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Short communication

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

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ABSTRACT

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.

Hypoxia is known as a major factor in tumor progression, metastasis and response to treatment, especially radiotherapy. It is well established that glucose transporters (GLUTs) are upregulated by chronic hypoxia [1]. As a consequence, it has been suggested that high [^{18}F]-fluoro-deoxy-glucose (^{18}F-FDG) uptake measured by Positron Emission Tomography (PET) may reflect the level of tumor hypoxia. Nevertheless conflicting results were reported and the debate is still ongoing [2]. Indeed, in tumors, high ^{18}F-FDG uptake can also arise under non-hypoxic condition through a process known as aerobic glycolysis or Warburg effect [2,3]. The objectives of the present study were twofold: (1) to assess the link between ^{18}F-FDG uptake and tumor oxygenation by comparing micro-PET data with Electron Paramagnetic Resonance (EPR) oximetry that provides absolute pO_2 measurements [4,5]; (2) to assess the influence of rapid changes in tumor oxygenation on the uptake of ^{18}F-FDG using different oxic conditions (carbogen or air breathing). The study was carried out in two different tumor xenografts, MDA-MB-231 and SiHa models selected according to their glycolytic [3] and oxidative [6] behavior respectively.

Materials 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.

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EPR oximetry

In vivo tumor pO_2 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 pO_2 [7], the EPR spectra line width was converted to pO_2. 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 pO_2 measurements correspond to an average of pO_2 values in the tumor volume. For air condition, basal measurements were performed. For carbogen condition, pO_2 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 ^{18}F-FDG (Betaplus Pharma, Brussels, Belgium). 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 size 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) for anatomical reference. The CT projections were reconstructed with a voxel size of $0.221 \times 0.221 \times 0.221$ mm^3. 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% 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. 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 $P < 0.05$ (*),<0.01 (**), or <0.001 (***) were considered significant. The scatter plots of measured pO_2 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 pO_2 values and the ^{18}F-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 ^{18}F-FDG. Basal measured pO_2 (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 pO_2 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

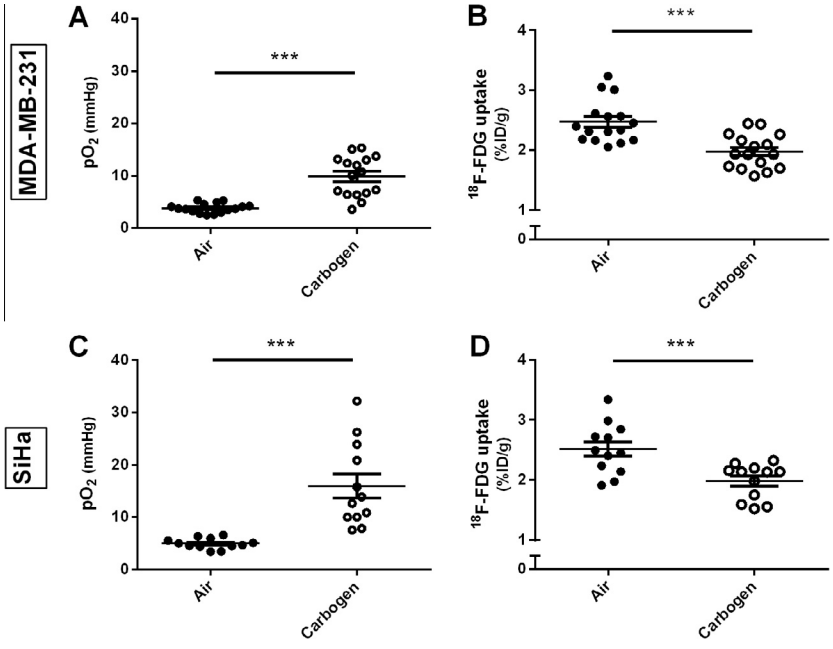

Fig. 1. pO_2 and ^{18}F-FDG uptake under air and carbogen breathing in MDA-MB-231 tumors (upper row) and SiHa tumors (lower row). A significant change of pO_2 was observed during the breathing challenge for each tumor model (A and C). A lower uptake of ^{18}F-FDG was observed under hyperoxic conditions compared to normoxic conditions in both tumor models (B and D).

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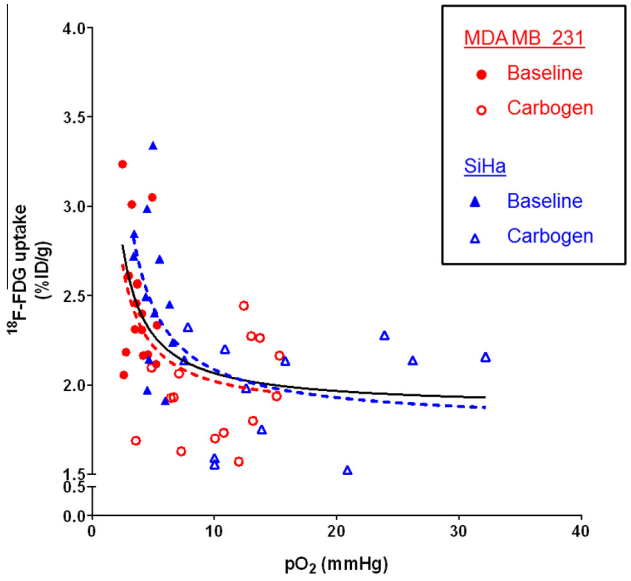

Fig. 2. Relationship between ^{18}F-FDG uptake and pO_2 values during air (filled symbol) and carbogen breathing (open symbol) presented as a non-linear fit for MDA-MB-231 (red line), SiHa (blue line), and pooled data (black line). The ^{18}F-FDG uptake was higher in hypoxic tumors compared to tumors with pO_2 larger than 10 mmHg. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tumors ($n = 12$). Similar results were found by expressing the ^{18}F-FDG uptake as SUV. Fig. 2 shows the relationship between ^{18}F-FDG uptake and pO_2 measurements obtained from individual tumors (mice breathing air or carbogen). According to CurveExpert software, the best fit were $y = 1.84e^{0.93/x}$, $y = 1.78e^{1.56/x}$, $y = 1.87e^{0.99/x}$ for MDA-MB-231, SiHa, and pooled data, respectively. In both tumor models, the ^{18}F-FDG uptake was higher in tumors with a pO_2 value inferior to 10 mmHg.

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 ^{18}F-FDG PET uptake and pO_2 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 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. A higher ^{18}F-FDG uptake has been found in tumors with a pO_2 value inferior to 10 mmHg, a status encountered at the basal level (air breathing) in most tumors belonging to both tumor models. This observation could be consistent with the upregulation of the expression of glucose transporters and glycolytic enzymes that are associated with hypoxia. However, we also found that the uptake of ^{18}F-FDG was lower after a short period of carbogen breathing. This observation emphasizes that the tracer uptake is not only dependent on transcriptional changes (like glucose transporters GLUTs). It also most likely relies on the rapid adaptation of the tumor cell metabolism when oxygen became available, a phenomenon known as the Pasteur Effect. This was true for both tumor models although their metabolic phenotype characterized in vitro indicate that the MDA-MB-231 tumor model is highly glycolytic [3] compared to the SiHa model that possess an oxidative phenotype [6].

^{18}F-FDG PET is a widespread technology approved in clinical practice for cancer diagnosis, staging and treatment monitoring. Beyond these applications, it has been suggested in the literature that ^{18}F-FDG uptake could also indirectly reflect the oxygenation status of tumors, a major factor involved in resistance to radiation therapy. This assertion has already been discussed in several studies providing discordant results [12–21]. Here, we found in both tumor models an inverse relationship between ^{18}F-FDG uptake and pO_2 values, a behavior that is rather comparable to what has been found with several nitroimidazoles such as EF_3 [22] and ^{18}F-FAZA [23]. Our present findings 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 [21]. However, this trend was found only when considering results on the whole tumor. Indeed, Christian et al. 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. Nevertheless, other studies have established a positive correlation between ^{18}F-FDG uptake and hypoxia using regional [24] or pixel by pixel analysis [25]. As commented by Pugachev and co-workers, the use of a thick section for autoradiography in Christian et al. study could explain the poor correlation reported [26].

Therefore, there is a need to take cautiously any relationship between oxygenation status and ^{18}F-FDG uptake, as the latter is dependent on other factors than oxygenation. Overall, our results highlight the rapid plasticity of the tumor cells to adapt their metabolism to the oxygen environment.

Conclusion

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 ^{18}F-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

[1] Brahimi-Horn MC, Chiche J, Pouyssegur J. Hypoxia and cancer. Mol Med 2007;85:1301–7.
[2] Dierckx RA, Van de Wiele C. FDG uptake, a surrogate of tumour hypoxia? Eur J Nucl Med Mol Imag 2008;35:1544–9.
[3] Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004;4:891–9.
[4] Gallez B, Jordan BF, Baudelet C, Misson PD. Pharmacological modifications of the partial pressure of oxygen in murine tumors: evaluation using in vivo EPR oximetry. Magn Reson Med 1999;42:627–30.
[5] Jordan BF, Baudelet C, Gallez B. Carbon-centered radicals as oxygen sensors for in vivo electron paramagnetic resonance: screening for an optimal probe among commercially available charcoals. MAGMA 1998;7:121–9.

[6] Sonveaux P, Vegran F, Schroeder T, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest 2008;118:3930–42.
[7] Gallez B, Baudelet C, Jordan BF. Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications. NMR Biomed 2004; 17:240–62.
[8] Bussink J, Kaanders JH, Rijken PF, et al. Vascular architecture and microenvironmental parameters in human squamous cell carcinoma xenografts: effects of carbogen and nicotinamide. Radiother Oncol 1999; 50:173–84.
[9] Secomb TW, Hsu R, Ong ET, Gross JF, Dewhirst MW. Analysis of the effects of oxygen supply and demand on hypoxic fraction in tumors. Acta Oncol 1995; 34:313–6.
[10] van der Sanden BP, Heerschap A, Hoofd L, et al. Effect of carbogen breathing on the physiological profile of human glioma xenografts. Magn Reson Med 1999;42:490–9.
[11] van Laarhoven HW, Bussink J, Lok J, Punt CJ, Heerschap A, van Der Kogel AJ. Effects of nicotinamide and carbogen in different murine colon carcinomas: immunohistochemical analysis of vascular architecture and microenvironmental parameters. Int J Radiat Oncol Biol Phys 2004;60:310–21.
[12] Busk M, Horsman MR, Kristjansen PE, van der Kogel AJ, Bussink J, Overgaard J. Aerobic glycolysis in cancers: implications for the usability of oxygen-responsive genes and fluorodeoxyglucose-PET as markers of tissue hypoxia. Int J Cancer 2008;122:2726–34.
[13] Busk M, Horsman MR, Jakobsen S, Bussink J, van der Kogel A, Overgaard J. Cellular uptake of PET tracers of glucose metabolism and hypoxia and their linkage. Eur J Nucl Med Mol Imag 2008;35:2294–303.
[14] Waki A, Fujibayashi Y, Yonekura Y, Sadato N, Ishii Y, Yokoyama A. Reassessment of FDG uptake in tumor cells: high FDG uptake as a reflection of oxygen-independent glycolysis dominant energy production. Nucl Med Biol 1997;24:665–70.
[15] Clavo AC, Brown RS, Wahl RL. Fluorodeoxyglucose uptake in human cancer cell lines is increased by hypoxia. J Nucl Med 1995;36:1625–32.
[16] Burgman P, Odonoghue JA, Humm JL, Ling CC. Hypoxia-Induced increase in FDG uptake in MCF7 cells. J Nucl Med 2001;42:170–5.
[17] Chan LW, Hapdey S, English S, et al. The influence of tumor oxygenation on (18)F-FDG (fluorine-18 deoxyglucose) uptake: a mouse study using positron emission tomography (PET). Radiat Oncol 2006;1:3.
[18] Gagel B, Piroth M, Pinkawa M, et al. pO polarography, contrast enhanced color duplex sonography (CDS), [18F] fluoromisonidazole and [18F] fluorodeoxyglucose positron emission tomography: validated methods for the evaluation of therapy-relevant tumor oxygenation or only bricks in the puzzle of tumor hypoxia? BMC Cancer 2007;7:113.
[19] de Geus-Oei LF, Kaanders JH, Pop LA, Corstens FH, Oyen WJ. Effects of hyperoxygenation on FDG-uptake in head-and-neck cancer. Radiother Oncol 2006;80:51–6.
[20] Li XF, Ma Y, Sun X, Humm JL, Ling CC, O'Donoghue JA. High 18F-FDG uptake in microscopic peritoneal tumors requires physiologic hypoxia. J Nucl Med 2010;51:632–8.
[21] Christian N, Deheneffe S, Bol A, et al. Is (18)F-FDG a surrogate tracer to measure tumor hypoxia? Comparison with the hypoxic tracer (14)C-EF3 in animal tumor models. Radiother Oncol 2010;97:183–8.
[22] Mahy P, De Bast M, Gallez B, et al. In vivo colocalization of 2-nitroimidazole EF5 fluorescence intensity and electron paramagnetic resonance oximetry in mouse tumors. Radiother Oncol 2003;67:53–61.
[23] Tran LBA, Bol A, Labar D, et al. Hypoxia imaging with the nitroimidazole 18F-FAZA PET tracer: a comparison with OxyLite, EPR oximetry and 19F-MRI relaxometry. Radiother Oncol 2012;105:29–35.
[24] Dearling JL, Flynn AA, Sutcliffe-Goulden J, et al. Analysis of the regional uptake of radiolabeled deoxyglucose analogs in human tumor xenografts. J Nucl Med 2004;45:101–7.
[25] Pugachev A, Ruan S, Carlin S, et al. Dependence of FDG uptake on tumor microenvironment. Int J Radiat Oncol Biol Phys 2005;62:545–53.
[26] Pugachev A, Axente M, Humm J. On autoradiographic studies comparing the distributions of ^{18}F- and ^{14}C-labeled compounds in tumor tissue specimens. Radiother Oncol 2010;97:609.

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