversibly and selectively binds to hemoglobin at a key NO binding site, the beta-cysteine 93 residue described above [62] (Fig. 3) and with glutathione [63,64] indirectly increasing oxidative stress [65]. While the RRx-001 glutathione adduct is rapidly excreted, RRx-001-bound hemoglobin remains in circulation for the duration of the lifetime of the red blood cell (Fig. 4).

NO release is biphasic: initial release occurs immediately on infusion, presumably due to the displacement of NO from the RRx-001 binding site on hemoglobin. The release is rapid and transient resulting in local vasodilation that resolves on cessation of infusion. Deoxygenated hemoglobin can act as an efficient nitrite reductase, catalyzing the reduction of serum nitrite to NO under hypoxic conditions and compensating for the inactivity of nitrite synthetase activity under hypoxia [67–69]. In the small sub-set of red blood cells that contain RRx-001 bound hemoglobin, the production of NO under hypoxic conditions is greatly potentiated [67], resulting in an overproduction of NO in deeply hypoxic tissue, particularly in tumors subject to acute hypoxia.

As has been shown above, NO can act as an efficient radiosenstizer. With RRx-001 delivering NO and ROS at the right time and in the right place through the amplification of hypoxia-mediated deoxyhemoglobin nitrite reductase activity, RRx-001 has been shown to possess marked radiosensitizing properties.

The radiosensitizing effects of RRx-001 were studied in cellular assays and in animal models [70]. RRx-001 was found to be a potent radiosensitizer *in vitro*, in multiple cell lines, with radiation dose modification factors (DMF) of 1.7 and 1.6 for HT29 and SCCVII cell lines respectively (Fig. 5). Radiation survival was studied under both hypoxic and normoxic conditions with RRx-001, and RRx-001 was found to increase the response of radioresistant hypoxic cells with a DMF of 1.9, suggesting an enhanced radiosensitizing effect of RRx-001 under hypoxia.

In vivo, the radiosensitizing effect of RRx-001 was studied in syngeneic tumor models [71]. Dosing RRx-001 at 5 or 6 mg/kg daily in combination with local tumor radiation at 250 or 400 cGy resulted in an enhancement of the effect of the local tumor irradiation, with a significant increase in the tumor growth delay time compared to untreated controls as well as mice treated with either RRx-001 or radiation alone (Fig. 6). Although as a single agent, RRx-001 inhibited tumor growth, the tumor growth delay observed in the combination therapy was significantly greater and was determined to be synergistic. Animals dosed with RRx-001 as

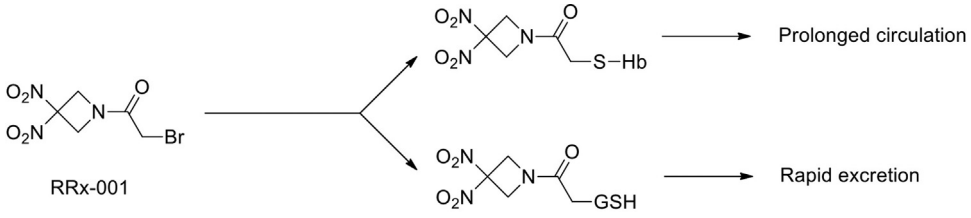

Fig. 2. RRx-001: structure and primary metabolites (Hb: hemoglobin, GSH: glutathione).

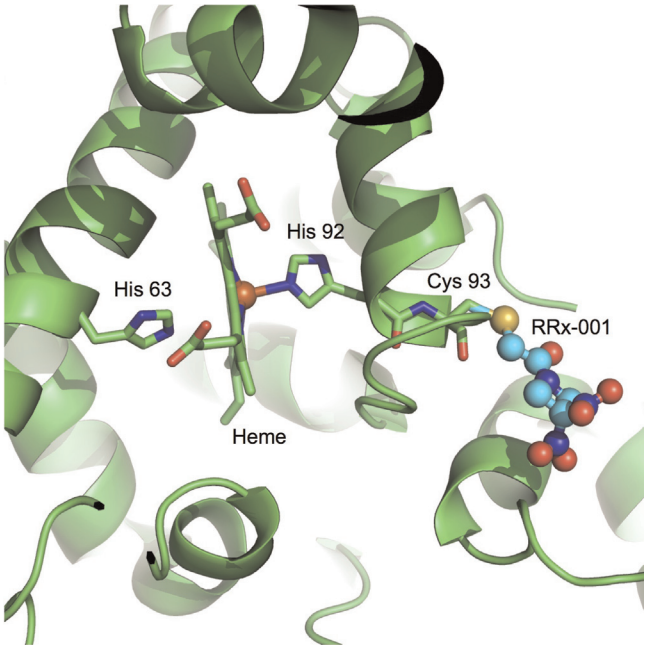

Fig. 3. Molecular model of RRx-001 bound to the beta-Cys-93 of deoxyhemoglobin showing proximity to heme.

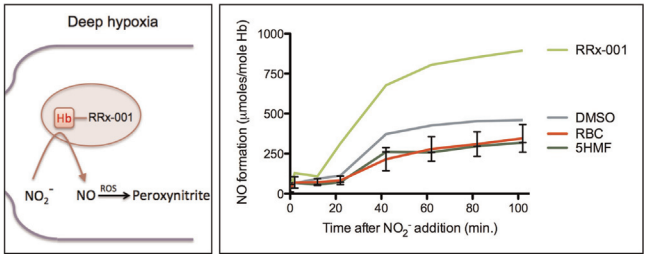

Fig. 4. NO release from RRx-001 treated RBCs. Left: schematic of reduction of serum nitrite to nitric oxide by RRx-001-bound hemoglobin. Right: RRx-001 potentiates the nitrite reductase activity of hemoglobin. Rate of NO formation of nitric oxide from nitrite compared to controls (Fens et al. [66]).

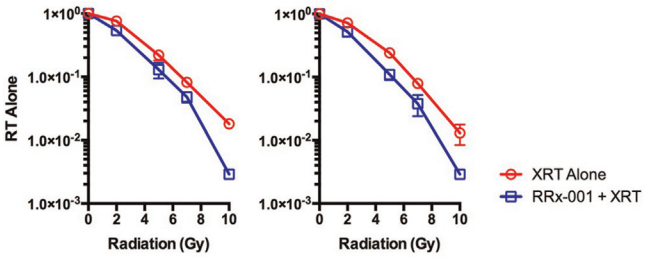

Fig. 5. Combination of RRx-001 and radiation. XRT alone or in combination with RRx-001 in HT-29 cells ($P<0.01$) (left) and SCCVII cells (right) ($P<0.01$).

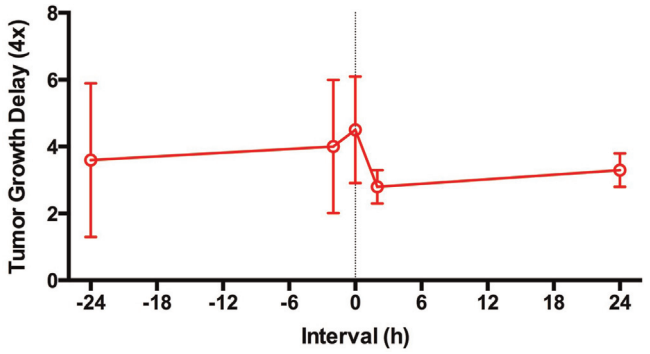

Fig. 7. Time and sequence dependence of the radiosensitizing effect of RRx-001 showing the relationship between 4× TGD and the dosing schedule of RRx-001 and radiation. RRx-001 was dosed at $t=0$, ± 2, and ± 24 h. Total body radiation (7 Gy) was administered at $t=0$ h.

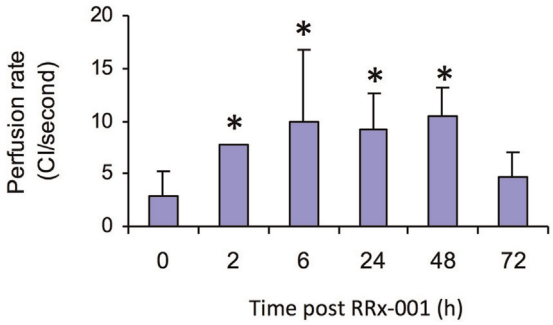

Fig. 8. Microbubble ultrasound imaging of tumor blood flow in mouse. Perfusion rate reaches a maximum at 6 h with the effect persisting for at least 48 h.

a single agent or in combination with radiation did not exhibit overt systemic toxicity as determined by weight loss and no significant hematological, biochemical, or histopathological changes were observed [70,71].

The effects of sequence and timing of RRx-001 radiosensitization were also investigated. In a SCCVII syngeneic mouse tumor model, RRx-001 was administered at the same time, or 2 and 24 h before and after a single dose of 7 Gy to the tumor. The maximal radiosensitizing effect was found to occur when RRx-001 was administered between 0 and 2 h before radiation (Fig. 7).

As discussed above, the radiosensitizing effects of NO and NO donor molecules can result from changes in tumor blood flow in addition to the direct effects of NO metabolites. RRx-001 was found to modify tumor blood flow. Using contrast-enhanced ultrasound, increases in tumor blood flow and perfusion were observed in mice bearing SCCVII tumors for up to 48 h post dose with flow peaking at 6 h (Fig. 8) [70].

Abdominal and pelvic tumors are often treated with radiotherapy but the dose of radiation that can be safely administered is limited by the sensitivity of the GI epithelium to radiation, which can result in both acute GI toxicity, as well as late effects such as fibrosis, obstruction and perforation [72]. The determination of

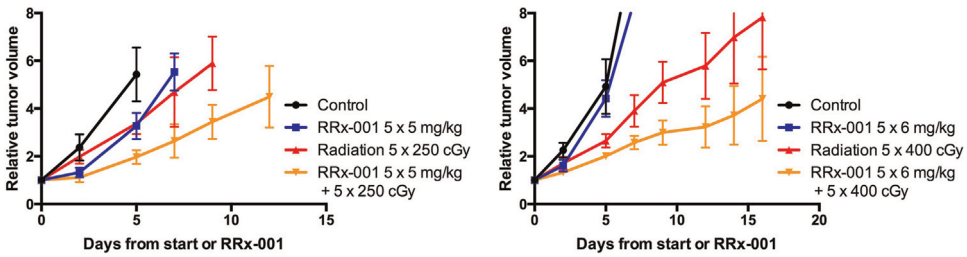

Fig. 6. Tumor growth curves in mice showing the effect of a combination of RRx-001 with radiation ($P<0.05$ between combination and single agent groups).

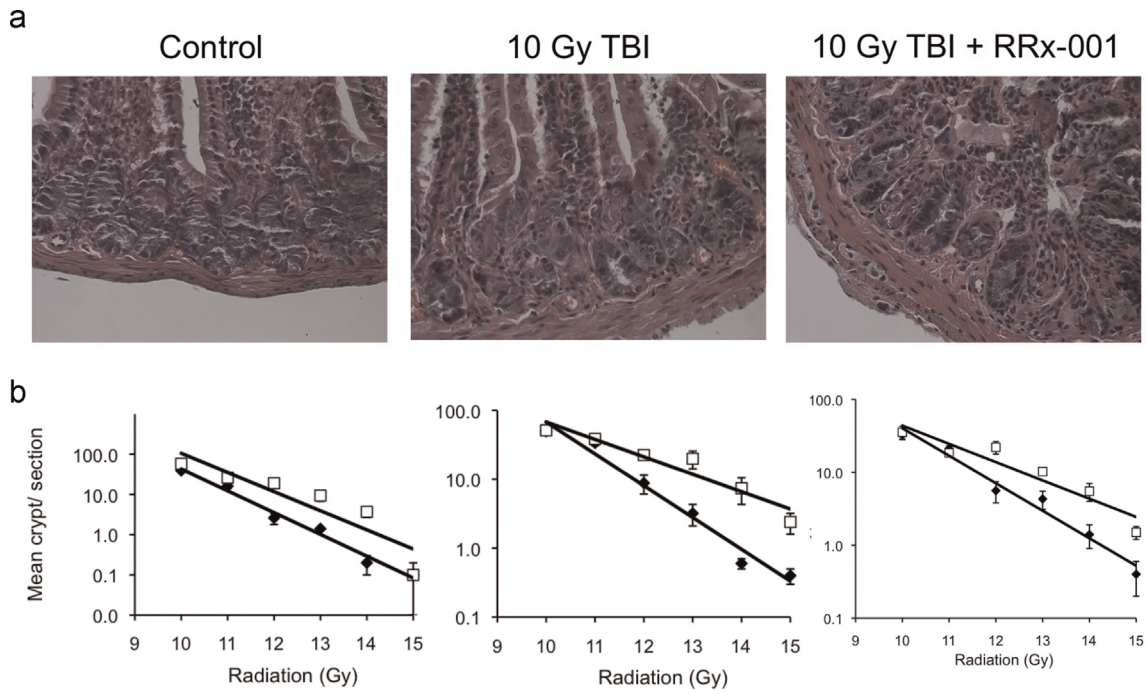

Fig. 9. Radioprotection of intestinal crypt cells by RRx-001. Mean crypt cells per duodenum (left) and jejunum (right) sections compared to TBI only dose. ♦=TBI; □=TBI+RRx-001.

whether or not a potential radiosensitizer could result in additional toxicity arising from sensitization of normal GI epithelium is therefore important. Since the survival of intestinal crypt cells is a very sensitive method for determining radiation-induced toxicity to the intestine, the intestinal crypt cell assay was used to determine the effect of RRx-001 on intestinal epithelium [73]. In these experiments, mice were exposed to total body radiation of 10–15 Gy in combination with RRx-001. RRx-001 was found to have a radioprotectant effect on stem cells in the duodenum, ileum and jejunum, with a higher number of viable crypt cells at the end of the experiment in the mice treated with RRx-001 prior to irradiation compared to mice treated with radiation alone (Fig. 9).

8. Conclusions

The roles of nitric oxide and oxygen in the context of radiosensitization are deeply intertwined with nitric oxide (NO), which can facilitate the permeation of oxygen into hypoxic areas and control oxygen function and reactivity. This close relationship suggests that nitric oxide donors can play an important role as radiosensitizers, and that manipulation of the balance of nitric oxide and oxygen can be an effective mechanism of tumor radiosensitization. However, given the often contradictory and confounding results from many studies, use of current NO donor chemotypes as radiosensitizers is fraught with difficulties, with mixed results and systemic toxicity. Therefore, further research into the mechanism of NO-mediated radiosensitization, as well as the discovery of a new generation of NO donors is needed to improve the therapeutic index of this approach. With the growing understanding of the context-specific activity of NO, research into novel NO donor radiosensitizers should focus on approaches that selectively increase the local concentration of NO in tumors. This approach would be anticipated to result in selective local effects in tumors, with little or no effect on systemic circulation or blood pressure, and would be widely applicable to different tumor types. Such a strategy could mitigate the effects of tumor hypoxia and associated downstream effects, thereby addressing an important unmet need in cancer therapy. Novel agents, such as the small molecule RRx-001, that deliver NO selectively to tumor tissue, while sparing normal tissue with negligible systemic side effects, represent an exciting new direction for the development of radiosensitizers in the future, that may have the potential to significantly increase the therapeutic index of radiation therapy.

Attributions

Figs. 5–9 are adapted (or reprinted) by permission from the American Association for Cancer Research [S. Ning, et al., Dinitroazetidines are a novel class of anticancer agents and hypoxia-activated radiation sensitizers developed from highly energetic materials, Cancer Res. 14(72) (2012) 2600–2608].

Acknowledgments

The authors would like to acknowledge collaboration with Drs. R. Wardle and L. Cannizzo at Orbital ATK (formerly ATK Aerospace Systems, ATK Thiokol) on the discovery of RRx-001. EpicentRx, Inc. provided funding for all studies related to RRx-001.

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