Measurement of Tumor Oxygenation

REGIONAL TUMOR OXYGEN TENSION: FLUORINE ECHO PLANAR IMAGING OF HEXAFLUOROBENZENE REVEALS HETEROGENEITY OF DYNAMICS

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Purpose: Therapeutic success could be enhanced if therapy were tailored to the characteristics of specific tumors. We have been developing novel approaches to measuring tumor oxygen tension in vivo, and recently reported a method based on 19F nuclear magnetic resonance (NMR) spin lattice echo planar imaging (EPI) relaxometry of hexafluorobenzene (HFB). We have now examined the feasibility of monitoring dynamic changes in regional tumor oxygenation in response to respiratory challenge. Preliminary data in one tumor show distinct differences before and subsequent to irradiation.

Methods and Materials: Dunning prostate adenocarcinoma R3327-AT1 was grown in the form of pedicles on the foreback of male Copenhagen rats. When the tumors reached ~1 cm diameter, HFB (40 μl) was administered by direct intratumoral injection deliberately dispersed to interrogate both central and peripheral regions. Local pO2 was determined using pulse burst saturation recovery 19F NMR EPI on the basis of the spin lattice relaxation rate.

Results: Interrogation of both central and peripheral regions of tumors showed bimodal distribution for oxygenation, including many voxels with pO2 < 15 torr. Altering the inspired gas to 100% O2 produced significant elevation for regions with initially high pO2 (p < 0.01), but the temporal course of dynamic changes varied for each voxel. Many voxels with low pO2 showed little response. Following irradiation (20 Gy), tumor oxygenation was significantly elevated and remained high for at least 10 h.

Conclusion: We believe this method provides a valuable new approach to investigate tumor oximetry that may extend our understanding of tumor physiology, and could have prognostic value. © 1998 Elsevier Science Inc.

INTRODUCTION

Tumor response to therapy is influenced by physiological parameters, as well as genetic characteristics (1). It has long been appreciated that hypoxic cells are relatively resistant to radiation therapy (2), and it has been suggested that measurement of tumor oxygen tension (pO2) could assist planning and delivery of treatment (3). Hypoxia is associated with genetic instability, angiogenesis and metastasis (4). Recent polarographic techniques (5) have shown significantly improved survival for patients whose tumors have median pO2 > 10 torr (6). However, current oximetry methods are highly invasive, and a recent NCI workshop called for the development of new techniques (3). We recently reported a new approach exploiting fluorine (19F) nuclear magnetic resonance (NMR) relaxometry of hexafluorobenzene (HFB), and demonstrated the ability to monitor regional tumor oxygen dynamics (7–9). We have now extended our investigations to examine changes in pO2 following a single high dose of radiation.

METHODS AND MATERIALS

Dunning prostate tumors (R3327-AT1) were implanted in skin pedicles on the foreback of 5 male Copenhagen rats and allowed to grow to ~1 cm diameter (potential volume doubling time, Tpot, 4–7 days) (10). Rats were anesthetized and maintained with O2/N2O/methoxyflurane (33%/66%/0.5%). Hexafluorobenzene (~ 40 μl) was injected directly into tumors at specific locations, both centrally and periph-
erally. Each rat was placed on a warming blanket in a 4.7 T magnet, with actively shielded gradients and a 2-cm coil around the tumor. Proton and corresponding $^{19}$F images were obtained to confirm distribution of the HFB. Tumor oxygenation was estimated on the basis of $^{19}$F EPI relaxometry of the HFB (8), with a typical in-plane resolution of $2.5 \times 2.5$ mm. Although images were acquired in projection mode, the distribution of HFB was limited to a thin slice of tissue by the mode of injection, and confirmed by traditional 3D MRI. Typically, $^{19}$F signal was observed from about 5–10% of each tumor. Following a pulse-burst saturation recovery preparation sequence with a variable recovery time, a single spin echo EPI sequence with blipped phase encoding (MBEST) was applied. $pO_2$ was estimated for each voxel on the basis of the spin lattice relaxation rate, $R_1$, using the relationship $pO_2$ (torr) = $[R_1(s^{-1}) - 0.074]/0.0016$ (8). Each relaxation (that is, $pO_2$) map required 6.5 or 20 min, depending on the extent of signal averaging. Measurements of $pO_2$ were repeated 3 times for baseline, and then again 3 times following alteration of the inhaled gas to 99.5% O$_2$ (+ 0.5% methoxyflurane). Subsequently, one tumor was irradiated. During irradiation, the anesthetized rat breathed 33% O$_2$. The beam was collimated and blocked to irradiate the tumor only. Bolus material was used to enhanced dose uniformity (20 Gy @ 4 MeV). 1-h, 4-h, and 10-h post-irradiation oxygen measurements were repeated.

**RESULTS**

Interrogation of both central and peripheral tumor regions by EPI relaxometry showed bimodal distribution for oxygenation, including many voxels with $pO_2 < 15$ torr, as shown for a representative tumor in Fig. 1a. Individual voxels had relatively stable $pO_2$ during 1 h of baseline measurement. Altering the inspired gas to 100% O$_2$ produced significant elevation for regions with initially high $pO_2$, but many voxels with low $pO_2$ showed little response. The temporal course of dynamic changes varied for each voxel. Overall mean and median $pO_2$ increased significantly in most cases (e.g., Fig. 1b) and the fraction of voxels with $pO_2 < 5$, 10, or 20 torr decreased (Fig. 2). One hour after irradiation, tumor $pO_2$ was significantly elevated ($p < 0.005$) and remained significantly elevated for at least 10 h. The fraction of tumor showing low $pO_2$ was reduced (Fig. 3). Four hours after irradiation, tumor $pO_2$ failed to respond to altered inhaled oxygen but, by 10 h, increasing the fraction of inhaled O$_2$ produced a significant increase, indeed, considerably greater than prior to irradiation.

**DISCUSSION**

Substantial differences in the hypoxic fractions of different tumor lines have been reported, e.g., using the paired-survival assay (11). More recently, hypoxic fraction has been estimated on the basis of tumor $pO_2$ measured directly, using electrode polarography. This has confirmed distinct intertumor heterogeneity, as well as great intratumoral heterogeneity, with a gradation of oxygenation ranging from hypoxic to well oxygenated (5). There is some evidence that $pO_2$ is directly related to “traditional” hypoxic fraction (3, 12) and significantly, results of clinical trials suggest better long-term survival for patients with tumors having median $pO_2 > 10$ torr (6). However, electrodes have several drawbacks: (a) vascular damage, particularly with repeated insertion; (b) oxygen consumption by the electrode; and (c) the inability to re-examine identical regions. In terms of understanding tumor response to interventions and therapy,
been demonstrated on the basis of 19 F NMR relaxometry by quantitative oximetry (14). Quantitative tumor oximetry has application in advanced cancer of the uterine cervix. Cancer Res 1996;56: 4509–4915.


