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PET imaging of hypoxia

Hypoxia imaging with the nitroimidazole ¹⁸F-FAZA PET tracer: A comparison with OxyLite, EPR oximetry and ¹⁹F-MRI relaxometry

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ABSTRACT

Background and purpose: ¹⁸F-FAZA is a nitroimidazole PET tracer that can provide images of tumor hypoxia. However, it cannot provide absolute pO_2 values. To qualify ¹⁸F-FAZA PET, we compared PET images to pO_2 measured by OxyLite, EPR oximetry and ¹⁹F-MRI.

Materials and methods: Male WAG/Rij rats grafted with rhabdomyosarcoma were used. Tumor oxygenation was modified by gas breathing (air or carbogen). The same day of PET acquisition, the pO_2 was measured in the same tumor either by OxyLite probes (measurement at 10 different sites), EPR oximetry using low frequency EPR or ¹⁹F-relaxometry using 15C5 on an 11.7 T MR system.

Results: There was a good correlation between the results obtained by PET and EPR (R = 0.93). In the case of OxyLite, although a weaker correlation was observed (R = 0.55), the trend for two values to agree was still related to the inverse function theoretically predicted. For the comparison of ¹⁸F-FAZA PET and ¹⁹F-MRI, no change in T_1 was observed.

Conclusions: A clear correlation between ¹⁸F-FAZA PET image intensities and tumor oxygenation was demonstrated, suggesting that ¹⁸F-FAZA PET is a promising imaging technique to guide cancer therapy. © 2012 Elsevier Ireland Ltd. All rights reserved. Radiotherapy and Oncology 105 (2012) 29–35

Tumor hypoxia has long been regarded as an important prognosis factor in oncology [1-4]. Experimental and clinical evidences have demonstrated relationship between this phenomenon and the probability of malignant progression, local recurrence and distant metastases [5-8]. Hypoxic fractions have also been known to be responsible for the reduced sensitivity of solid tumor to surgery [6], possibly to certain chemotherapeutic agents and especially to ionizing radiation [9-11]. Tumor oxygenation and perfusion are therefore the relevant parameters that are associated with treatment outcome. Quantitative information and distribution of oxygen concentration would be valuable for the improvement of treatment methods to kill maximum tumor cells with minimum side effects. Given the importance of this aspect, many techniques have been reported to be useful for hypoxia detection in tumors [12,13]; however, a limited number of methods have been implemented into clinical practice.

Positron emission tomography (PET) is one of methods currently available for human application. For PET hypoxia imaging, 2-nitroimidazoles are recognized to be specific for hypoxic cells [14]. These compounds enter cells and undergo a succession of

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reduction steps. In the presence of oxygen, the first step is reversible; consequently, the reduced nitroimidazole is immediately reoxidized and washed out from tissues. Under hypoxic condition. the reoxidation is slow, and that allows further reduction to occur: the compound can thereby bind covalently to intracellular macromolecules and be retained inside cells. Due to the need for enzyme system to reduce and bind nitroimidazole, these tracers accumulate selectively only in viable hypoxic cells. The other advantages of PET are its non-invasive and non-toxic nature; its capacity to perform repeated measurements (providing enough time is given between two tracer injections) and the resulting overall hypoxia images in three dimensions [15]. However, it is an indirect method that cannot provide absolute value of pO₂. Therefore, in order to evaluate the potential of PET hypoxia images, we decided to compare PET hypoxia imaging to other oximetry techniques: OxyLite[®], Electron Paramagnetic Resonance (EPR) spectroscopy and Nuclear Magnetic Resonance Imaging by fluorine relaxometry (¹⁹F-MRI).

Oxylite measures directly pO_2 using the principle that the lifetime of fluorescence is inversely proportional to oxygen pressure at the probe tip. This method was compared with "gold standard" Eppendorf electrode and reported to have good correlation [16,17]. Nevertheless, OxyLite had never become a routine clinical assay because it is invasive, only feasible for accessible tumors and cannot distinguish viable and necrosis tissue. EPR spectroscopy determines oxygen level through the changes in EPR spectral linewidth

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caused by the interaction between paramagnetic molecular oxygen and paramagnetic sensor. This technique appeared as a direct, noninvasive and highly sensitive tool to measure absolute oxygen concentration *in vivo* [12,18–22]. Another technique also used in this comparative study was ¹⁹F-MRI that is based on the linear relationship between the spin–lattice relaxation rates of ¹⁹F nuclei in perfluorocarbons and oxygen concentration in tissue. However, these two MR-based techniques share the same major limitation in term of non-clinical implementation [23].

For this comparison study, we chose ¹⁸F-fluoroazomycin-arabinoside (¹⁸F-FAZA) as the PET hypoxia-specific tracer. This marker has recently demonstrated to have superior biokinetics in comparison to other PET hypoxia tracers [24–26] as well as to be feasible and safe for clinical hypoxia imaging [27–30]. Herein we accessed the correlation between PET image intensities and measured pO₂ values as the mean for the validation of ¹⁸F-FAZA PET imaging.

Materials and methods

Animal and tumor model

Male adult WAG/Rij rats (Harlan Nederland, Horst, The Netherlands) weighting between 200 and 250 g were used for this study. Fragments of about 1 mm³ from a syngenic rhabdomyosarcoma were grafted subcutaneously in both thighs of each rat under general anesthesia by using intraperitoneal injection of a mixed solution of ketamine and xylazine (80 and 10 mg/kg, respectively). The experiments were performed when tumors grew to be 20–25 mm in diameter (at this size of tumor, the proportion of necrotic fraction semiquantified on histological section was less than 8%). In all comparisons, animals were randomly divided into groups, breathing either room air or carbogen (5% CO_2 in oxygen).

Experimental design

Each tumor was assessed by ¹⁸F-FAZA PET imaging to obtain the tumor to background (T/B) ratio. On the same day, the pO_2 value of these tumors was also measured either by OxyLite, EPR spectroscopy or ¹⁹F-MRI. The detail of comparative assay between PET imaging and every oximetry technique is presented in Fig. 1.

PET imaging

For PET studies, rats were injected intravenously with 18.5– 29.6 MBq ¹⁸F-FAZA that was produced as previously described [31]. Rats breathing carbogen were kept in a chamber equilibrated with carbogen, beginning 1 h before tracer administration and continuing to the end of the study; while other animals were kept in normal conditions. The purpose of carbogen challenge was to get a higher pO_2 in the tumor tissue during the distribution phase of ¹⁸F-FAZA before the PET image acquisition. PET scans were performed 3 h after tracer injection on a dedicated small-animal PET scanner (MOSAIC, Philips) with a spatial resolution of 2.5 mm (FWHM). Rats anesthetized with 2% isoflurane underwent first a 10 min emission scan followed by a 10 min transmission scan using a 370-MBq ¹³⁷Cs source. After the correction with attenuation factors obtained from the transmission scan, images were reconstructed using a fully 3D iterative algorithm (3D-RAMLA) in



Fig. 1. Experimental protocol described in chronological order from left to right.

a $128 \times 128 \times 120$ matrix, with a voxel of 1 mm³. The result of tracer distribution in tumor was expressed as T/B ratio, calculated as the mean activity in the tumor region divided by the mean activity in the background region. Regions of interest (ROI) were manually delineated on consecutive transversal slices, creating a volume of interest (VOI) encircling the whole tumor. The background VOI of 1 cm³ was chosen in pelvic region to be representative for the tracer presence within non-tumor tissue.

OxyLite

We used an OxyLite 2000 system (Oxford Optronix, Oxford, UK) consisting of a 230- μ m diameter optical fiber for oxygen measurement and a thermocouple to monitor the temperature of tumor. This system converts automatically the measured signals to pO₂ values using individual probe calibration supplied by the manufacturer. The probe was inserted thanks to a 24-gauge needle that was then withdrawn, leaving the probe inside the tumor. The results were read only after the signals reached the stabilization. The median pO₂ value of each individual tumor was obtained from the measurement at 10 independent sites within the tumor. As the OxyLite measurement is temperature-sensitive, an infrared lamp was used to maintain the body temperature of animals constant during the experiment.

EPR oximetry

For EPR spectroscopy, charcoal wood power (CX0670-1, EM Science, Gibbstown, NJ) was used as oxygen sensitive sensor. About 200 µL of charcoal suspension (100 mg/ml) was introduced within tumor at a depth of 3-6 mm using a 23-gauge needle. EPR acquisitions were performed 24 h after probe implantation by using an Lband EPR spectrometer (Magnettech, Berlin, Germany) equipped with a low-frequency microwave bridge operating at 1.2 GHz and a loop-gap resonator. The modulation frequency was 100 kHz and the amplitude modulation was less than one-third of the peak-to-peak linewidth to avoid peak distortion. Animals were anesthetized with isoflurane during EPR readings and placed on such a way that the tumor was in the center of the loop resonator. In the carbogen group, the rats inhaled the gas at least 30 min before EPR spectrum was recorded so that pO₂ reached a maximum. The linewidth of the first-derivative EPR spectrum that was the average of five scan accumulations was then converted to pO₂ using a calibration curve [32].

¹⁹F-MRI

For these experiments, the marker 15C5 (perfluoro-15-crown-5ether, Fluorochem Ltd., Derbyshire, UK) was injected intratumorally along three tracks (3 \times 50 μ L). Rats were maintained under general anesthesia and their body temperature was monitored with a rectal probe at 37 ± 1 °C by a warm water blanket. To have the effect of carbogen, rats were subjected to respiratory challenge about 20 min before the acquisition. MRI was performed on an 11.7 T, 16 cm inner diameter bore system (Bruker Biospec) with a tunable ¹H/¹⁹F surface coil for signal transmission and reception. MRI scout images were obtained for both ¹H (500.31 MHz) and ¹⁹F (470.71 MHz) to reveal the distribution of marker within the tumor. Parametric images of the spin-lattice relaxation time (T_1) were acquired using a Fast Imaging with Steady state Precession (FISP) sequence in FID mode. The acquisition parameters were $TR/TE/FA/BW/NA/matrix = 4 ms/1.2 ms/5^{\circ}/100 kHz/2/64 \times 64$, 4 segments and the total acquisition time was 1 min 36 s. Images were treated using a homemade program in Matlab (The Math-Works, Inc., Natick, MA). The values of pO₂ were then determined from T_1 by an *in vitro* calibration curve as reported elsewhere [33].

Statistics and data analysis

One-way analysis of variance (ANOVA) was performed to assess statistically significant differences between groups. Results were expressed as mean value of parameter \pm SEM. For all tests, P < 0.05 was considered statistically significant.

To validate the overall correlation between two methods, the scatter plots of measured $pO_2(y)$ versus T/B ratio (x) were traced using data from all tumors of both groups. The process of finding the best fit was done by letting CurveExpert software (version 1.4) compare data to each model function and automatically choose the best curve. We also evaluated ¹⁸F-FAZA PET according to the oxygen-nitroimidazole binding curve theoretically predicted by Chang et al. by fitting our data to the modified hyperbolic function [34]:

$$y = -a + \frac{ax_{\max}}{x} \tag{(*)}$$

where "*y*" is the measured pO₂ value, "*x*" is the T/B ratio derived from ¹⁸F-FAZA PET, " x_{max} " is the maximum T/B ratio achieved in each comparative assay and "*a*" is the regression coefficient.

Results

Comparison between ¹⁸F-FAZA PET imaging and OxyLite oximetry

In this comparison (Fig. 2), animals were transferred to OxyLite system immediately after the PET scans. The mean ± SEM pO₂ measured by OxyLite for the tumors used in normoxic condition was 1.89 ± 0.60 mm Hg, n = 11. A significant increase of this value was observed in the tumors of carbogen group ($pO_2 = 7.12 \pm$ 1.74 mm Hg, n = 13). Similarly for PET imaging, ¹⁸F-FAZA exhibited a significant lower uptake in the case of carbogen (T/B ratio = 2.18 ± 0.71) than that of room air (T/B ratio = 2.90 ± 0.75). The best trend for two values to agree was a reciprocal logarithm function (Eq. (1) - Sup.Table 1) with a correlation coefficient R = 0.55. The user-defined fitting function according to the modified hyperbolic curve (Eq. (*)) was then evaluated with the correlation R = 0.50 (Eq. (2) – Sup.Table 1). On the other hand, the results from OxyLite indicated highly heterogeneous distributions of hypoxia in this tumor model (Sup.Table 2). The carbogen group displaying particularly a larger range of pO₂ demonstrated that the response to carbogen varied widely among the measurement sites. Indeed, during respiratory challenge, it seemed that the pO₂ enhancement in poorly-oxygenated region changed smaller and slower than that in initially better-oxygenated region (Fig. 3).

Comparison between ¹⁸F-FAZA PET imaging and EPR oximetry

The oxygen status of tumors was assessed by using first EPR spectroscopy and followed by PET imaging. The mean ± SEM of T/ B ratio on PET images reached 2.51 ± 0.14 for the room air group (n = 13), while this value for the carbogen group was 1.93 ± 0.08 (n = 12). In accordance with PET signal intensities, the results measured by EPR showed a significant increase in oxygen concentration under carbogen condition $pO_2 = 8.68 \pm 0.59$ mm Hg compared to $pO_2 = 5.28 \pm 0.73$ mm Hg under normal condition. Fig. 4 presents the association between the values obtained from EPR spectroscopy and ¹⁸F-FAZA-PET. The best fit was a logarithm function with the correlation R = 0.93 (Eq. (3) – Sup.Table 1). Shown on the right of the same figure, the theoretically predicted curve displays the slightly weaker coefficient R = 0.86 (Eq. (4) – Sup.Table 1).



Fig. 2. Comparison between ¹⁸F-FAZA PET and OxyLite. *Upper row:* The mean values \pm SEM of pO₂ measured by OxyLite and T/B ratio derived from PET for the room air group (n = 11) and the carbogen group (n = 13). *Significant difference between two groups. *Lower row:* The scatter plots of pO₂ versus T/B ratio. Each point was representative for one individual tumor subjected either to room air (open symbol) or to carbogen (filled symbol). The curve on the left was the best fit for the data. The right one was the curve found when fitting data to the user-defined function (Eq. (*)).



Fig. 3. Simultaneous monitoring of oxygenation by OxyLite at two independent sites within one individual tumor.

Comparison between ¹⁸F-FAZA PET imaging and ¹⁹F-MRI relaxometry

¹⁸F-FAZA was also validated by comparison with 15C5 marker in ¹⁹F-MR images (Sup.Fig. 1). The results from PET experiments always revealed the effect of carbogen; T/B ratio in room air and in carbogen was respectively 2.51 ± 0.25 (n = 5) and 1.91 ± 0.12 (n = 7). However, the spin–lattice relaxation time T_1 registered by ¹⁹F-MRI did not change significantly between two groups as expected, $T_1 = 472.85 \pm 2.37$ ms in room air and 471.13 ± 10.41 ms in carbogen.

Discussion

Considering the important role of tumor oxygenation, many hypoxia-targeting therapies have emerged and are being developed [35]. These therapeutic strategies, however, cannot be fully utilized unless we have a suitable method to assess hypoxia in routine clinical procedure. The current standard method [36] (Eppendorf mea-

surements) is invasive and cannot provide three-dimensional heterogeneity of oxygenation. Moreover, these systems are not produced anymore by Eppendorf, and, consequently this technique is no more a clinical option for the institutions that do not possess these systems. Alternatively, imaging techniques with MRI and PET that can overcome these limitations are becoming appealing. Recently, the use of PET imaging with specific-hypoxia tracer was concluded as a plus for radiation therapy planning [37]. In spite of that, the non-specific binding of tracer and its slow wash-out from non-target tissue are still the major problems of PET hypoxia imaging. ¹⁸F-FAZA belongs to a new generation PET hypoxia tracer recently recommended for preclinical and clinical studies. Its specific accumulation in hypoxic region was reported in in vitro experiments [38-40] as well as in vivo studies [24,25,41-44]. ¹⁸F-FAZA also exhibited a better tumor-to-reference contrast than other tracers because of its faster clearance thanks to its low lipophilic characteristic. Further, the autoradiography of ¹⁸F-FAZA distribution was compared with the immunohistochemical image of hypoxia marker pimonidazole and it was demonstrated that ¹⁸F-FAZA PET was able to map accurately and quantitatively hypoxia with the underlying microscopic reality [43]. Nevertheless, in order to be applied in practice, all new oxygenation techniques should be validated based on reference method [45-49]. That is the reason why it was still necessary to assess further ¹⁸F-FAZA in this comparative study. Up to date, the direct link between uptake of PET hypoxia tracer and oxygen tension has not vet been elucidated. According to the theory proposed by Chang et al., oxygen level is inversely related to nitroimidazole concentration by a rectangular hyperbolic function [34]. Therefore, to analyze the correlation between ¹⁸F-FAZA PET and the other oximetry techniques, we not only found the best fit for our data but also fit them to a user-defined model as presented in the theoretically predicted function.

The mean pO_2 values of this tumor model measured by OxyLite and EPR were in range of 0–10 mm Hg, which are the



Fig. 4. Comparison between ¹⁸F-FAZA PET and EPR. *Upper row:* The mean values \pm SEM of pO₂ measured by EPR and T/B ratio derived from PET for the room air group (n = 13) and the carbogen group (n = 12). *Significant difference between two groups. *Lower row:* The scatter plots of pO₂ versus T/B ratio. Each point was representative for one individual tumor subjected either to room air (open symbol) or to carbogen (filled symbol). The curve on the left was the best fit for the data. The right one was the curve found when fitting data to the user-defined function (Eq. (*)).

radiobiologically relevant regions and always showed a significant increase for the breathing carbogen group. Consistent with that, the PET scans revealed clearly the ¹⁸F-FAZA uptake 3 h post injection in all images, confirming the tracer accumulation in hypoxic tumors; also, the T/B ratio under high oxygen condition displayed about 1.3-fold decrease compared to that under normoxic condition in all comparative assays. Fig. 5 shows the representative images of two animals subjected to different atmospheres. The proportion 1.3 was similar to that previously reported for A431 mouse tumor model [25]. However, the PET T/B ratios and the measured pO₂ values were not fully correlated, especially in the case of OxyLite, the correlation coefficient was quite weak. This may relate to some limitations in this kind of analysis as discussed below.

Firstly, this is an *in vivo* study that was obviously influenced by parameters such as biodistribution and elimination of tracer compounds. Indeed, the fact that physiology (blood flow, tracer delivery, washout, etc.) and pharmacokinetic (binding, association, etc.) affect PET images was already reported for some PET tracers [50,51]. Moreover, it should be noted that pO_2 may vary during the prolonged period of carbogen breathing [52]. Yet, as the time necessary to have the accumulation of PET tracer in hypoxic tissue is unknown, the breathing challenge was prolonged over the whole distribution phase of ¹⁸F-FAZA before the PET acquisition. Hence, the information of ¹⁸F-FAZA PET images could not exclude the complex effect of these factors. Secondly, oximetry methods cannot differentiate necrosis and hypoxic area, the necrotic zones are identified as being hypoxic where the pO₂ recorded may be zero or very low. In contrast, ¹⁸F-FAZA is not retained inside necrotic cells. Consequently, the expected inverse correlation of pO₂ value and PET signal intensity was adversely affected in this critical region. The third reason for this disagreement is the discrepancies of information content achieved from the methods. The T/B ratio of PET imaging was representative for the entire tumor volume. whereas the pO₂ value of tumor was measured only in 10 measurement sites for the case of OxyLite and in where the charcoal sensor was distributed for the case of EPR spectroscopy. Furthermore,



Fig. 5. Representative images in two different experimental conditions illustrating higher uptake of ¹⁸F-FAZA in rat breathing room air than that breathing carbogen. (Backgrounds normalized to have the same intensity; ROI encircling tumor marked by red curve on the transversal slice.)

both OxyLite (Sup.Table 2) and PET imaging (the coefficient of variation of the voxel activity for each individual tumor was generally around 40% – data not shown here) demonstrated a large

heterogeneity of hypoxia; in particular, the values in carbogen group were more variable because of the large response to gas. A good correlation would not be easy to observe in tumors with such highly variable intratumoral oxygenation levels. In term of temporal information, the data from PET scans were due to the integration during 3 h from tracer injection to image acquisition, thus, they visualized chronic hypoxia rather than acute hypoxia. Whereas OxyLite and EPR spectroscopy yielded pO₂ at discrete time points, thereby reflected both acute and chronic hypoxia. Additionally, although animals were transferred to the next method immediately after the registration was finished on the first one, the results obtained from two systems in each comparative experiment could not be considered as being recorded at the same time. In this regard, the temporal change of tumor oxygenation due to acute hypoxia could compromise the results [53,54].

Considering the above explanations, R = 0.93 achieved from the best fit curve of T/B ratio versus pO₂ measured by EPR was really an excellent correlation coefficient. In the comparison of PET and Oxy-Lite, a weaker correlation was found. Given the nature of OxyLite method, it is, however, not surprising. In fact, OxyLite is an invasive method that was demonstrated to be less consistent than EPR oximetry [55]. It should also be noted that the single-measurement OxyLite probe could not be expected to report the same correct median values as Eppendorf technique with needle moving step by step.

Another interesting observation was the proximity in value of the correlation coefficient R between the best fit curve and the modified hyperbolic curve. Compared to the best fit, the values of *R* for the user-defined fit were just slightly smaller. This observation supports the oxygen-nitroimidazole binding curve previously established [34]. According to this theoretical hyperbolic function, the more pO₂ decreases, the more substantial the accumulation of tracer is; and this shift is most sensitive at the zone of low oxygen pressure that may be critical in terms of radiation resistance. This steep may also make a problem for the effect of ¹⁸F-FAZA at higher oxygen level. But fortunately, due to the definite increase in radiosensitivity, the dependence of treatment response on pO₂ would be much less relevant in this region [56]. With all data achieved from this study, it seemed that T/B ratio = 1.5 was a pertinent value to discriminate "hypoxic" area from "normoxic" area; the values pO₂ measured in most of tumors possessing T/B ratio >1.5 were in range of 0-10 mm Hg. Interestingly, some clinical studies selected this ratio (1.5) arbitrarily as the threshold to contour potentially hypoxic tumor tissue [27,28]. To have the larger range of oxygen level for the correlation analysis, we investigated the experimental model in which animals breathed either normal air or high oxygen atmospheres. This tumor model, however, responded to carbogen not very strongly; we did not have many tumors with T/B ratio <1.5 and hence could not verify the correlation at higher oxygen concentration. This will require further investigations using other tumor models.

In the case of ¹⁹F-MRI, there was no decrease in T_1 -values under high oxygen condition despite greatly decreased T/B ratio in PET images (Sup.Fig. 1). This could be explained by the lower sensitivity of ¹⁹F-relaxometry compared to EPR and OxyLite. The spin–lattice relaxation rate R_1 of ¹⁹F nuclei is related to oxygen tension according to a linear function; the higher the slope is and/or the smaller the intercept value is, the greater sensitivity we can achieve. Therefore, the ratio η = slope/intercept has been proposed as a sensitivity index [57]. This ratio derived from the ¹⁹F-relaxometry calibration curve of our group was 0.0036 [33]. Whereas when examining the EPR calibration curve in range of 1–10 mm Hg, where the relationship between two variables could be considered as linear [32], the corresponding sensitivity index was 0.0461, considerably much better than that of ¹⁹F-MRI. On the other hand, the response of 15C5 was already known to be temperature-sensitive, 1 °C error could lead to 3 mm Hg error in the value of pO_2 [58]. During the experiment, the body temperature of animals was always maintained around 37 °C, however, the temperature change in such a large tumor of rats could be quite important. As can be seen from the measurement of OxyLite and EPR, the pO_2 enhancement due to respiratory challenge was only about 3–5 mm Hg for this rat tumor model, ¹⁹F-MRI may not be capable enough to tackle this small change of pO_2 . In order to verify that, we tried to monitor the oxygenation within individual tumors during carbogen challenge; indeed, no significant change of T_1 -value was observed (data not shown).

In conclusion, our data corroborate with the results obtained in previous studies that ¹⁸F-FAZA is an effective surrogate of hypoxia fractions. Obviously, ¹⁸F-FAZA PET imaging still suffers some weaknesses. As stated before, this method measures predominantly chronic hypoxia and may be insensitive to transient hypoxia: while the precise timing of hypoxia targeting is also a question that should be refined. Another obstacle relating to inherence of PET is that the voxel size analyzed by PET scans is much larger than the scale of hypoxia heterogeneity [59]. These limitations should be carefully taken into account when applying PET images in practice. In spite of that, ¹⁸F-FAZA PET imaging with excellent correlation to oxygen level as demonstrated herein would be valuable for clinical application to target areas of hypoxia [60-63]. Future studies are needed to investigate further the relationship between ¹⁸F-FAZA uptake and other hemodynamic parameters such as R_2^* of BOLD-MRI (Blood Oxygen Level Dependent), K_{trans} of DCE-MRI (Dynamic Contrast Enhancement); as well as the prognostic value of ¹⁸F-FAZA PET imaging and its usefulness in guided-therapy for cancer.

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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.2012. 04.011.

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