

Chapter 25

Impact of Oxygenation Status on ^{18}F -FDG Uptake in Solid Tumors

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Abstract The influence of changes in tumor oxygenation (monitored by EPR oximetry) on the uptake of ^{18}F -FDG tracer was evaluated using micro-PET in two different human tumor models. The ^{18}F -FDG uptake was higher in hypoxic tumors compared to tumors that present a pO_2 value larger than 10 mmHg.

Keywords EPR • ^{18}F -FDG • PET • Carbogen • Tumor oxygenation

1 Introduction

High fluorodeoxyglucose (^{18}F -FDG) uptake may be a direct consequence of the upregulation of the glucose transporters (GLUTs) stimulated by hypoxia. However, high ^{18}F -FDG uptake can also arise even under non-hypoxic condition through a situation known as aerobic glycolysis or the Warburg effect. The issue of the relationship between glucose uptake, GLUTs expression and hypoxia within tumors has been debated in the literature [1–4]. To assess the influence of manipulation of tumor oxygenation on the uptake of ^{18}F -FDG, we evaluated the uptake of ^{18}F -FDG using micro-PET imaging under different breathing conditions in parallel with pO_2 measurements with EPR oximetry in two different human tumor models, MDA-MB-231 and SiHa models.

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2 Material and Methods

A total of 10^7 MDA-MB-231 cells (ATCC, Manassas, USA) or 10^7 SiHa cells (ATCC, Manassas, USA), amplified in vitro, were collected by trypsinization, washed three times with Hanks balanced salt solution and resuspended in 200 μ L 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, Le Genest-Saint-Isle, France). The experiments were performed when tumors reached 7 mm in diameter.

Mice were scanned twice for the breathing challenge, air versus carbogen breathing, with 1 day between each condition. The details of the protocol are presented in Fig. 25.1. For each breathing condition, EPR measurements were performed before PET imaging and final anatomical images were acquired by CT scan. Mice were fasted overnight before measurements. Animals were anesthetized by inhalation of isoflurane (Forene, Abbot, England) mixed with either air (21 % oxygen) or carbogen (5 % CO₂ in oxygen), depending on the breathing condition tested, in a continuous flow (2 L/min). Fasted animals were warmed (approximately 35 °C) throughout the anesthesia period.

In vivo tumor pO₂ was monitored by EPR oximetry using charcoal as the oxygen-sensitive probe [5, 6]. EPR spectra were recorded using a 1.1 GHz EPR spectrometer (Magnetech, Berlin, Germany). A charcoal suspension (100 mg/mL) was injected intratumorally (60 μ L) 24 h before experiments. For EPR reading, the tumor under study was placed in the center of the extended loop resonator. For air condition, basal measurements were performed. For carbogen condition, pO₂ measurements were started after a 10 min inhalation period. According to calibration curves [5], the EPR line width was converted to pO₂.

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 μ L intraperitoneally with 300–400 μ Ci of ¹⁸F-FDG (Betaplus Pharma, Brussels, Belgium).

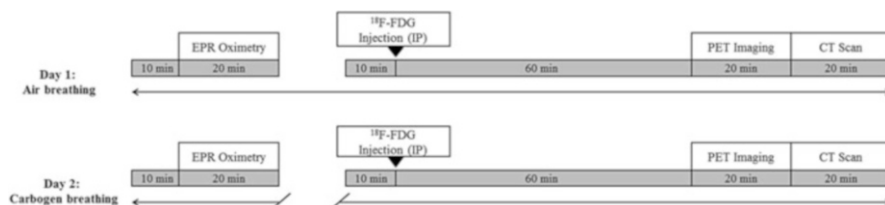


Fig. 25.1 Experimental Protocol

A 10 min transmission scan was first obtained in a single mode using a 370 MBq ^{137}Cs 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 $128 \times 128 \times 120$ matrix, with a voxel of 1 mm^3 . 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). The CT projections were reconstructed with a voxel size of $0.221 \times 0.221 \times 0.221 \text{ mm}^3$.

Regions of Interest (ROIs) were delineated on fused PET/CT images using PMOD software (PMODTM, version 3.403, PMOD technologies Ltd, Zurich, Switzerland). 2D ROIs were established on consecutive transversal slices using a 50 % isocontour 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. The global tracer uptake was assessed in tumors and expressed as standardized uptake values (SUV).

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 $\pm$ SEM. For all tests, results with p-values < 0.05 (*), < 0.01 (**), or < 0.001 (***) were considered significant. The scatter plots of measured pO_2 versus SUV 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).

3 Results

For each breathing condition, air or carbogen breathing, the oxygen status of MDA-MB-231 tumors ($n = 16$) and SiHa tumors ($n = 11$) was assessed by using EPR spectroscopy, followed by PET imaging for tracer uptake study.

A significant change in pO_2 was observed during the breathing challenge for each tumor model (Fig. 25.2). Basal pO_2 measured was $4 \pm 1 \text{ mmHg}$ for MDA-MB-231 tumors and $5 \pm 1 \text{ mmHg}$ for SiHa tumors. Under carbogen breathing, MDA-MB-231 and SiHa tumors reached pO_2 values around $10 \pm 1 \text{ mmHg}$ and $16 \pm 1 \text{ mmHg}$, respectively.

Acute changes in global ^{18}F -FDG uptake linked to carbogen challenge were found in this study. In Fig. 25.3, we can observe a significant decrease in the uptake of ^{18}F -FDG under carbogen compared to air breathing in both tumor models. SUV measured on PET images were 0.675 ± 0.023 under air and 0.548 ± 0.017 under carbogen for MDA-MB-231 tumors, and 0.678 ± 0.023 under air and 0.553 ± 0.019 under carbogen for SiHa tumors. There were no differences in ^{18}F -FDG uptake between the two tumor models.

Fig. 25.2 The mean values $\pm$ SEM of pO_2 measured by EPR during the breathing challenge in MDA-MB-231 tumors (light grey) and SiHa tumors (dark grey)

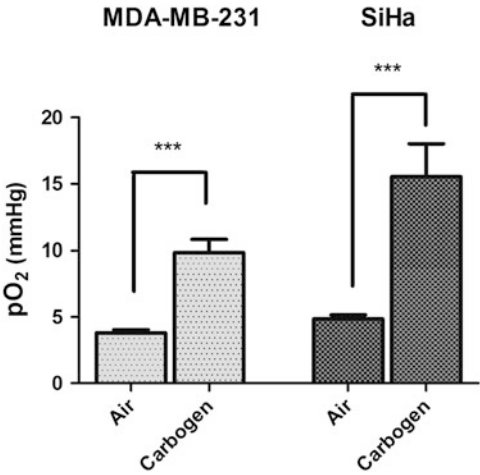
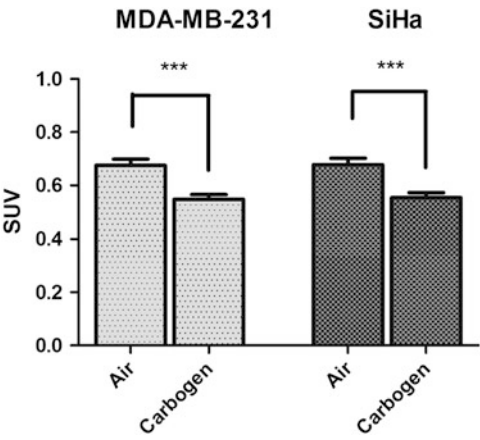
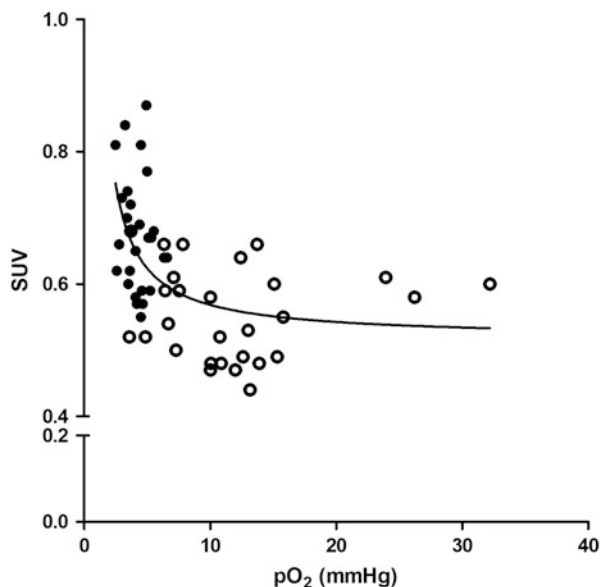


Fig. 25.3 ^{18}F -FDG uptake under normoxia and hyperoxia in MDA-MB-231 tumors (light grey) and SiHa (dark grey). A lower uptake of ^{18}F -FDG was observed under hyperoxic conditions compared to normoxic conditions in both tumor models



In Fig. 25.4, the relationship between global ^{18}F -FDG uptake and pO_2 measurements obtained from individual tumors (mice breathing air or carbogen) is presented as a non-linear fit (modified exponential, $r = 0.557$). The ^{18}F -FDG uptake was higher in hypoxic tumors compared to tumors with pO_2 larger than 10 mmHg.

Fig. 25.4 Relationship between ^{18}F -FDG uptake and pO_2 values during air (filled symbol) and carbogen breathing (open symbol). The ^{18}F -FDG uptake was higher in hypoxic tumors compared to tumors with pO_2 larger than 10 mmHg. According to CurveExpert software, the best fit is $y = 0.52 e^{0.93/x}$



4 Discussion

In this study, carbogen breathing was used as a modulator of hypoxia in the two tumor models and pO_2 variation has been assessed by EPR oximetry. To our knowledge, it is the first time that EPR, a highly sensitive method to measure pO_2 values in vivo [7], has been associated to global ^{18}F -FDG uptake measurement in vivo. Furthermore, the experimental design was built as a dynamic follow-up of the tumors during a breathing challenge. Here we assessed the correlation between global PET uptake and pO_2 values for each tumor during the breathing challenge.

We found that the uptake of ^{18}F -FDG was higher in tumors with a pO_2 value inferior to 10 mmHg. This observation is consistent with the upregulation of GLUT-1 that is associated with hypoxia. Nevertheless, we also found that the uptake of ^{18}F -FDG was lower after a short period of carbogen breathing. This observation emphasizes that the uptake is not only depending on the GLUT-1 expression, but depends on the rapid adaptation of the metabolism of the tumor cells when oxygen became available as well, phenomenon known as the Pasteur Effect (glycolysis inhibition in presence of oxygen) [8]. Furthermore, as mentioned by Thews et al., GLUT-1 expression is also controlled by other microenvironmental parameters not only oxygen dependent [4]. The change in ^{18}F -FDG uptake was true for both tumor models although their metabolic phenotype characterized in vitro indicate that the MDA-MB-231 tumor model is highly glycolytic [8] compared to the SiHa model that possess an oxidative phenotype [9].

Our results could also suggest that, beyond tumor delineation based on metabolism evaluation, ^{18}F -FDG uptake could also indirectly reflect the oxygenation

status of tumors, a major factor involved in tumor progression and resistance to therapy. The fact that ^{18}F -FDG could indirectly reflect the level of hypoxia has already been discussed in several studies [4, 10–19]. Our results are consistent with the study of Christian et al. that showed that the ^{18}F -FDG uptake was higher under severe hypoxia compared to normoxic conditions [19]. However, this trend was found only when considering the whole tumor, as authors also found that the correlation at the microscopic level was poor when considering the co-localization of ^{18}F -FDG uptake and of the nitroimidazole ^{14}C -EF3 [19]. On the other hand, Thews et al. found that ^{18}F -FDG uptake did not vary with the tumor volume in DS-sarcoma, whereas the oxygenation status was determined to be impaired with increasing tumor volume in this tumor model [4]. However, no dynamic follow up of the tumors was assessed in this study in order to evaluate the impact hypoxia on ^{18}F -FDG uptake in the same animals over time. The fact that ^{18}F -FDG could also be used as a hypoxia biomarker could be attractive, but our results should not be over-interpreted. They just highlight the rapid plasticity of the tumor cells to adapt their metabolism to the oxygen environment.

In conclusion, our study showed that global ^{18}F -FDG uptake was higher in hypoxic tumors compared to tumors with pO_2 values above 10 mmHg, and that acute changes in ^{18}F -FDG uptake were observed during carbogen challenge, demonstrating a rapid metabolic adaptation in both tumor models investigated.

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