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NITRIC OXIDE AS A RADIOSENSITIZER: EVIDENCE FOR AN INTRINSIC ROLE IN ADDITION TO ITS EFFECT ON OXYGEN DELIVERY AND CONSUMPTION

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Different nitric oxide (NO)-mediated treatments (e.g., isosorbide dinitrate, insulin and electrical stimulation of the host tissue) have been investigated for their effects on tumor oxygenation and radiation sensitivity. We further address the issue of the role played by modulation of the NO-pathway in tumor radiosensitivity. For this purpose, the local concentration of NO was monitored after treatment in FSaII tumors and a comparison between the sensitivity of LLC tumors implanted both on eNOS$^{-/-}$ and wild-type (WT) mice was carried out. First, we demonstrate the central role played by eNOS in the radiosensitizing effect after application of insulin treatment and electrical stimulation: a significant increase in tumor NO content is induced by these treatments and the increase in tumor oxygenation, as well as the radiosensitizing effect are abolished in eNOS knock-out mice, in contrast to WT mice. Second, by comparing the level of oxygen and NO achieved in tumors after NO-mediated treatments and carbogen, we provide evidence that these NO-mediated treatments are not simply acting by a single oxygen effect. These treatments induced significant regrowth delays compared to carbogen, despite a smaller increase in tumor oxygenation. For the NO-mediated treatments, there was a direct correlation between the NO content and the radiosensitizing effect. These data strongly suggest that NO is a complementary factor additive to oxygen in determining the sensitivity to irradiation and we therefore propose that NO acts as an intrinsic radiosensitizer *in vivo*.

Key words: *tumor; radiation sensitivity; eNOS$^{-/-}$; nitric oxide; oxygen*

Tumor oxygenation and blood flow are of fundamental importance to many forms of cancer therapy. The partial pressure of oxygen (pO_2) plays an important role in the response of tumors to cytotoxic treatments such as chemotherapy, radiotherapy and photodynamic therapy. Both oxygen diffusion and oxygen consumption by tumor cell metabolism contribute to the occurrence of hypoxia. Oxygen deficiency is caused by an insufficient oxygen supply as a result of inadequate tumor perfusion (diffusion limited hypoxia) and fluctuations in red cell flux (acute hypoxia).[1] A promising approach in cancer therapy consists of the manipulation of tumor blood oxygen delivery and oxygen consumption to improve either radio- or chemotherapeutic response. Vasoactive agents,[2] modifiers of tumor cell oxygen consumption[3] or carbogen breathing[4] that is already being tested in Phase III clinical studies, are currently being evaluated for their potential therapeutic interest.

We documented recently that nitric oxide (NO)-dependent modifiers of tumor hemodynamics were able to radiosensitize experimental tumors *in vivo*. Three strategies were considered for that purpose: (*i*) administration of a NO donor (isosorbide dinitrate),[5,6] (*ii*) slow infusion of insulin,[7] and (*iii*) electrical stimulation of the host tissue.[8] The first treatment acted by a direct release of exogenous NO whereas the others were actually shown to be mediated by the activation of endogenous NO synthase.[5–8] Accordingly, isosorbide dinitrate, insulin, and electrical stimulation of the host tissue are able to modulate the level of oxygenation in TLT and FSaII tumors. It was suggested that this increase in tumor oxygenation was achieved via alterations in blood flow and oxygen consumption through NO-mediated pathways.[5–8]

Whether NO also had direct radiosensitizing effects was, however, not addressed in these studies. Besides the well-known 'oxygen effect' that is involved in tumor radiation response induced by these NO-mediated modulators,[5–8] NO produced in the tumor may also contribute to the radiosensitization of tumors. In that respect, previous reports suggested the potential role of NO as an intrinsic radiosensitizer using *in vitro* models, due to the likely fixation of radiation-induced damage in DNA.[9–13]

To further investigate the contribution of NO to the hemodynamic and radiosensitizing properties of these treatments, we used eNOS knock-out (eNOS$^{-/-}$) mice bearing experimental tumors in comparison with their wild type (WT) littermates. The effects of insulin and electrical stimulation in these 2 groups of mice were compared, in terms of regrowth delays after irradiation. We also monitored tumor oxygenation over time before, during and after treatment administration. Finally, the level of NO that was achieved in tumors after treatment was monitored with an EPR spin-trapping method. Two relatively hypoxic ($pO_2 \sim 3$–5 mm Hg) tumor models were used for our study. A fibrosarcoma (FSaII) and the Lewis Lung carcinoma (LLc) tumor models were implanted on C3H and C57Bl6 mice, respectively.

MATERIAL AND METHODS

Animal tumor models

The syngeneic FSaII (mouse fibrosarcoma)[14] and LLc (mouse lung carcinoma)[15] tumor models were implanted in the thigh of C3H/He (Charles River Laboratories, Brussels, Belgium) and C57Bl/6J (Elevage Janvier, Le Genest-St.-Isle, France) (WT and eNOS$^{-/-}$) mice, respectively. C57BL/6J eNOS$^{-/-}$ mice were originally from the Jackson Laboratory (JAX GEMM Strain, Bar Harbor, ME) and were inbred at the FATH laboratory (UCL, Brussels, Belgium). The measurements were carried out when the tumor diameter was 8.0 ± 0.5 mm. Each procedure was approved by the local authorities according to national animal care regulations.

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Treatments

To restrain the mice during the experiments, anesthesia was first induced by an i.p. injection of ketamine (80 mg/kg)/xylazine (8 mg/kg) and maintained with ketamine alone (30 mg/kg). Mouse temperature was maintained at 37°C using a homeothermic blanket. Three independent NO-mediated treatments were tested in our study: isosorbide dinitrate administration, insulin infusion and electrical stimulation of the host tissue. Isosorbide dinitrate (Cedocard, Byk Belga, 1 mg/ml) was diluted in saline (1 mg/40 ml) and administered i.p. at a dose of 0.2 mg/kg.[5] Insulin (Actrapid HM, Novo Nordisk, Bagsvaerd, Denmark) was infused i.v. at a rate of 16 mU/kg/min for 25 min.[7] To stimulate the host tissue with electric pulses, platinum electrodes were placed around the right sciatic nerve. A 15-min "exercise" protocol was achieved by electrical stimulation at 5 Hz, with pulses of 0.2 msec duration.[16] Carbogen (5% CO_2/95% O_2) breathing (5 L/min) was used as a reference treatment. When used, the NOS inhibitor, Nω-nitro-L-arginine methyl ester (Sigma, Steinheim, Germany) was injected i.p. at 15 μmol/kg^{-1}, 1 hr before administration of the treatment.[17]

Irradiation and tumor regrowth delay assay

Irradiation was first carried out on the FSaII tumor model to determine the radiosensitizing properties of each NO-mediated treatment. All treatments (isosorbide dinitrate, insulin and electrical stimulation of the host tissue) were evaluated in independent experiments. Isosorbide dinitrate was administered 15 min before irradiation; insulin was infused 30 min before irradiation; electrical stimulation was carried out during (Protocol I), or 30 min before irradiation (Protocol II). A preliminary study indicated that each treatment alone had no effect on tumor growth. To avoid tumor cure but still achieve a measurable regrowth delay, a single irradiation dose of 16 Gy of X-rays was selected. A positive control (carbogen breathing) was used in all irradiation experiments and allowed a comparison between regrowth delays obtained from distinct experiments. Carbogen was flushed during irradiation. Subsequently, an irradiation experiment including all groups was carried out on LLc tumors implanted on eNOS$^{-/-}$ mice and on WT mice. The effects of insulin and electrical stimulation were compared between mice with and without eNOS. The tumor-bearing leg was locally irradiated with 16 Gy of 250 kV X-rays (RT 250, Philips Medical Systems). Mice were anesthetized and the tumor was centered in a 3-cm-diameter circular irradiation field. When tumors reached 8.0 ± 0.5 mm in diameter, the mice were randomly assigned to a treatment group and irradiated. After treatment, tumors were measured every day until they reached a diameter of 15 mm, at which time the mice were sacrificed. For each tumor, transverse and antero-posterior measurements were obtained. An average tumor diameter was then calculated. Upon tumor regrowth (1–3 days after irradiation), a linear fit could be obtained between 8–15 mm, which allowed us to determine the time to reach a particular size for each mouse.

pO_2 measurements

Local tumor oxygenation measurements were carried out using 2 non-oxygen consuming and independent techniques: electron paramagnetic resonance (EPR) oximetry and a fiber-optic device, OxyLite (Oxford Optronix, Oxford, UK). EPR spectra were recorded using an EPR spectrometer (Magnettech, Germany) with a low frequency microwave bridge operating at 1.2 GHz and extended loop resonator. Charcoal (CX0670-1, EM Science, Gibbstown, NJ) was used as the oxygen sensitive probe in all experiments. Calibration curves were made by measuring the EPR line width as a function of the pO_2.[2] For this purpose, the charcoal was suspended in a tumor homogenate and EPR spectra were obtained on a Bruker EMX EPR spectrometer (9 GHz) between 0–21% O_2. Nitrogen and air were mixed in an Aalborg gas mixer (Monsey, NY), and the oxygen content was analyzed using a servomex oxygen analyzer OA540. Mice were injected into the center of the tumor using the suspension of charcoal (100 mg/ml, 50 μl injected, 1–25 μm particle size). The EPR measurements were started 2 days after the injection. The tumor under study was placed in the center of the extended loop resonator of which the sensitive volume extended 1 cm into the tumor mass, using a protocol described previously.[2] The OxyLite allows pO_2 measurement that is based on the oxygen-quenched lifetime of a luminescent ruthenium dye.[18,19] This system was used for simultaneously and continuously monitoring local tissue oxygenation and temperature at the same location, before, during and after treatment. FSaII tumors were monitored with both techniques and for all treatments, whereas LLc tumors were studied with EPR oximetry alone for the treatments that induce endogenous production of NO (insulin and electrical stimulation) on control and eNOS$^{-/-}$ mice.

Ex vivo *nitric oxide spin trapping experiment*

The detection of NO production in FSaII tumors was conducted using the NO trapping method with the water soluble iron-MGD complex (iron-*N*-methyl-D-glucamine dithiocarbamate). MGD and iron form a paramagnetic complex with NO (NO-Fe^{2+}(MGD)$_2$), which gives a characteristic anisotropic triplet EPR spectrum.[20,21] The sodium salt of MGD was from Alexis Biochemicals (Brussels, Belgium). For the control group (n = 7), 200 μl of a solution of freshly prepared iron sulfate (20 mM), and MGD (100 mM) was administered i.v. to anesthetized tumor-bearing mice. Thirty minutes later, mice were sacrificed and the tumor was taken, homogenized, and immediately frozen in liquid nitrogen. The timing of treatment application for each group is illustrated in Figure 1. For the isosorbide dinitrate group (n = 6), the drug was administered 10 min after injection of the spin-trap agent. For the insulin group (n = 7), the drug was first infused for 25 min and the spin-trap agent was injected 5 min later. The "exercise" group was electrically stimulated 15 min after the administration of the spin-trap agent (Protocol I, n = 6). A second "exercise" group was first electrically stimulated for 15 min and then injected with the spin-trap agent at the end of the stimulation period (Protocol II, n = 6). For the carbogen group (n = 8), the gas was flushed at 5 L/min for 15 min just before resection of the tumor. Finally, 2 complementary groups were added in which L-NAME was administered 1 hr before insulin infusion and electrical stimulation, respectively. In all groups, the mice were sacrificed 30 min after the administration of MGD-Fe(II). The EPR spectra were recorded at 77K on a Bruker EMX EPR spectrometer operating at 9.4 GHz. Typical spectrometer conditions were: incident microwave power, 40 mW; modulation amplitude, 2 Gauss; sweep width, 150 Gauss; time constant, 5.12 msec; 200 scans accumulation. The signal/noise ratio (maximum signal intensity/mean noise) was calculated for each group (treatment). This ratio was normalized for the tumor protein content. As the experimental conditions were strictly identical between the different groups, a direct comparison of the effect of the treatments on the relative NO availability in tumors was possible.

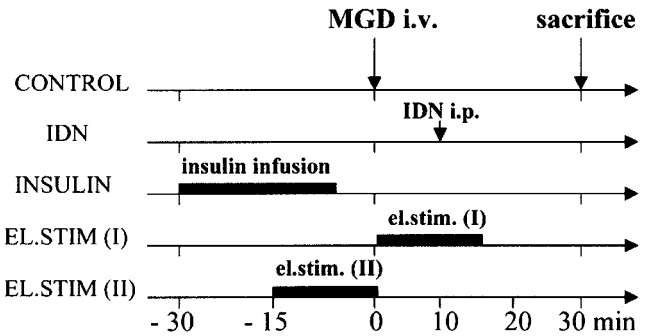

Figure 1 – Timing of treatment application, spin-trap agent injection, and mouse sacrifice for the *ex vivo* determination of the level of NO in FSaII tumors. IDN: isosorbide dinitrate; El.Stim: electrical stimulation (Protocol I or II). Timing of drug administration or of exercise protocol was determined with regard to the increase in tumor oxygenation induced by the treatment.[5–8]

Data analysis

Data are reported as mean ± SEM; Student's *t*-test or ANOVA were used where appropriate to compare pO_2 values, relative increase in NO levels and mean regrowth delays to reach 12 mm in tumor diameter between different treatments.

RESULTS

Sensitivity of FSaII tumors to irradiation in response to NO-dependent treatments

To determine whether the treatments had an effect on the tumor response to radiotherapy, FSaII tumor bearing mice were treated with irradiation alone or with the combination of a given treatment (n = 6/group) and irradiation, and the tumor regrowth delays were measured. Isosorbide dinitrate, insulin infusion, and electrical stimulation were tested in distinct irradiation experiments, as detailed in Table I. Treatment with isosorbide dinitrate 15 min before irradiation increased the tumor radiation response by a factor of 1.4, similar to the effect of carbogen breathing in the same experiment. Interestingly, insulin infusion further increased tumor radiation response, improving tumor regrowth delay by a factor of 2.1, significantly superior to the factor obtained for carbogen in the same experiment. Finally, electrical stimulation (15 min stimulation) of the host tissue improved tumor radiosensitivity when it was carried out during irradiation (Protocol I) (similar effect to that of carbogen breathing) but was not effective when applied from 45–30 min before irradiation (Protocol II) (no significant increase in tumor regrowth delay). Calculated factors of increase in regrowth delay (F), derived from the regrowth delays to reach 12 mm of mean tumor diameter, are summarized in Table I. The F factors were also normalized in function of the value obtained for carbogen (positive control) in each independent experiment; *F normalized* for a given treatment is the result of the factor of increase in regrowth delay for the treatment (F) multiplied by the ratio [mean Fcarbogen/ Fcarbogen] to allow comparison of F between independent experiments (Table I , Fig. 2*a*). Of interest, the administration of a NOS inhibitor (L-NAME) 1 hr before treatment abolished the radiosensitizing effect induced by insulin or electrical stimulation.

Evaluation of the tumor radiosensitivity in $eNOS^{-/-}$ mice

An irradiation experiment including insulin and electrical stimulation treatments was carried out on tumor-bearing $eNOS^{-/-}$ mice and WT mice. As for the FSaII tumor model, insulin radiosensitized LLc tumors implanted in control C57Bl6J mice with more efficiency than carbogen breathing (factors of increase in regrowth delay [F] of 3.9 and 2.6, respectively, n = 6/group). Electrical stimulation (protocol I) had almost similar properties to carbogen (F = 2.3). On the contrary, tumors implanted in eNOS knock-out mice were not radiosensitized with insulin or with electrical stimulation (F = 1.3 for both treatments, n = 4/group), confirming that the NO pathway involved in these treatments is responsible for the tumor radiosensitization. An illustration of growth curves of tumors after irradiation and insulin treatment for control and eNOS knock-out mice is presented in Figure 3. All regrowth delays for each given treatment are summarized in Table II.

Evolution of the tumor oxygenation during treatment

To determine whether an increase in tumor oxygenation could be involved in the radiosensitizing properties of these treatments, we monitored tumor pO_2 using 2 independent techniques. We have demonstrated previously that all of these treatments increase tumor pO_2 for a prolonged time in FSaII tumor-bearing mice. They improved tumor oxygenation with about the same efficacy, increasing FSaII tumor pO_2 from ~3 mm Hg to ~9 mm Hg (Fig. 2*b*).[6–8] This increase in tumor pO_2 was abolished by the administration of a NOS inhibitor (L-NAME) one hour before treatment. Carbogen breathing (positive control) increased tumor oxygenation up to 21 mm Hg. In our present study, we found that the level of oxygenation was similarly improved in LLc tumors after treatment with insulin or electrical stimulation (up to 17 mm Hg and 8 mm Hg, respectively, Table III). On the contrary , LLc tumors implanted in eNOS knock-out mice were not responsive to these oxygenating treatments. LLc tumor pO_2 indeed remained at the basal level during and after treatments on $eNOS^{-/-}$ mice. All results on FSaII tumors were confirmed using OxyLite . Table III summarizes all pO_2 data. The sample volumes of those 2 techniques are quite different but both techniques allow a continuous monitoring of tumor oxygenation; comparing these complementary techniques validates the data between them.

Changes in tumor nitric oxide after treatment

To compare the relative levels of NO produced in FSaII tumors after a given treatment, we carried out *ex vivo* EPR spin-trapping after i.v. injection of the spin-trap agent MGD. These results are presented in Figure 2*c*. We observed a significant increase in the relative tumor NO content after isosorbide dinitrate administration (+46.8%, n = 6, $p < 0.01$). Insulin infusion further increased the tumor NO content (+81.8%, n = 7, $p < 0.001$). Interestingly, the tumor NO content was rapidly increased by the electrical stimulation (Protocol I) (+39.2%, n = 5, $p < 0.05$) but returned to the basal level when measured 30 min after the end of the stimulation period (Protocol II). Carbogen breathing did not significantly alter the tumor NO content (+11.1%, n = 8, $p > 0.05$). Finally, 2 additional groups were studied in which L-NAME was administered 1 hr before insulin or stimulation treatment. Tumor NO content did not increase in these cases (+13.4%, n = 4, $p > 0.05$ and −8.4%, n = 4, $p > 0.05$).

DISCUSSION

Role of the NO pathway in tumor radiosensitization

We have documented previously that the 3 treatments used in our study modify hemodynamic properties of experimental tumors in a NO-dependent manner. Isosorbide dinitrate, a NO donor, is able to improve tumor oxygenation by increasing blood flow and decreasing oxygen consumption.[5] Insulin infusion and electrical stimulation of the host tissue have the ability to activate eNOS.[7,8] We therefore postulated that the NO produced in tumors is responsible for the decreased mitochondrial respiration of tumor cells and so will lead to an increase in tumor pO_2 in various tumor types.[7,8]

In our present study, we definitely demonstrate the central role played by eNOS-derived NO in the enhanced radiosensitizing effect induced by insulin treatment or electrical stimulation. First, we directly demonstrate an increase in the tumor NO content induced by these treatments. Second, we show that the increase in

TABLE I – EFFECT OF DIFFERENT NO-MEDIATED TREATMENTS ON FSAII TUMOR RADIOSENSITIVITY[1]

Type of treatment	Factor of increase in regrowth delay			Potentiation of tumor radiation response	L-NAME inhibition	Reference
	Treatment	Carbogen	*F* normalized			
Isosorbide dinitrate	1.4[2]	1.5[2]	1.5[3]	Yes	ND	6
Insulin	2.1[2]	1.6[2]	2.1[4]	Yes	Yes	7
Electrical stimulation (I)	1.7[2]	1.8[2]	1.5[3]	Yes	Yes	8
Electrical stimulation (II)	1.1	1.5[2]	1.2[5]	No	ND	This study

[1]The regrowth delays to reach 12 mm mean tumor diameter are measured after irradiation with 16 Gy of X-rays combined or not with a given treatment (n = 6/group).–[2]$p < 0.05$, a significant increase compared to irradiation alone (*t*-test).–[3]Effect is similar to carbogen.–[4]Effect is significantly > carbogen.–[5]Effect is significantly < carbogen (*t*-test). ND, not determined.

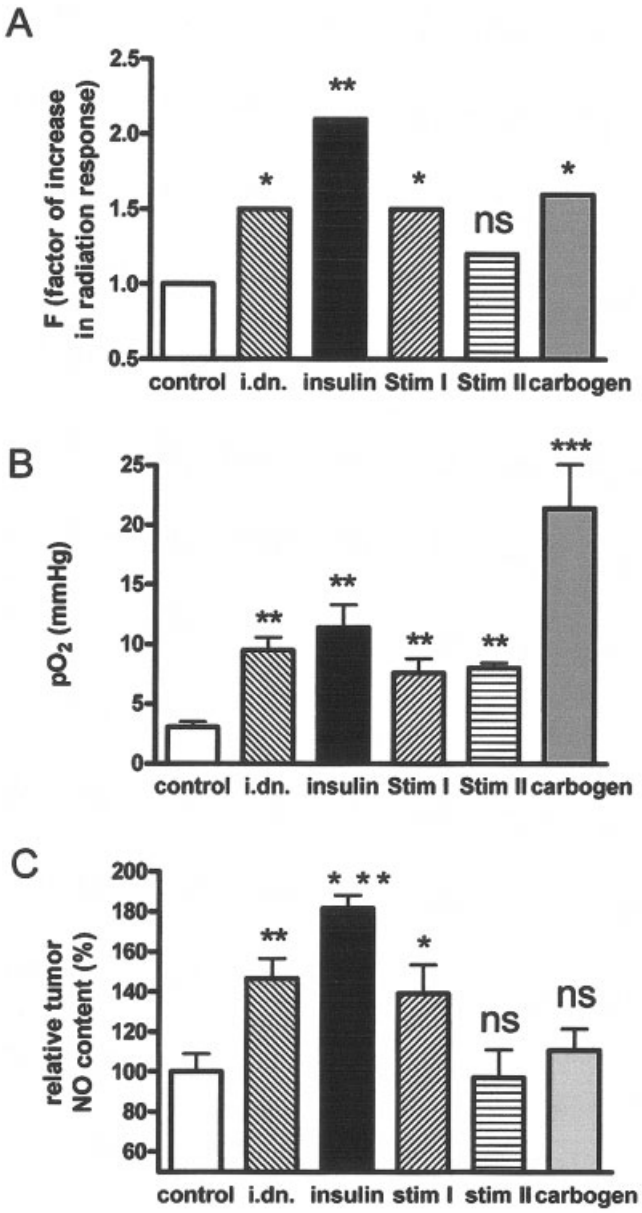

FIGURE 2 – (*a*) Effect of each treatment on the FSaII tumor radiation sensitivity. Each bar represents the factor of increase in regrowth delay (F) determined from each irradiation experiment. (*b*) Effect of each treatment on the oxygenation of FSaII tumors. Each bar represents the mean value ± SEM (mm Hg). (*c*) Effect of each treatment on the relative increase in FSaII tumor nitric oxide content. This was carried out by *ex vivo* EPR spin-trapping after i.v. injection of the spin-trap agent MGD. The value obtained for the control group was considered as a 100% value. Each bar represents the mean value ± SEM (%). i.dn., isosobide dinitrate; stim I, electrical stimulation (Protocol I); stim II, electrical stimulation (Protocol II). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; ns, not significant.

tumor oxygenation, as well as the radiosensitizing effect, are completely abolished in eNOS knock-out mice.

Additive effects of oxygen and nitric oxide

Although all treatments are efficient at radiosensitizing tumors, they diverge in the extent to which they increase pO_2. Isosorbide dinitrate and electrical stimulation of the host tissue (Protocol I) radiosensitized tumors to the same extent as carbogen breathing, even though the latter induced a more significant increase in tumor oxygenation. Moreover, insulin induced regrowth delay compared to carbogen, despite a smaller increase in tumor oxygenation. A second protocol of electrical stimulation (Protocol II) did not improve tumor radiation response, although pO_2 was increased at the time of irradiation (Fig. 2). Taken together, this suggests that oxygen alone is not the only factor that accounts for tumor radiosensitization by these NO-dependent treatments.

Based on our current study, we propose that NO has intrinsic radiosensitizing properties *in vivo*. The relative increase in tumor NO content could be correlated with the efficacy of irradiation. No increase in NO (stimulation, Protocol II) was indeed related to a lack of sensitivity, even in the presence of a high oxygen level. In addition, insulin induced the most important increase in tumor NO level, and was also the most efficient radiosensitizer (Fig. 2). It has been suggested previously that the NOS pathway could represent an approach to exploit the radiosensitizing properties of NO *in vitro*.[9–13] The activation of inducible NOS was shown to increase hypoxic cell radiosensitivity[10] and NO derived from NO donors (DEA/NO, GSNO, SNAP) sensitized hypoxic cells to ionizing radiation to a similar extent to oxygen.[12] We demonstrate that administration of

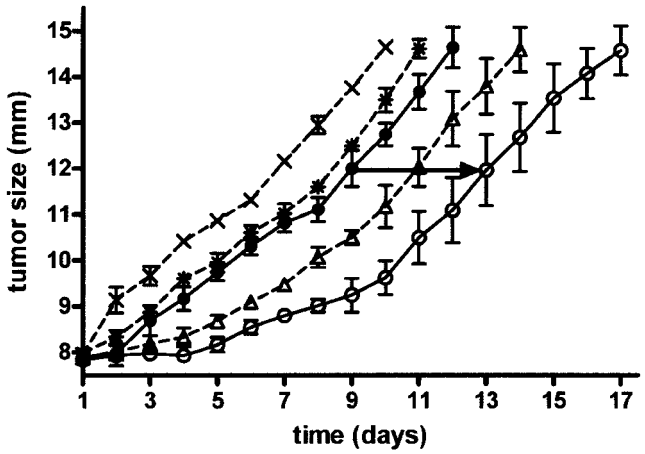

FIGURE 3 – Effect of the combination of insulin and irradiation on LLc tumor regrowth. Comparison between control and eNOS$^{-/-}$ mice. C57Bl6 WT mice: untreated (±), treated with 16 Gy of X-rays alone (✡), infused with insulin and irradiated with 16 Gy of X-rays (○), or treated with carbogen during irradiation with 16 Gy of X-rays (△). Each point represents the mean tumor size ± SEM of 6 tumors. C57Bl6 eNOS$^{-/-}$ mice: treated with 16 Gy of X-rays alone (not shown, similar to ✡), or infused with insulin and irradiated with 16 Gy of X-rays (●). Each point represents the mean tumor size ± SEM of 4 tumors. Arrow: difference (days) between control and eNOS KO mice to reach a 12 mm mean tumor diameter after treatment with insulin. Note that tumor radiosensitization by insulin is abolished when using eNOS knock-out mice. Similar data were obtained with the electrical stimulation protocol (data not shown).

TABLE II – COMPARISON OF THE RADIOSENSITIVITY BETWEEN eNOS$^{-/-}$ LLC TUMOR BEARING MICE AND WT MICE[1]

Type of treatment	Littermate	*F* ratio	Potentiation of tumor radiation response?
Insulin	WT	3.9	Yes
	eNOS$^{-/-}$	1.3	No
Electrical stimulation (I)	WT	2.3	Yes
	eNOS$^{-/-}$	1.3	No
Carbogen	WT	2.6	Yes

[1]The regrowth delays to reach 12 mm mean tumor diameter are measured after irradiation with 16 Gy of X-rays combined or not with a given treatment ($n = 4$/group).

TABLE III – EFFECT OF EACH TREATMENT ON THE TUMOR OXYGENATION[1]

Tumor type	Littermate	Treatment	ΔpO_2 (mm Hg)		References
			EPR oximetry	OxyLite™	
FSaII	C3H	Isosorbide dinitrate	6.4 ± 1.2[2]	5.2 ± 2.7[2]	5,6
		Insulin	8.0 ± 2.3[2]	8.0 ± 2.5[2]	7
		Electrical stimulation (I)	4.5 ± 1.6[2]	4.5 ± 0.8[2]	8
		Electrical stimulation (II)	4.9 ± 0.8[2]	4.0 ± 1.7[2]	8
		Carbogen	18.7 ± 4.2[2]	19.0 ± 9.2[2]	5–8
LLC	C57Bl6	Insulin	16.9 ± 0.5[2]	ND	This study
		Electrical stimulation (I)	6.7 ± 0.7[2]	ND	This study
		Electrical stimulation (II)	7.5 ± 0.3[2]	ND	This study
	C57Bl6-eNOS$^{-/-}$	Insulin	2.4 ± 0.5	ND	This study
		Electrical stimulation (I)	1.8 ± 0.7	ND	This study
		Electrical stimulation (II)	1.4 ± 1.8	ND	This study

[1]Local tumor oxygenation measurements were carried out using EPR oximetry and OxyLite.–[2]Tumor oxygenation is significantly increased after treatment. $p < 0.05$ by *t*-test, $n = 5$/group for FsaII tumors and $n = 3$/group for LLc tumors. ND, not determined.

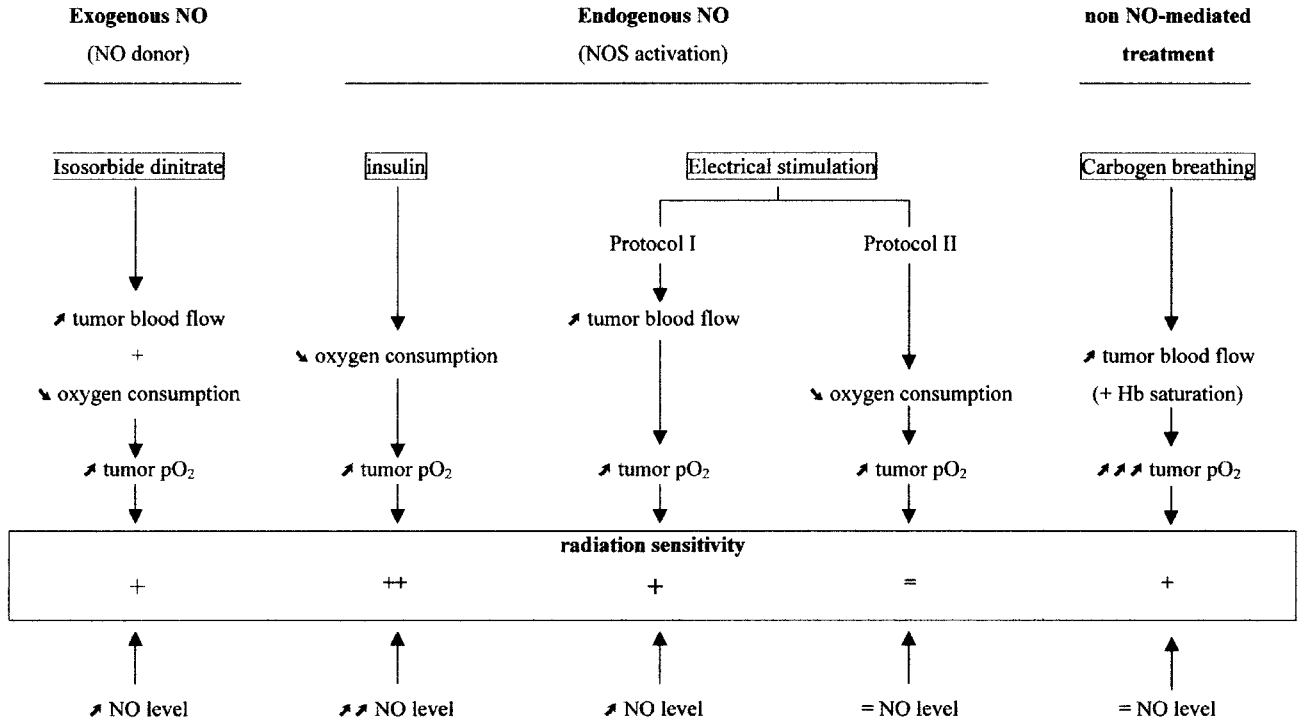

FIGURE 4 – Summary of the effects contributing to tumor radiosensitization on the FSAII tumor model.

NO or activation of the eNOS pathway could result in experimental tumor radiosensitization *in vivo*. We further show that inhibition of the eNOS pathway abolished this effect and that NO is involved in enhanced tumor sensitivity to irradiation.

The intrinsic role played by nitric oxide as a radiosensitizer is clearly not exclusive, but seems to be combined with the oxygen effect when using NO-dependent treatments. Carbogen breathing indeed sensitized both tumor models to X-rays without improving the level of nitric oxide inside tumors. Accordingly, in this case, the primary sensitizing effect is that of oxygenation. In consequence, it is likely that the efficacy of radiation therapy could be dependent either on oxygenation or on a combined effect of oxygenation and nitric oxide (other microenvironmental factors not being excluded). Figure 4 summarizes the potential effects contributing to tumor radiosensitization by the treatments considered here. Our study opens new possibilities for tumor radiotherapy by modulation of the NO pathway. Within this field, the effect of upregulation of the NO pathway on tumor response to radiation has been considered in other recent studies.[22,23]

CONCLUSION

Our study provides new arguments in favor of an intrinsic role for NO *in vivo* as a radiosensitizer. The hemodynamic properties of NO make it impossible to discriminate between the effect of an improvement in tumor pO_2 and an intrinsic role of nitric oxide itself *in vivo*. Nevertheless, the use of mice lacking endothelial NO synthase and the relative quantification of NO after treatment in tumors suggest that modulation of the NOS pathway could be a useful tool in modifying tumor radiation response. A key point for further pre-clinical studies is to selectively target hypoxic cells with this approach.

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