Oxygen deficiency in tumors reduces the efficacy of nonsurgical treatment modalities such as conventional radiotherapy and chemotherapy. Since tumor perfusion is directly affected by the vascular resistance to flow of vessels feeding the tumor, vasodilator drugs might be a way to increase tumor blood flow and oxygenation. The effects of nitric oxide (NO) donor administration on tumor oxygenation, perfusion and radiation sensitivity were studied in the FSAII tumor model. Local tumor oxygenation was measured using electron paramagnetic resonance oximetry and a fiberoptic probe, OxyLite. We concomitantly measured the modulation of tumor blood flow by laser Doppler flowmetry. We determined FSAII tumor regrowth delay after isosorbide dinitrate administration and irradiation compared to carbogen breathing before irradiation and with X-rays alone. Administration of the NO donor improved the FSAII tumor pO2 concomitant with an increase in tumor blood flow. We also demonstrated an increase in FSAII tumor radiation sensitivity after isosorbide dinitrate administration, which was similar to the effect of carbogen breathing in the same tumor model. Administration of isosorbide dinitrate could be considered in terms of improvement in tumor blood flow and a possible concomitant increase in accessibility of chemosensitizing agents to the tumor, particularly in terms of modification of the tumor response to irradiation.

**MATERIAL AND METHODS**

**Animal tumor model and treatment**

The syngeneic FSAII tumor model was implanted in the thigh of C3H/He mice. To restrain mice during the experiments, anesthesia was first induced by an i.p. injection of ketamine (80 mg/kg) and xylazine (8 mg/kg) and maintained with ketamine alone (30 mg/kg). Isosorbide dinitrate (Cedocard, 1 mg/ml; Byk Belga, Brussels, Belgium) was diluted in saline (1 mg/40 ml) and administered i.p. at a dose of 0.2 mg/kg. Carbogen (5% CO2/95% O2) breathing (5 V/min) was used as a reference treatment.

**PO2 and blood flow measurements**

Local tumor oxygenation measurements were carried out using 2 independent techniques: electron paramagnetic resonance (EPR) oximetry and the fiberoptic probe OxyLite. We used OxyFlo probes to assess blood flow inside the tumor. Mice were maintained at 37°C using an infrared lamp and a probe combined with a temperature control unit.

**EPR oximetry**. EPR spectra were recorded using an EPR spectrometer (Magnettech, Berlin, Germany) with a low-frequency microwave bridge operating at 1.2 GHz and an extended loop resonator. Charcoal (charcoal wood powder, 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 pO2. For this purpose, the charcoal was suspended in a tumor homogenate and EPR spectra were obtained on a Bruker EMX EPR spectrometer (Karlsruhe, Germany) (9 GHz) at 0–21% O2. Nitrogen and air were mixed in an Aalborg gas mixer (Monsey, NY), and the oxygen content was analyzed using the sidewave oximeter analyzer OAS40 (Analytic System, Brussels, Belgium).

**Key words**: nitric oxide donor; oxygenation; radiosensitization; isosorbide dinitrate; FSAII tumor

Cells in experimental and human tumors can become deprived of oxygen through abnormal tumor blood supply and rapid tumor cell growth relative to vascular endothelial cell proliferation. Both oxygen diffusion and oxygen consumption by metabolism in tumor cells 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). The partial pressure of oxygen (pO2) plays important roles in the response of tumors to cytotoxic treatments such as chemotherapy, radiotherapy and photodynamic therapy. The importance of tumor vascular development in relation to the interdependence between tumor growth and angiogenesis is of main concern. Clinical trials utilizing strategies to either inhibit tumor vascular growth or directly attack the tumor vasculature are under way. A different approach to the problem consists in the manipulation of tumor blood flow and oxygen delivery to improve either radio- or chemotherapeutic response. The use of vasoactive agents, modifiers of tumor cell oxygen consumption,7–9 or carbogen breathing,4–5 which is already used in phase III clinical studies, have improved radiation response.

We previously demonstrated that the nitric oxide (NO) donor isosorbide dinitrate had a dose-dependent effect on transplantable liver tumor (TLT) tumor pO2, and that it was the consequence of an increase in tumor blood flow, as demonstrated by magnetic resonance imaging (MRI). Here, we evaluated the effect of isosorbide dinitrate on a second tumor model, which is well characterized for its response to ionizing radiation. Using 2 independent techniques, we monitored tumor pO2 after administration of isosorbide dinitrate to FSAII tumor-bearing mice. We concomitantly measured the modulation of tumor blood flow by laser Doppler flowmetry. We further determined the FSAII tumor regrowth delays after isosorbide dinitrate administration and irradiation in comparison to carbogen breathing before irradiation and with X-rays alone.

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(8 mm diameter) using a suspension of charcoal (100 mg/ml, 50 μl injected, 1–25 μ particle size). EPR measurements were started 2 days after the injection. The tumor under study was placed in the center of the extended loop resonator, the sensitive volume of which extended 1 cm into the tumor mass, using a protocol previously described.10,14

Oxylite/Oxyflo technique. We used the Oxylite in conjunction with Oxyflo (Oxford Optronix, Oxford, UK) for simultaneously and continuously monitoring tissue blood flow, oxygenation and temperature at the same location.15,16 Fiberoptic microprobes combining a laser Doppler system, an oxygen sensor and a thermocouple were inserted into the tumor. Data were collected continuously at a sampling frequency of 20 Hz, before and after isosorbide dinitrate administration. Oxylite pO2 measurements are single-point values, and the volume sampled was confined to the sensor tip (230 μm diameter).

Irradiation and tumor regrowth delay assay

The tumor-bearing leg was locally irradiated with 16 Gy of 250 kV X-rays (RT 250; Philips, Hamburg, Germany). 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, mice were randomly assigned to a treatment group and irradiated. After treatment, tumors were measured every day until they reached a diameter of 16 mm, at which time the mice were killed. A linear fit could be obtained between 8 and 16 mm, which allowed us to determine the time to reach a particular size for each mouse. For each tumor, transversal and anteroposterior measurements were obtained. An average tumor diameter was then calculated.

RESULTS

Effect of isosorbide dinitrate on tumor oxygenation

The 2 techniques we used are intended for continuous measurement of the local pO2 without altering the local oxygen concentration and allow real-time study of the oxygen fluctuations in tissues.

EPR oximetry relies on the oxygen-dependent broadening of the EPR line width of a paramagnetic oxygen sensor implanted in the tumor.14,17 The fiberoptic Oxylite probes allow pO2 measurement, based on the oxygen-quenched lifetime of a luminescent ruthenium dye.15,16 We demonstrated previously that the effect of isosorbide dinitrate on TLT tumor pO2 was dose-dependent and that a dose of 0.2 mg/kg was the most efficient.10 Using the same dose, the basal FSaII tumor pO2 was 3.1 ± 0.1 mm Hg and increased to a maximum value 30 min after injection of isosorbide dinitrate (12.5 ± 1.2 mm Hg, n = 5) (Fig. 1). Since the measurement volumes are different for EPR oximetry and the Oxylite probe, individual tumor pO2 values measured with these 2 techniques may be quite different. Nevertheless, mean pO2 values for a group of mice do not differ significantly from one technique to another. Isosorbide dinitrate was proportionally more effective at increasing oxygenation in the FSaII tumor model than on the previously studied TLT tumor model. Carbogen breathing, used as a positive control, induced an increase of 18.7 mm Hg in the FSaII tumor model (n = 10).

Effect of isosorbide dinitrate on tumor blood flow

We used the OxyFlo technique, based on laser Doppler flowmetry, to assess blood flow inside the tumor, which allows relative measurements of blood flow in arbitrary units (blood perfusion units). We demonstrated a slight increase in tumor blood flow of 34.8% ± 9.5% (n = 3) after isosorbide dinitrate administration. However, great variability was observed between results, probably due to the confinement of the measurement volume around the probe and to the inter- and intratumor heterogeneity of tumor vascular networks. A typical experiment is shown in Figure 2.

Effect of isosorbide dinitrate on sensitivity of tumors to irradiation

To determine whether isosorbide dinitrate had an effect on the tumor response to radiotherapy, FSaII tumor-bearing mice were treated with irradiation alone or with the combination of isosorbide dinitrate and irradiation and the tumor regrowth delays measured. As the FSaII tumor model is known to be radiosensitized by carbogen,7,11 we compared the effect of carbogen breathing during irradiation to the effects of isosorbide dinitrate administration. Four groups of 6 mice were used. To avoid tumor cure but still achieve a measurable regrowth delay, a single irradiation dose of 16 Gy was selected as the radiation dose (RX) after preliminary tests. The regrowth delay to reach 12 mm tumor diameter was 5.1 ± 1.1 days for RX alone, 7.4 ± 0.6 days for carbogen and RX (p < 0.05) and 7.1 ± 0.4 days for isosorbide dinitrate and RX (p < 0.05) (Fig. 3). These data indicate that isosorbide dinitrate increased the sensitivity of the tumor to X-ray irradiation, increasing regrowth delay by a factor of 1.39 compared to a factor of 1.45 for
is as efficient at radiosensitizing FSaII tumors as carbogen when
in tumor model. We can only speculate as to why isosorbide dinitrate
which was similar to the effect of carbogen breathing in the same
model.

event of Gd-DTPA contrast-enhanced MRI in the TLT tumor
showed an increase in tumor blood flow after NO donor adminis-
tration using OxyFlo. We already
increase in FSaII tumor oxygenation is the result of an increase in
tumor blood flow, as demonstrated using OxyFlo. We already
showed an increase in tumor blood flow after NO donor adminis-
tration using Gd-DTPA contrast-enhanced MRI in the TLT tumor
model.

We also demonstrated in the present study an increase in FSaII
tumor radiation sensitivity after isosorbide dinitrate administration,
which was similar to the effect of carbogen breathing in the same
tumor model. We can only speculate as to why isosorbide dinitrate
is as efficient at radiosensitizing FSaII tumors as carbogen when
carbogen induces a greater increase in tumor pO2. Besides the
radiosensitizing effect due to oxygen, an additional effect of NO
itself cannot be excluded since NO is a radiosensitizer of hypoxic
cells in vitro and in vivo.

Administration of isosorbide dinitrate could be considered in
terms of improvement in tumor blood flow and a possible consec-
utive increase in accessibility of chemosensitizing agents to the
tumor, particularly in terms of modification of the tumor response
to irradiation.

Since tumor perfusion is directly affected by the vascular resist-
tance to flow of vessels feeding the tumor, vasodilator drugs might
be a way to increase tumor blood flow and oxygenation. Changes in
tumor blood flow caused by external factors are greatly influ-
enced by the structural relationship between vascular beds of
tumor and surrounding normal tissues. When the vascular beds in
tumors parallel those in normal tissues, changes in perfusion would
be opposite: an increase in normal tissue blood flow due to vaso-
dilation would shunt away the blood flow from the tumor to the
normal tissue, resulting in a decrease in tumor blood flow (steal
effect). However, if these vessels are located in series, dilation of
host tissue vessels may improve tumor blood flow. In many
tumors, series and parallel types may be mixed or combined, and
modifications of tumor perfusion vary depending on the relative
contribution of these 2 types. Because many tumors lack vascular
smooth muscle cells, modifications of tumor blood flow are mainly
due to changes in blood flow in adjacent normal tissues. This is the
main reason that the potential effect of vasodilators on tumor
perfusion is unclear.

The effect of NO donors on the tumor microenvironment was
previously investigated by several groups. Wood et al.33 described
an improvement of the bioenergetic status and the radiosensitivity
of experimental tumors after application of the NO donor SIN-1.
We also found that several NO donors (molsidomine, sodium
nitroprusside, nitroglycerin and isosorbide dinitrate) increased pO2
in TLT tumors.10,14 On the contrary, Thews et al.22 described
decreased tumor perfusion in rats bearing s.c. DS sarcomas, which
 correlated with a fall in mean arterial blood pressure, resulting in
a decrease in red blood cell flux by 40%. Although contradic-
tory, these results lead to an interesting question concerning the
level of NO effectively delivered to the tumor. Different NO
donors may have different efficacy and kinetics in terms of NO
delivery. Moreover, the i.p. injection we performed for adminis-
tration of isosorbide dinitrate does not guarantee the same dose in
the blood circulation and should induce only a short increase in
NO levels compared to the 30 min i.v. infusion used by Thews et al.22 Like the opposite effects described with different doses of
hydralazine administration, NO donors could have different ac-
tions at high and low doses.

A likely additional effect of isosorbide dinitrate on blood rhe-
ology should also be considered. Indeed, the effect of nitrovaso-
dilators on blood viscosity has already been studied in vitro and in vivo.
Both blood cell shape and blood viscosity are influenced in
vitro by some nitrovasodilators.23,24 This effect could be important
by decreasing the transient occlusion of vessels (acute hypoxia).

Finally, we previously found that NO can also modulate the pO2
in tumors by decreasing the oxygen consumption of tumor cells
after insulin infusion through regulation of mitochondrial respira-
tion.7

In conclusion, we found that isosorbide dinitrate increased pO2
and blood flow in FSaII tumors. This effect induced a radiosensi-
tizing effect on the tumor. Thus, appropriate modulation of NO
levels in tumors may lead to enhancement of the response of
tumors to irradiation.

REFERENCES

supply, and metabolic microenvironment of human tumors: a review.

D, Hong K, Dewhirst MW. Fluctuations in red cell flux in tumor
microvessels can lead to transient hypoxia and reoxygenation in tumor


4. Jain RK. Normalizing tumor vasculature with anti-angiogenic ther-
apy: a new paradigm for combination therapy. Nat Med 2001;7:
987–9.

5. Biaglow JE, Manevich Y, Leeper D, Chance B, Dewhirst MW,
Jenkins WT, Tuttle SW, Wroblewski K, Glickson JD, Stevens C,
Evans SM. MIBG inhibits respiration: potential for radio-
and hyperthermic sensitization. Int J Radiat Oncol Biol Phys 1998;42:
871–6.

J, Brizel DM, Dewhirst MW. Simultaneous administration of glucose
and hyperoxic gas achieves greater improvement in tumor oxygen-

FIGURE 3 - Effect of the combination of isosorbide dinitrate and
radiation on FSaII tumor regrowth. Mice were untreated (open trian-
gles), treated with 16 Gy of RX alone (open circles), treated with
isosorbide dinitrate 15 min before 16 Gy of RX (solid circles) or
-treated with carbogen 15 min before and during irradiation with 16 Gy
of RX (solid squares). Each point represents the mean tumor size ± SEM of 6 tumors. Regrowth delays to reach 12 mm tumor diameter
were 5.1 ± 1.1 days for RX alone and 7.1 ± 0.4 days for isosorbide
dinitrate and RX (p < 0.05). Isosorbide dinitrate increased the re-
growth delay by a factor of 1.39.


