Measurement of Nitric Oxide in Mice using EPR and Spin Trapping with Fe-(DETC)$_2$ After Irradiation

J.A.O'Hara, O.Y.Grinberg, and H.M.Swartz
EPR Center for the Study of Viable Systems, Dept. of Diagnostic Radiology, Dartmouth Medical School Hanover NH. USA

INTRODUCTION

The short -lived free radical nitric oxide (NO) has complex and incompletely understood effects as a signalling molecule, potential reactant and perhaps as a signal of oxidative stress. The occurrence and role of NO in tumors after irradiation is not well worked out but it has been implicated in many processes that influence tumor progression and response to treatment (1). Previously, using electron paramagnetic resonance (EPR) oximetry and MRI we have established time courses for altered tumor pO_2 (2) and altered vascular permeability (3) after irradiation, both likely to be influenced or regulated by NO (1). In this work we detected NO in tissues after whole body irradiation and after local irradiation to the tumor, using the administration of the nitric oxide specific spin trap, Fe-(DETC)$_2$ (diethyl dithiocarbamate) with EPR both in frozen samples and *in vivo*, whole body measurements.

METHODS

a. Mice and Tumor Model. Mice were Balb/c, athymic nude mice or C3H/HeJ mice. Tumors were MTG-B growing s.c. on C3H/HeJ female mice.

b. Irradiation: Whole body (WBI)or local irradiation to the tumor only using a GE-Maxitron orthovoltage machine as previously described (2).

c. Measurements of NO: Mice were given ferrous sulfate (50 mg/kg) and disodium citrate dihydrate (250 mg/Kg) in saline (s.c.), and then DETC (500 mg/kg) (i.p) 75 minutes before sacrifice (4) .

In vivo: EPR spectra recorded repeatedly (every 2 min) on an L-band spectrometer with a whole body resonator operating at 1.1GHz from 30 min to 75 min post-DETC injection. NO-Fe-(DETC)$_2$ has a characteristic EPR spectrum that can be detected directly from tissue (4). ***Ex-vivo:*** EPR spectra were recorded at X-band (9.5 GHz) on tumors and liver tissue frozen in liquid nitrogen which increases sensitivity by a factor of at least ten-fold over L-band. We compared signal intensities (corrected for weight of the tissues and instrument settings) with a reference sample. (The reference sample, a frozen solution of NO-FeMGD complex in propylene glycol/water 50:50, was made for us by Jesse A. Fecker, Dartmouth Chem. Dept.

RESULTS AND DISCUSSION

In liver tissue, we observed 1) NO was elevated compared to baseline after 10-50 Gy (WBI) in a dose dependent manner; 2) the peak occurred 3.5-4 hr after irradiation which agrees with the work of others (5) ; 3) baseline NO levels varied about 5 fold among mouse strains studied. Balb/c>athymic nude>C3H. NO was elevated in liver but was not detected in tumors of C3H mice after local irradiation of MTG-B tumors.

CONCLUSIONS: Further work will investigate radiation induced production of NO and the implications of inhibition of NO production on tumor pO_2. Since the signal from the oxygen sensitive material used to assess pO_2 in EPR oximetry (2,4) does not interfere with the NO-Fe-(DETC)$_2$ signal we will measure pO_2 and NO in the same animals.

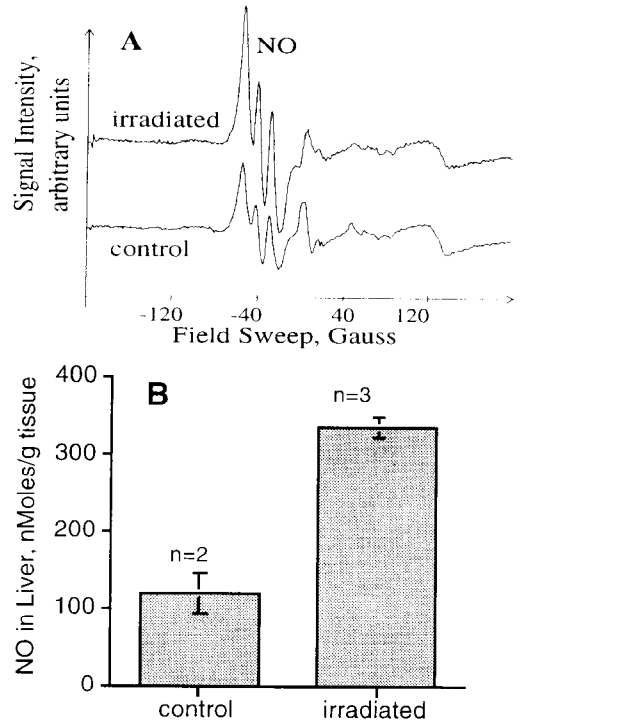

Fig.1. A. EPR spectrum of liver from unirradiated and irradiated animals (50Gy WBI). NO is about 3fold elevated 4 hrs after irradiation. **B**: average ±SD of NO in livers of Balb/c mice.

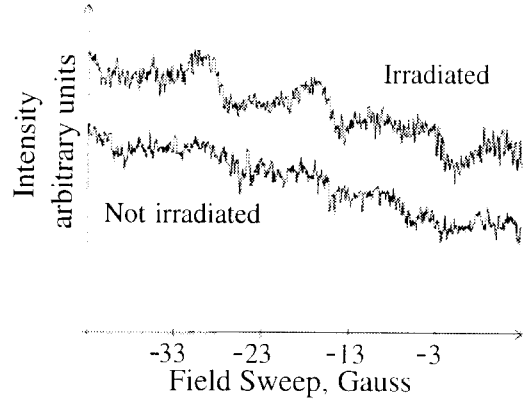

Fig. 2. Triplet characteristic of NO-Fe-(DETC)$_2$ **in a living Balb/c mouse *in vivo* four hours after WB I.**

REFERENCES

1. D. Fukumura, R.K. Jain, *Canc Metastasis Rev* **17,** 77-89 (1998).
2. J.A. O'Hara, F. Goda, E. Demidenko, H.M. Swartz, *Radiation Research* **150,** 549-556 (1998).
3. F. Goda, G. Bacic, J.A. O'Hara, B. Gallez, H.M. Swartz, J.F. Dunn, *Cancer Res* **56(14),** 3344-3349 (1996).
4. P.E. James, K.J. Liu, H.M. Swartz, *Adv Exp Med Biol* **454,** 181-187 (1998).
5. N.V. Voevodskaya, A.F. Vanin, *Biochem Biophys Res Commun* **186(3),** 1423-8 (1992).