Hypoxia

The increase in tumor oxygenation under carbogen breathing induces a decrease in the uptake of $[^{18}F]$-fluoro-deoxy-glucose

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A B S T R A C T
We investigated the impact of oxygenation status (measured by EPR oximetry) on the uptake of $[^{18}F]$-FDG (measured by PET) in two different tumor models during a carbogen breathing challenge. We observed a significant drop in $[^{18}F]$-FDG uptake under carbogen breathing that suggests a rapid metabolic adaptation to the oxygen environment.

Material and methods

Animal and tumor models

A total of $10^7$ MDA-MB-231 cells (ATCC) or $10^7$ SiHa cells (ATCC), amplified in vitro, were collected by trypsinization, washed three times with Hanks balanced salt solution and resuspended in 200 μL of a 1:1 mixture of Matrigel (BD Biosciences) and Hanks balanced salt solution. The tumor cells were inoculated subcutaneously into the hind thigh of nude NMRI female mice (Janvier). The experiments were performed when tumors reached 7 mm in diameter to limit partial volume effects (at this tumor size, necrosis was less than 5% at this stage of development as characterized by Hematoxylin Eosin staining). A total of 16 MDA-MB-231 tumors and 12 SiHa tumors were used in the study.

Experimental design

Mice were fasted overnight before the measurements. Animals were anesthetized by inhalation of isoflurane (Forene, Abbot, England) mixed with either air (21% oxygen) or carbogen (5% CO$_2$/95% oxygen), depending on the breathing condition tested, in a continuous flow (2 L/min). Animals were warmed (approximately 35 °C) throughout the anesthesia period. Mice were scanned twice for the breathing challenge, air versus carbogen breathing, with one day between each condition (crossed conditions tested). The protocol is summarized in Supplementary Fig. 1. For each breathing condition, EPR measurements were performed before PET imaging in the same animals.
EPR oximetry

In vivo tumor pO2 was monitored by EPR spectroscopy using charcoal as the oxygen-sensitive probe [4,5]. EPR spectra were recorded using a 1.1 GHz EPR spectrometer (Magnettech, Berlin, Germany). According to calibration curves made by measuring the EPR line width as a function of the pO2 [7], the EPR spectra line width was converted to pO2. A charcoal suspension (100 mg/mL) was injected intratumorally (60 µl) 24 h before experiments. For EPR readings, the tumor under study was placed in the center of the extended loop resonator whose sensitive volume extends 1 cm into the tumor mass. The pO2 measurements correspond to an average of pO2 values in the tumor volume. For air condition, basal measurements were performed. For carbogen condition, pO2 measurements were started after a 10 min inhalation period.

PET/CT imaging

Whole-body PET imaging was performed on a dedicated small-animal PET scanner (Mosaic, Philips Medical Systems, Cleveland, USA) with a spatial resolution of 2.5 mm (FWHM). The PET scans were followed by whole-body acquisitions using a helical CT scanner (NanoSPECT/CT Small Animal Imager, Bioscan Inc., DC, USA). For each breathing condition, anesthetized mice were injected 120 µl intraperitoneally with 300–400 µCi of 18F-FDG (Betaplus Pharma, Brussels, Belgium). A 10 min transmission scan was first obtained in a single mode using a 370 MBq 137Cs source for attenuation correction. A 10 min static PET acquisition was then performed after a 60 min resting period. After the correction with attenuation factors obtained from the transmission scan, images were reconstructed using a fully 3D iterative algorithm (3D-RAMLA) in a 128 × 128 × 120 matrix, with a voxel size of 1 mm³. After PET acquisition, anesthetized animals were transferred on the same bed from the PET scanner to the CT scanner (X-ray tube voltage: 55 kVp; number of projections: 180; exposure time 1000 ms) for anatomical reference. The CT projections were reconstructed with a voxel size of 0.221 × 0.221 × 0.221 mm³. Regions of Interest (ROIs) were delineated on PET images using PMOD software (PMOD™, version 3.403, PMOD technologies Ltd, Zurich, Switzerland). 2D ROIs were established on consecutive transversal slices using a 50% isointens contour tool (ROI including the pixel values larger than 50% of the maximum pixel) that semi-automatically defined a 3D Volume of Interest (VOI) around the tissue of interest. To avoid overestimation of the uptake within the VOI, PET/CT fused images where used to discriminate hot pixels coming from the neighboring tissues like urinary bladder. Using the mean uptake within this VOI, the global tracer uptake was assessed in tumors and expressed as percentage of injected dose per gram of tissue (%ID/g).

Statistics analysis

Paired t-tests were used to compare mean changes between groups (air vs. carbogen) for each tumor model and non-paired t-tests were used to compare mean changes between the two tumor models. Analysis was performed using the Graphpad software. Results were expressed as mean value of parameter ± SEM. For all tests, results with *P < 0.05, **P < 0.01, or ***P < 0.001 were considered significant. The scatter plots of measured pO2 as a function of %ID/g were traced using data from all tumors of both groups. The process of finding the best fit was done by using CurveExpert software (version 1.4).

Results

The pO2 values and the 18F-FDG uptake (%ID/g) measured in both tumor models under both breathing conditions are presented in Fig. 1. In both tumor models, we found that carbogen breathing led to a significant increase in tumor oxygenation and a significant decrease in the uptake of 18F-FDG. Basal measured pO2 (mean ± SEM) were 3.8 ± 0.2 mmHg for MDA-MB-231 tumors and 4.9 ± 0.3 mmHg for SiHa tumors. Under carbogen breathing, MDA-MB-231 and SiHa tumors reached pO2 around 9.9 ± 0.95 mmHg and 16.0 ± 2.3 mmHg respectively. %ID/g measured on PET images (mean ± SEM) were 2.47 ± 0.09 under air and 1.98 ± 0.07 under carbogen for MDA-MB-231 tumors (n = 16) and 2.52 ± 0.12 under air and 1.98 ± 0.08 under carbogen for SiHa tumors.
Discussion

In this study, the experimental design was built as a dynamic follow-up of the tumors during a breathing challenge to determine the correlation between global 18F-FDG PET uptake and pO2 values for each tumor tested under different breathing conditions. Carbogen breathing influences several physiological parameters in tumors like oxygenation status, blood flow, extracellular pH, proliferation and energy status [8–11], changes described as tumor-dependent. In this study, carbogen has been investigated as a modulator of hypoxia in the two tumor models and pO2 variation has been assessed by EPR oximetry. To our knowledge, it is the first time that EPR, a highly sensitive method to measure pO2, has been associated to global 18F-FDG uptake measurement in vivo. A higher 18F-FDG uptake has been found in tumors with a pO2 value inferior to 10 mmHg, a status encountered at the basal level (air breathing) in most tumors belonging to both tumor models. In conclusion, our longitudinal study demonstrates a rapid metabolic adaptation during the carbogen challenge in both tumor models investigated according to the significant drop of 18F-FDG uptake under carbogen breathing compared to air breathing.

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Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2015.04.023.

References


