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BIOLOGY CONTRIBUTION

EFFECT OF INTRATUMORAL HETEROGENEITY IN OXYGENATION STATUS ON FMISO PET, AUTORADIOGRAPHY, AND ELECTRODE Po_2 MEASUREMENTS IN MURINE TUMORS

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Purpose: To explore conflicting results obtained when tumor hypoxia is assessed with Eppendorf electrode Po_2 measurements and with positron emission tomography (PET) by use of [^{18}F]fluoromisonidazole (FMISO).
Methods and Materials: We compared the 2 methods in conjunction with 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) PET, dual-tracer *ex vivo* autoradiography (FMISO and 2-deoxy-D-[1-^{14}C]glucose (2DG)), and histology in 2 murine tumor models, the C3H mammary carcinoma and the SCCVII squamous cell carcinoma.
Results: 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG)-PET showed tumor-to-reference tissue ratios of 3.5 in both tumor models after 2 hours. C3H mammary carcinoma reached an FMISO PET ratio of 11 after 3.5 hours. Autoradiography showed large confluent areas of FMISO and 2DG uptake. Median Po_2 was 7 mm Hg and necrotic fraction was 10% to 30%. SCCVII squamous-cell carcinoma reached an FMISO PET tumor-to-reference tissue ratio of 2 after 2.5 hours. Autoradiography showed homogeneous 2DG uptake and scattered foci of high FMISO uptake. Median Po_2 was 1 mm Hg and necrotic fraction was below 5%.
Conclusions: *Ex vivo* dual-tracer autoradiography documented the ability of *in vivo* FMISO PET to distinguish between confluent areas of either viable tissue or necrosis. Electrode Po_2 measurements could not be ascribed to specific areas in the tumors. Less uptake of FMISO in SCCVII squamous-cell carcinoma than in C3H mammary carcinoma could be caused by scattered foci versus confluent areas of viable hypoxic tissue in the 2 tumors, respectively. © 2005 Elsevier Inc.

Hypoxia, FMISO, Deoxyglucose, Positron emission tomography, Autoradiography.

INTRODUCTION

Tumor hypoxia has been shown to be an important prognostic factor for response to cancer therapy (1–4), rate of tumor cell proliferation (5), and incidence of distant metastases (6) in studies that directly measure the oxygen partial pressure in tumors with the Eppendorf Po_2 histograph electrode (7). This method is, however, invasive and can only be readily applied to tumors accessible from the body surface. Furthermore it does not distinguish between viable hypoxic cells, nonhypoxic cells, blood vessels, and stroma (8–10). Positron emission tomography (PET) recording by use of hypoxia markers labeled with radioactive isotopes is an attractive method of assessing tumor hypoxia because it is noninvasive and not limited in applicability to superficial tumors. Tracers such as [^{18}F]-fluoroerythronitroimidazole (FETNIM) (11), copper-diacetyl-*bis*(N^4-methylthiosemicarbazone) (Cu-ATSM) (12, 13), [^{18}F]-fluoroazomycinarabinofuranoside (FAZA) (14), and [^{18}F]-fluoromisonidazole (FMISO) (15, 16) are trapped in living tissue with low oxygen content. [^{18}F]-labeled fluoromisonidazole (FMISO), a nitroimidazole, is the most widely investigated tracer for this purpose. It diffuses freely into cells and undergoes reduction where oxygen tension is low. The product then binds irreversibly to cellular macromolecules (15). Chemical reduction of FMISO is catalyzed by nitroreductases, and is, thus, preferentially trapped in viable hypoxic tissue (17). However, in a recent FMISO PET study of soft-tissue

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sarcoma in humans (18), FMISO uptake was increased in only 2 of 7 malignant tumors, despite prevailing low Eppendorf Po_2 measurements.

In the present study, we investigated FMISO PET assessment of tumor hypoxia in comparison with Eppendorf Po_2 measurements. In addition, 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) PET was performed to image viable tissue *in vivo* (19, 20). We also utilized the high spatial resolution of the *ex vivo* autoradiography technique to evaluate the PET results, by performing dual-tracer studies with [^{14}C]-labeled deoxyglucose (2DG) and FMISO. The purpose of contrasting results with FDG/2DG and FMISO was to distinguish viable normoxic from viable hypoxic tissue. Experiments were performed in mice with C3H mammary carcinoma or SCCVII squamous-cell carcinoma, the former characterized by a larger necrotic fraction and a higher radiobiological hypoxic fraction than the latter (21, 22). The experimental design allowed us to evaluate the impact of individual tumor heterogeneity on FMISO PET imaging of hypoxic tumors.

METHODS AND MATERIALS

Animals and tumor models

Tumors were grown subcutaneously on the backs of 12-week-old to 16-week-old female mice. C3H mammary carcinoma was grown in CDF1 mice and SCCVII squamous-cell carcinoma was grown in C3H/K_m mice. Derivation and maintenance of the cell lines have been described previously (23, 24). Body weight ranged from 21 to 32 g (median: 25 g) for the CDF1 mice and from 20 to 36 g (median, 27 g) for the C3H/K_m mice. Tumor sizes ranged from 350 to 450 mm^3 (median: 400 mm^3) and were calculated as $D1\times D2\times D3\times \pi/6$; D1, D2, and D3 are the 3 orthogonal dimensions of the tumor measured with a slide gauge. Experiments were performed in accordance with European Union, nationally, and institutionally approved guidelines for animal welfare.

Radiopharmaceuticals

2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) and [^{18}F]-fluoromisonidazole (FMISO) were prepared in our own laboratory, as previously described (25, 26). Radiochemical purities were higher than 95% for both tracers. 2-Deoxy-D-[1-^{14}C]-glucose (2DG) was purchased from Amersham Biosciences (Hørsholm, Denmark) and came in an aqueous solution that contained 3% ethanol. It was diluted with isotonic saline (0.9% NaCl) to a final radiochemical concentration of 92.5 kBq/mL.

PET study

The mice were deprived of food for at least 3 hours before tracer injection but had free access to water. Two hours before the PET recording, the mice were anesthetized with 0.3 mL mebumal (4.76 mg/mL saline, i.p.) and had a venflon catheter (0.8 mm/22-gauge; length, 25 mm) placed in the bladder. The mice were positioned individually in lucite plastic jigs and secured with tape to immobilize the tumor. During the PET recordings, the mice were no longer anesthetized. Six plastic jigs were placed in a custom-built plastic device to assure a uniform distance from each tumor to the center of the tomograph, a Siemens ECAT EXACT HR (961) with a field of view of 15 cm (Siemens AG, Munich, Germany). By use of the tomograph's laser facility, the exact positions of the mice in the field of view were determined and a 15-minute transmission scan was performed. A rotating Ge-68 source was used for attenuation correction of the subsequent emission recordings. After the transmission scan, the jigs were removed from the tomograph, and the tracer (FDG or FMISO) was injected. Six mice of each tumor type were scanned with FDG, and another 6 mice of each tumor type were scanned with FMISO. Of the 6 mice per group, 3 had the tracer injected i.v. via a tail vein and the other 3 were injected i.p. Gamma radioactivities in the syringes were counted in a well counter before and after the injection to determine the injected tracer doses. Doses ranged from 2.0 to 2.3 MBq for FDG and from to 2.1 to 5.3 MBq for FMISO, and the time from first injection to start of the emission recording ranged from 8 to 13 minutes (median: 9.5 minutes). After the jigs were replaced in the tomograph by use of the laser guide to place them in the same position as during the transmission scan, a dynamic PET recording of 5 hours duration was made that had a frame structure of 20 frames of 30 seconds each, followed by 29 frames of 10 minutes each. The bladder catheters were flushed with 0.9% NaCl at 60, 90, 120, 180, and 240 minutes after start of the recording to minimize radiation spillover from the bladder. On the basis of earlier experiments with this method, body temperature was expected to remain stable (25). One C3H mammary carcinoma mouse from the FDG study and 1 C3H mammary carcinoma mouse from the FMISO study had to be excluded because of technical problems.

Dynamic recordings were reconstructed as images that consisted of 47 transaxial planes of 3.1 mm thickness. A ramp filter with a cut-off frequency of 0.5 was used for the reconstruction, which resulted in an almost uniform resolution and a full width at half maximum (FWHM) of 4.5 mm. Measurements were corrected for radioactive decay to the time of the tracer injection. The PET images were analyzed by ECAT-7 software. For each recording, a summation of emission frames that ranged from 30 to 60 minutes (the interval of highest tumor activity) was made. Each plane was then examined to find the highest radioactivity concentration within each tumor, and a region of interest (ROI) was drawn in every plane with a boundary value of 75% of the maximal value. The tumor ROIs were summed into a volume of interest (tumor VOI) that was applied to the dynamic recordings. A time-activity curve (tumor TAC) was extracted that showed the time course of mean radioactivity concentration per mL of tissue. The tumor radioactivities were normalized to the injected dose and body weight of each animal.

For reference tissue measurements, 3 mice of each strain without tumors were scanned with FDG and another 3 were scanned with FMISO. The mice were prepared and scanned exactly as the tumor-bearing mice, except that tracer was administered i.v. in all the animals. ROIs were drawn in every plane from the neck to midabdomen, while skin was carefully avoided. Time-activity curves were examined and ROIs with tracer accumulation deleted until 2 to 3 regions remained with TACs similar in form to blood TACs, as reported by Kubota *et al*. (27). These ROIs were all located in the thoracic region. They were summed into a reference VOI, and a reference TAC was extracted from the dynamic-emission sequence and normalized to injected dose and body weight. For both FDG and FMISO, the triplicate determinations of