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FLT-PET Imaging of Radiation Responses in Murine Tumors

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Abstract

Background—3'-[F-18]Fluoro-3'-deoxythymidine (FLT) traces thymidine phosphorylation catalyzed by thymidine kinase during cell proliferation. Knowing the rate of cell proliferation during cancer treatment, such as radiation therapy, would be valuable in assessing whether tumor recurrence is likely and might indicate the need for additional treatments. However, the relationship between FLT kinetics and the effects of radiation is not well-understood. Nor has the method for optimal quantification of FLT uptake within the irradiated tumor microenvironment been extensively examined.

Materials and Methods—We performed dynamic FLT-positron emission tomography (PET) studies (60 min) on 22 mice implanted subcutaneously with syngeneic mammary MCaK tumors bilaterally in the shoulder area. A day before the FLT-PET imaging, the tumor on the right side was irradiated with a single dose (0, 2.5, 5, 10, or 20 Gy) or with fractionated exposures (4×2.5 Gy given in 12 h intervals). Standardized uptake value (SUVs) of FLT on tumors at 10 and 60 min post injection were calculated; model fitting was used to estimate the kinetic parameters. Significant radiation-induced changes were shown by comparing the irradiated tumor with the control tumor in the same animal and by comparing it to nonirradiated mice. The effect of radiation on MCaK cell cycle parameters and FLT uptake was also examined *in vitro*.

Results—*In vivo* FLT kinetics were sensitive to radiation doses of 5 Gy and higher (administered 1 day earlier), as judged by SUV semiquantitative measures and by modeling. Single irradiation with 10 Gy had greater impact on SUVs and kinetic parameters than fractionated exposures. Overall, the uptake constant K_i appeared to be the best marker for these radiation effects. FLT uptake by irradiated cells *in vitro* at various doses gave similar findings, and the *in vitro* FLT uptake correlated well with K_i . Radiation-induced G2/M arrest appeared to influence FLT uptake, and this was more pronounced after single than fractionated doses.

Conclusion—The kinetics of FLT uptake into murine mammary tumors was altered 1 day after radiation treatment. The dose-dependent response correlated well with *in vitro* FLT cellular uptake. Parameters (e.g., K_i) derived from FLT kinetics are expected to be useful for assessing the efficacy of irradiation treatment of tumors.

Keywords

3'-Deoxy-3'-18F fluorothymidine (FLT); Positron emission tomography; MCaK tumor; Kinetic modeling; Quantitation; Radiation treatment; Cell proliferation

Introduction

Glucose metabolic imaging using 2-deoxy-2-[F-18] fluoro-D-glucose (FDG) is currently the mainstay of clinical positron emission tomography (PET) imaging for tumors, but other PET tracers are undergoing preclinical or clinical evaluation for their ability to image cellular proliferation, amino acid, protein and membrane biosynthesis, tissue hypoxia, angiogenesis, and tumor receptor expression. One of the most frequently used PET imaging agents for cellular proliferation is the thymidine analog, 3'-[F-18]fluoro-3'-deoxythymidine (FLT), which is accumulated after its phosphorylation by cellular thymidine kinase-1 (TK1). TK1 is a key enzyme of the pyrimidine salvage pathway of DNA synthesis whose activity is three to four times higher in malignant than in benign cells [1,2]. Since this enzyme is functional only during the S-phase of the cell cycle, uptake of FLT is tied to cell proliferation [3]. This has been substantiated by the finding of correlations of FLT uptake with both TK enzyme activity and cell proliferation, as measured by Ki-67 (MIB-1) and proliferating cell nuclear antigen (PCNA) expression [4-9].

Results from *in vivo* cancer models have suggested that FLT may be superior to FDG in monitoring tumor response to treatment with chemotherapy, antiproliferative agents, or kinase inhibitors [2,10-13]. However, only a limited number of reports have addressed the use of FLT-PET for monitoring tumor response to radiotherapy (RT). Sugiyama et al. suggested that FLT may be superior to FDG because RT causes minimal changes in tumor glycolysis while inducing inflammation, which increases overall glucose utilization [14,15]. Recently, Molthoff et al. [16] studied RT effects on mouse tumors using FDG and FLT tracers. Their results suggested that FLT could be a good indicator for radiation-induced changes in proliferation. A similar conclusion was reached in other comparative studies on irradiated tumor cells *in vitro* and *in vivo* [17].

Multifractionation regimens, commonly used in conventional RT, produce better tumor control for a given level of normal tissue toxicity than a single large dose. A value of being able to measure tumor cell proliferation during or soon after RT could lie in early assessment of accelerated tumor repopulation which can occur during RT, especially if treatment is prolonged. Recognition of this occurrence would allow for treatment adjustment in an attempt to gain better local control.

The use of FLT-PET to monitor early therapy-induced changes in tumor behavior could be problematic if only simple semiquantitative measurements, like standardized uptake value (SUV) [18-21], tumor to background ratio, or injected dose per gram of tumor (%ID/g) [21] taken from a single delayed static image are used. Full characterization of the kinetics of tracer uptake into tissue will be more sensitive and this requires kinetic analysis to model the tissue uptake/clearance of tracer. Also, the imaging parameters will be less variable and provide a better correlate to patient outcome when kinetics is taken into account than those not utilizing the kinetic information [22].

The aim of the current study is to investigate changes in the rate of cell proliferation in a murine tumor model after RT using FLT and microPET and to evaluate the sensitivity of kinetic analysis over semiquantitative measures for monitoring radiation responses. The FLT kinetic findings were supported by radiation studies on *in vitro* FLT uptake by tumor cells and cell cycle analysis.

Materials and Methods

FLT PET image acquisition was performed 1 day after irradiation to single doses of 0, 2.5, 5, 10 to 20 Gy or 4×2.5 Gy. Semiquantitative and quantitative methods were applied to analyze

the acquired PET images. Image-derived *in vivo* results were also compared to *in vitro* FLT uptake study under compatible irradiation regimens.

Radiopharmaceuticals

For the synthesis of FLT, fluoride ion was produced by 11-MeV proton bombardment of 95% ^{18}O -enriched water via $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction. The aqueous [^{18}F]-fluoride ion (~ 18.5 GBq) solution was released from the cyclotron target into a reaction vessel and treated with potassium carbonate and Kryptofix 2.2.2 (Merck). Water was evaporated by azeotropic distillation with acetonitrile. The dried K^{18}F /Kryptofix residue was subsequently reacted with 5'-O-[4,4'-dimethoxytrityl]-2,3'-anhydrothymidine and then hydrolyzed with dilute HCl. The radiochemical yield and purity were 6-12% (after decay correction) and >99%, respectively [23].

Animals and Tumor Models

Six- to 8-week-old $\text{C}_3\text{H}/\text{Sed}/\text{Kam}$ mice ($n=22$) were maintained in a strict defined-flora, pathogen-free environment in the AALAC-accredited Animal Facilities of the Department of Radiation Oncology, UCLA. All local and national guidelines for the care of the animals were adhered to. The syngeneic murine mammary carcinoma cell line MCK [24] was cultured in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics-antimycotic solution. MCK cells were injected subcutaneously as single-cell suspensions in phosphate buffer saline (PBS; 5×10^5 to 10^6 cells in 100 μl) bilaterally in the dorsal shoulder region of the mice. When the diameters of the tumors were approximately 5 mm, the tumor on the right side was irradiated, while the tumor on the left side acted as the control.

Irradiation Protocols

Mice were anesthetized with i.p. injection of ketamine/xylazine and positioned in a Lucite jig with lead shielding the body except for the right tumor area. Radiation was delivered at a dose rate of approximately 67 cGy/min (^{137}Cs source; Atomic Energy of Canada, Ottawa, Canada). For dose response studies, the right tumors were irradiated with 2.5, 5, 10, and 20 Gy (three, five, four, and four studies, respectively). For examination of the effect of dose fractionation, two daily fractions of 2.5 Gy were applied with a 12-h interval for 2 days ($n=4$) to give a total dose of 10 Gy. In a baseline study, two mice were scanned without prior irradiation ($n=2$).

In Vitro FLT Uptake and Cell Cycle Distribution of Tumor Cells after Irradiation

For *in vitro* FLT uptake experiments, cells were incubated with 0.07 MBq/ml of FLT at 37°C for 1 h, washed three times with DMEM, and radioactivity taken up in the cells was measured with a calibrated well counter. To test the effect of irradiation, the same doses as in *in vivo* experiments (see above) were applied 1 day before FLT incubation. For the fractionated experiment, the incubation FLT concentration was at 0.028 MBq/ml. All experiments were done in triplicates (i.e., $n=3$).

For cell cycle analysis, 10^6 cells were trypsinized and collected by centrifugation at 2,000 rpm for 5 min. The cells were then fixed with ice-cold ethanol at -20°C overnight, washed three times with PBS, resuspended in isoton, treated with RNase A (0.5 mg/ml) for 15 min, and stained with propidium iodide (0.05 mg/ml) for 30 min at room temperature. Fluorescence was measured in a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) and cell cycle data were analyzed by ModFit version 3.1 (Verity Software House, Topsham, ME, USA).

MicroPET Imaging

MicroPET scans were performed 1 day after completion of the irradiation, using a microPET® Focus™ 220 tomograph (Siemens Preclinical Solutions, Knoxville, TN, USA). This scanner has a 7.6 cm axial field of view (FOV). Mice were maintained under anesthesia with 2% isoflurane and placed on a heating imaging chamber at 35°C during scanning. A 60-min dynamic scan was performed beginning at the time of tracer injection (8.14 ± 0.74 MBq bolus via tail vein). For image reconstruction, list-mode data were first sorted into three-dimensional (3D) sinograms, followed by Fourier rebinning and two-dimensional (2D) filtered backprojection reconstruction using a ramp filter with a cutoff at the Nyquist frequency. The list-mode data were reframed according to the sequence: 8×15 , 3×60 , 5×300 , 3×600 s. The spatial resolution of the reconstructed images is ~ 1.75 mm at full-width at half-maximum.

Data Analysis

Kinetic Modeling—Images were analyzed with the AMIDE 0.8.8 software [25]. The 3D isocontour regions of interest (ROIs) were manually defined for bilateral tumors in each mouse on 60 min images with the same thresholds applied to both tumors. An elliptical ROI ($2.0 \times 1.5 \times 1.5$ mm) was drawn on the left ventricle (LV) region at the first time frame (1–15 s) and projected to all dynamic images to provide the LV time activity curve (TAC) which was used as the input function for modeling analysis. The tumor ROIs were also copied to the entire dynamic image sequence in order to obtain TACs that represented the kinetics of FLT in tumor tissues. A two-tissue compartment model (Fig. 1) was used to describe the kinetics of FLT in tumor tissue [11,26]. The individual rate constants of the model represent the forward transport of FLT from plasma to tissue (K_1), the reverse transport (k_2), the phosphorylation (k_3), and dephosphorylation (k_4) steps. The values of the rate constants were estimated using the kinetic imaging system (KIS) [27], which is an Internet-based kinetics simulation and model fitting program. All the parameter values were derived from fitting the tissue TAC data using the LV TAC as the input function. The FLT model configuration is similar to the FDG model [27, 28], adapted from the DG model used originally by Sokoloff et al. [29]. It is also consistent with the model proposed by Mankoff et al. [30] for thymidine analogs. The ratio K_1/k_2 representing the concentration ratio of FLT in tissue relative to that in plasma when the phosphorylation rate is small is referred to as the distribution volume of the tracer in tissue. The value of the FLT uptake constant, K_i , was calculated as:

$$K_i = K_1 \times k_3 / (k_2 + k_3).$$

Semiquantitative Analyses—For semiquantitative analyses, ROIs for both tumors were generated for each study as described above. To avoid contribution from the background region and to minimize sensitivity to how the ROI was defined, only voxels with image values above 70% of the highest voxel value within the ROI were included for the calculation of the average ROI value [31]. Several semiquantitative measures were calculated.

1. SUVs at 60 and 10 min for both irradiated and control tumor were calculated as:

$$\text{SUV}_{\text{irrad}_60} = (\text{ROI of irradiated tumor at 60 min}) / (\text{injected FLT dose/body weight})$$

$$\text{SUV}_{\text{irrad}_10} = (\text{ROI of irradiated tumor at 10 min}) / (\text{injected FLT dose/body weight})$$

$$\text{SUV}_{\text{contl}_60} = (\text{ROI of control tumor at 60 min}) / (\text{injected FLT dose/body weight})$$

$$\text{SUV}_{\text{contl}_10} = (\text{ROI of control tumor at 10 min}) / (\text{injected FLT dose/body weight}).$$

2. Normalizing the irradiated tumor activity at 10 and 60 min to the corresponding value of the control tumor:

$$\text{SUVR}_{60} = \text{SUV}_{\text{irrad}_60} / \text{SUV}_{\text{contl}_60}$$

$$\text{SUVR}_{10} = \text{SUV}_{\text{irrad}_10} / \text{SUV}_{\text{contl}_10}$$

3. Ipsilateral normalization using the 60-min ROI value divided by the value at 10 min:

$$\text{SUVN}_{\text{irrad}} = \text{SUV}_{\text{irrad}_60} / \text{SUV}_{\text{irrad}_10}$$

$$\text{SUVN}_{\text{contl}} = \text{SUV}_{\text{contl}_60} / \text{SUV}_{\text{contl}_10}$$

Statistical Analysis—Data for each measure/parameter were grouped according to the irradiation dose level and all were stated as mean±SD. For each dose group, the values of individual kinetic parameters and of semiquantitative measures (except the standard uptake value ratios [SUVRs]) of the irradiated tumor (right side) were compared with those of the control tumor (left side) in the same mouse using a paired *t* test. *p*<0.05 was considered statistically significant. For SUVs, the values of each dose group were tested to see if they are different from 1.0. Values of all the parameters/measures of each dose group were also compared with those of the 0-dose group, using unpaired *t* test. In this set of comparisons, since tumors on both sides of the animal of the 0-dose group could be considered as control, the values on tumors of both sides were pooled together as a single group for statistical tests. Statistical analyses were performed using the statistical tools in Microsoft Excel 2000.

Results

Dose Response Changes

A typical FLT PET image is shown in Fig. 2a. Results of the kinetic and semiquantitative analyses are summarized below.

Kinetic Patterns of Tumor Time Activity Curves—Representative tumor FLT TACs 1 day after 2.5 and 20 Gy doses are shown in Fig. 3. After a bolus injection of FLT, tumor uptake in the control tumor or in tumors receiving 2.5 or 5 Gy radiation rose rapidly within the first 10 min and continued to rise, but more slowly thereafter (Fig. 3a). A distinctly different FLT kinetic pattern was observed for tumors irradiated with 10 Gy or higher. The pattern was characterized by a rapid, more marked increase within 10 min that was not sustained (Fig. 3b).

Semiquantitative Data Analysis—These distinct uptake patterns following irradiation are also seen in values of semiquantitative measures of FLT uptake that are summarized in Table 1. Most measures showed a gradual dose-dependent decrease after irradiation. However, not all reached statistical significance. $\text{SUV}_{\text{irrad}_60}$, $\text{SUV}_{\text{irrad}_10}$, and $\text{SUVN}_{\text{irrad}}$ had significantly lower values than those of the control side (paired *t* test, *p*<0.05) for 5 and 10 Gy doses. For the case of 20 Gy, none of the measures, except SUVR_{60} , reached the significance level. The values of SUVR_{60} were significantly less than 1.0 for 5, 10, and 20 Gy doses. The value of SUVR_{10} was significantly less than 1.0 for 5 Gy dose.

In the comparisons to the 0-dose radiation level, SUVR_{60} showed significant decrease at 10 and 20 Gy and $\text{SUV}_{\text{irrad}_10}$ showed difference at 2.5 Gy. Somewhat surprisingly, $\text{SUV}_{\text{contl}_60}$ and $\text{SUV}_{\text{contl}_10}$ for 5 and/or 10 Gy doses were significantly increased from those of the 0-dose group. SUVNs of the irradiated tumor showed significant decreases only at the 10-Gy level. Excluding those for $\text{SUV}_{\text{contl}}$, all the significant differences in the comparison to the 0-dose group (except $\text{SUV}_{\text{irrad}_60}$ at 20 Gy) were also revealed in the comparison between irradiated and control tumors within one animal. The differences judged significant by the

paired comparison to the control tumor, but were missed by the comparison to the 0-Gy dose, were SUV_{irrad_60} and $SUV_{\text{N}_{\text{irrad}}}$ at the 5-Gy dose level.

Quantitative Data Analysis—Values of the rate constants (K_1 , k_2 , k_3 , and k_4) and K_i (FLT uptake constant) as estimated by model fitting to the tumor TACs in various dose groups are summarized in Table 2. Only values of k_3 and K_i showed a clear gradual trend for dose-dependent changes, but not all values at all dose levels reached statistical significance. The rate constant k_3 , representing the rate limiting step of FLT phosphorylation, was lower in the irradiated than in the control tumor for 5 and 20 Gy doses. The equilibrium distribution volume of FLT (K_1/k_2) was not significantly different between the irradiated and the control sides, except at 20 Gy where it is higher on the irradiated side. Compared to the control side, significant decreases in K_i values for 5 and 20 Gy levels paralleled those of k_3 .

In the unpaired comparison to control 0 Gy values, k_3 and K_i of the irradiated tumor at both 10 and 20 Gy were significantly decreased. The K_i value of the control tumor at 20 Gy was also significantly decreased, suggesting some systemic effect.

Dose Fractionation

Kinetic TACs Patterns—The tumor TACs after exposure to fractionated doses did not have the characteristic pattern associated with TACs of a single dose of 10 Gy or higher, but was similar to those of lower single doses, indicating that the dose effect on the TAC pattern was not cumulative.

Semiquantitative Analysis—Semiquantitative analyses results are shown in Table 1. Among these measures, only SUV_{irrad_60} was found significantly different between the control tumor and the irradiated one (with fractionated regimen of 4×2.5 Gy). This was confirmed by the finding that the value of SUV_{R60} was significantly different from 1.0. However, this result is different from visual inspection of the kinetic TAC pattern (results of the “Kinetic TACs Patterns” section) and from the quantitative analysis below.

Quantitative Analysis—Results of the quantitative analyses are included in Table 2. Though the values of k_3 and K_i tended to be lower in the irradiated tumor than in the control, none of the decreases was statistically significant. This is consistent with the findings of visual inspection of the kinetic TAC pattern that the 4×2.5 Gy doses in the fractionation regimen did not show a cumulative effect. The K_i value for tumors receiving dose fractionation was significantly higher than those in the 10-Gy single dose group ($p < 0.05$), but was not significantly different from the single 2.5-Gy dose group, indicating that the fractionated dose gave irradiation effects equivalent to somewhere between 2.5 and 10 Gy single doses.

In Vitro FLT Uptake

In vitro studies were undertaken to determine if tracer uptake by tumor cells changes following irradiation that might explain *in vivo* imaging observations. The *in vitro* study results also served as a standard to evaluate the qualitative and quantitative measures derived from *in vivo* data.

Dose Response Changes—*In vitro* FLT uptake in MCK cells after irradiation are shown in Table 3. Compared to the 0-Gy controls, FLT uptake was significantly decreased for cells 1 day after a single dose of 10 or 20 Gy. Correlations of *in vitro* FLT uptake with *in vivo* measures/parameters are shown in the last column of Tables 1 and 2 and in Fig. 4. The strongest correlation was with K_i values ($r=0.86$), followed by SUV_{irrad_60} ($r=0.76$), and k_3 ($r=0.74$).

Dose Fractionation—The FLT uptake values in MCK cells after 4×2.5 Gy fractionated doses of radiation delivered b.i. d. are in Table 4. Fractionated doses were again shown to be less effective in suppressing FLT uptake than a single comparable total dose. Compared to the nonirradiated (i.e., 0 Gy) control group, a single dose of 10 Gy decreased FLT uptake by 22% ($p<0.05$), which is consistent with the results shown in Table 3 (indicating good agreement between the two *in vitro* FLT uptake experiments performed at two different times and with different incubation FLT concentrations), while the 4×2.5 Gy regimen only decreased FLT uptake by 5% (the difference is insignificant, $p>0.05$). The lack of significant difference between the *in vitro* fractionated dose and zero dose is also consistent with the *in vivo* kinetic parameters results (Table 2) as well as with the kinetic TAC pattern of the *in vivo* experiments, but is somewhat different from the semiquantitative measures, SUV_{irrad_60} and $SUVR_{60}$, in Table 1.

Cell Cycle Phase Determination—Since the activity of thymidine kinase that is responsible for the phosphorylation of FLT is cell cycle-dependent during normal condition, the effects of irradiation on the cell cycle status of MCK cells were studied using flow cytometry. The results of the fractions of cells in various cell cycle phases are shown in Table 5. A single dose of 10 Gy caused G2/M arrest (37.3% compared to 10.4% in controls). Dose fractionation (4×2.5 Gy) also increased the percentage of cells in G2/M, though to a lesser extent (28.1%). These increases were largely due to decreases in cells in the G1 phase with only a small drop in S-phase cells following single or fractionated doses of radiation (Table 5). The results indicated that the cell cycle was arrested in G2/M after irradiation more so with a single large dose than with fractionated doses (i.e., single 10 Gy is more than 4×2.5 Gy).

Discussion

PET, using FDG, is a routine functional imaging modality in oncology practice. In recent years, several additional tracers have been developed to image different aspects of tumor physiology, including cellular proliferation. After being transported across the cell membrane by a carrier-mediated mechanism [3], FLT undergoes monophosphorylation catalyzed by the cytosolic TK1 enzyme. It implies that TK1 activity ultimately governs FLT activity in tissue measured by PET and this presumption has been supported by *in vitro* experiments in A549 human lung carcinoma cells where FLT uptake by cells and TK1 activity were correlated [32]. Since TK1 activity is expressed in the S-phase and is thought to reflect the rate of cellular proliferation [33,34], imaging this surrogate marker may provide a reasonable measure of tumor proliferation kinetics [8]. The fact that kinetic changes we measured *in vivo* following irradiation correlated well with FLT uptake *in vitro* demonstrates that parameters from PET measurement reflect biological activity in cells rather than simply tissue-related extracellular changes like perfusion. According to our cell cycle studies, G2/M arrest might be involved in decreasing FLT uptake after irradiation. This is consistent with previous findings on radiation-induced G2/M arrest [35]. Although the overall fraction of cells in the S-phase decreased less than as expected, one can still infer that the TK1 activity was suppressed when the cell cycle was arrested or with a prolonged cycling time post irradiation, thus leading to reduced uptake of FLT. We, therefore, believe that changes in the FLT kinetics in tumors provide insight into changes in cell cycle status.

Results of the present study show that tumor FLT kinetics is sensitive to radiation effects of 5 Gy or higher with some discrepancies between comparisons to different control groups, i.e., intramouse and intermouse. For each animal, the irradiated tumor and the control tumor were fed by the same plasma FLT concentration and over the same time course. Thus, variations in the plasma FLT TAC would not influence the intraanimal results. If the irradiated and the control tumors on an animal were of identical size, the partial volume effect (PVE) [36] due to small object size in imaging would also be minimized. However, in practice, it is difficult

to have equal sizes for the irradiated and the control tumors, and the PVE could not be eliminated by the use of the control tumor. The pooling of both tumors of the animal in the 0-Gy group may have artificially increased the statistical power of the comparison to that group because the FLT uptakes in two tumors of an animal are not completely independent. However, using a single tumor of 0 Gy but adding the nonirradiated tumor of the animals with 2.5 Gy to form the control group ($n=5$) of 0 Gy did not change much the significance of the comparison to the control group. Only the values for SUV_{irrad_10} at 2.5 Gy, SUV_{contl_60} at 5 Gy, and SUV_{contl_10} at 5 and 10 Gy in Table 1 became insignificantly different from the nonirradiated group, and the values of K_1 and k_2 (in Table 2) of the irradiated tumor at 20 Gy became significantly different. However, the following discrepancies between the comparison to the control group (interanimal) and the intraanimal control tumor remain unchanged. Comparing against the control tumor in the same mouse, SUV_{irrad_60} , k_3 , and K_i revealed irradiation effects at 5 Gy that were not indicated by comparison to the 0-Gy control mouse (different mouse). Use of the control tumor also revealed the increase in K_1/k_2 due to 20 Gy irradiation, while it failed to detect the effects of 20 Gy by SUV_{irrad_60} and K_i of the control as well as the effect at 10 Gy by the parameters k_3 and K_i . One of the reasons for having missed these effects by intramouse comparison that were indicated by comparison to the 0-Gy control mouse may be systemic effects of irradiation, which can be experienced by tissue that are outside the radiation field, known as abscopal effects [37,38]. It might also explain why the overall effectiveness of radiation was less obvious when compared to the control tumor on the contralateral side. Hence, the combined use of both types of comparison is more revealing than either one alone.

There are also differences between the use of the semiquantitative measures and the use of the modeling kinetic parameters which have been debated in the literature. Both have their advantages and limitations. We calculated the SUV values at 10 and 60 min because, based on the FLT tumor kinetics, the SUV values at those two time points well-characterized the magnitude and shape of the tissue kinetics. The value at 10 min is roughly related to the early uptake of FLT; the value at 60 min is related to the retained amount of FLT. SUVR is calculated to have a built-in comparison to a control tumor in the same animal and is useful only in the preclinical setting. By normalizing the value at 60 min by that at 10 min, SUVN is like an index of the slope of the kinetics between 10 and 60 min. It is less dependent on the magnitude and the PVE, but, like SUV, does not account for across-animal variations in the plasma FLT TAC.

Among the semiquantitative measures, SUV_{irrad_60} or $SUVR_{60}$ were decreased above 5 Gy including in the fractionated regimen. In contrasts, the increases seen in SUV_{irrad_10} (or decrease in $SUVR_{10}$) at 2.5-10 Gy (or 10 Gy) are harder to interpret. At the 5-Gy level, higher SUV_{irrad_10} values imply an irradiation-induced increase in early FLT uptake, yet the decrease in $SUVR_{10}$ implies the opposite. Somewhat surprising is that $SUVN_{\text{irrad}}$ was less sensitive than SUV_{irrad_60} in showing irradiation effects at 20 Gy. This might be due to an increase in variability resulting from taking the ratio of two noisy measurements.

Compared to the semiquantitative measures, the kinetic parameters were more consistent. Based on the FLT model of Fig. 1, the decreases in k_3 at the 5-, 10-, and 20-Gy levels reflect less FLT phosphorylation. Due to the small rate of dephosphorylation (k_4), this decrease in k_3 was propagated to the value of K_i . At 20 Gy, the decrease in the K_i value of the control tumor and the increase in K_1/k_2 of the irradiated tumor were consistent with abscopal effects. The high correlation of K_i with *in vitro* FLT uptake further indicated that the K_i value can be used to quantify radiation effects which has the added advantage that K_i can be estimated as the slope of the Patlak plot (a graphical method) [39] without going through the model fitting procedure. The results of this study showed that, for FLT studies between semiquantitative and kinetic modeling, K_i is probably the best measure in terms of ease and reliability to monitor tumor radiation responses *in vivo*. In this study, K_i demonstrated most accurately dose-dependent change in irradiated tumors.

PVE [36,40] due to tumor size was not corrected in the present study. However, the tumor size was difficult to estimate from microPET images. Also, the tracer uptake in tumor could be quite heterogeneous, especially for largesized tumors with necrotic cores. The PVE probably accounted for a large share of the variability of the different measures obtained in this study. The measures affected directly by PVE include K_1K_i , K_1/k_2 , SUVs, and SUVRs. For SUVNs, if the relative distributions at 10 and 60 min were the same, the measures would not be affected by PVE, but this condition rarely occurred. The spillover of background activity to the tumor kinetics could also affect the estimates of k_2 , k_3 , and k_4 . However, due to the small background level of FLT uptake in normal tissue, the PVE on these parameters are relatively small.

There are other limitations in the current study, including the use of the TAC obtained directly from an ROI defined on the LV chamber region of the early image as the input function. It is technically challenging to take serial blood samples in mouse studies to give the input function. Some technical breakthroughs in mouse blood sampling [41] have emerged recently, but were not widely available when the present study was performed. The use of ROI on the LV chamber on dynamic microPET images to derive the input function has also been demonstrated [42, 43] and is the approach adopted in this study. However, the method has some limitations. Even though the myocardial uptake of FLT is low (see Fig. 2b), the kinetics in the myocardium is different than in the blood. This would give different spillovers from the myocardium at early times versus at later times and could potentially cause the shape of the LV ROI curve to deviate from the true blood curve, thus the input function. In a set of six studies, serial blood samples were taken from the tail artery and compared to the corresponding LV ROI curves. The area under the curves (AUC) were not found to be different ($p>0.05$) between the two sets of curves (after a PV correction of the LV ROI curves with a RC of 0.8). The difference between the plasma and whole blood concentrations of FLT was also reported before to be insignificant [11]. In this study, we did not correct for any labeled metabolites in the blood [11,26] because FLT is metabolically stable *in vivo*. In mice, Barthel et al. demonstrated that parent FLT comprised 96% and 90% of the total radioactivity in plasma and liver samples, respectively [11]. Therefore, although the LV ROI curve was used to approximate the input function in the present study and the estimates of the rate constants in the modeling approach might contain some systematic bias, the effects on the evaluation of radiation response is expected to be relatively small (since all contained similar biases). However, future studies on these issues are warranted.

Ultimately, the all important question will be whether FLT can be used to monitor the impact of dose response and dose fractionation in the clinic. In this study, FLT was shown to be sensitive to radiation effects shortly after irradiation and without the “flare” response of FDG [15]. We further show that coupling the FLT data with the data from the flow cytometry indicate that radiation-induced changes in the K_i value indeed correlate with cell cycle redistribution, thus a change in cell proliferation rate.

For the formulation of individual therapeutic plans, measures of cellular proliferation, in addition to size, location, and extent of the tumor, could further guide therapy and evaluate treatment response early during the course of treatment, but this measure needs to perform with high sensitivity and reliability. Several research groups have investigated different aspects of FLT uptake in tumors and reported promising results. The majority of them have focused on the correlation of FLT uptake and tumor response to chemotherapy or kinase inhibitor agents [11-13]. However, low contrast between tumor uptake and background is one of the major drawbacks in the interpretation of FLT-PET images. We show that kinetic analysis is a potential solution to illuminate the associated biological changes in tumors in response to treatment.

Conclusion

The ability to measure tumor response soon after initiation therapy may provide an early indicator of the treatment efficacy. In the current clinical setting, patients are most often treated with a fixed therapy protocol when the success or failure of treatment is indicated only by the appearance of anatomic changes which may only be detected weeks or months after treatment begins. The results of the present study showed that FLT is a promising imaging marker for the prediction of tumor responses early, namely, 24 h after irradiation. The value of K_i , best correlated with *in vitro* FLT uptake in tumor cells, is expected to be useful as an indicator for early assessment of radiation treatment efficacy that can guide the therapeutic protocol to tailor to individual patient/tumor regarding radiation frequency and radiation doses administered or the timing to add adjuvant therapies.

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Fig. 1.

Compartmental model used to describe the kinetics of FLT in tumor tissue. *Cp* blood concentration of FLT, *Ce* exchangeable FLT concentration in tissue, *Cm* phosphorylated FLTs (mainly FLT-monophosphate) in tissue. ^{18}F -metabolites in blood are negligible and do not enter tumor cells. The rate constants, K_1 , k_2 , k_3 , and k_4 , represent the forward transport of FLT from blood to tissue, the reverse transport, the phosphorylation, and dephosphorylation steps, respectively.

Fig. 2.

a Coronal section PET image of FLT in a mouse with implanted tumor at 60 min post tracer injection was fused with the CT image (in black and white) of the same section. The FLT PET image was displayed in color with the color scale shown on its right side. The animal had implanted tumors bilaterally in the shoulder region. The tumor on the right side (also the right side of the image) was irradiated (10 Gy) 24 h before PET imaging. b Transaxial section of FLT PET across the heart of a mouse at 60 min post FLT injection. The PET image is with the same color scale as in a and is fused with the CT image of the same section. The image shows the low uptake of FLT in the myocardium. The LV ROI that was defined based on early frame PET images and the fused CT image to obtain the LV TAC in this study is also shown on the image.

Fig. 3.

Representative tumor FLT TACs at 1 day after irradiation. a The two TACs are for a 2.5-Gy irradiated tumor and its corresponding control tumor from the same animal. The scaling difference between the two curves is due to the size difference between the irradiated and the control tumors. The similarity in shape of the two curves is representative of TACs with irradiation doses of less than or equal to 5 Gy. b The TAC of the irradiated tumor (20 Gy) was noticeably different from the one in the control tumor of the same animal. This is typical for cases with irradiation doses of 10 Gy or higher.

Fig. 4.

Scatter diagram of the value of K_i (the uptake constant of FLT from *in vivo* PET imaging) versus *in vitro* FLT uptake (in cell line experiments) at various irradiation levels, showing a good correlation ($r=0.89$) between the *in vivo* kinetic parameter and the *in vitro* FLT uptake.

Table 1

Values of semiquantitative measures

Irradiation dose in Gy	0 (n=2)	2.5 (n=3)	5 (n=5)	10 (n=4)	20 (n=4)	4×2.5 (n=4)	Correlation coefficient w/ <i>in vitro</i> FLT uptake (r)
SUV _{irrad_60}	1.607±0.308	1.934±0.552	1.606±0.238*	1.195±0.110*,**	0.900±0.263**	1.852±0.495*	0.76
SUV _{irrad_10}	1.362±0.129	1.563±0.041**	1.472±0.127*	1.440±0.092*	1.012±0.339	1.371±0.268	0.65
SUV _{contl_60}	1.692±0.428	2.689±1.391	2.444±0.199**	1.987±0.391	1.185±0.380	2.721±0.893	0.51
SUV _{contl_10}	1.351±0.112	1.662±0.221	1.676±0.132**	1.646±0.144**	1.001±0.346	1.574±0.034	0.53
SUV _{R50}	0.919±0.006	0.803±0.248	0.656±0.070*,**	0.621±0.140*,**	0.798±0.204*	0.687±0.132*,**	0.16
SUV _{R10}	0.956±0.085	0.949±0.102	0.878±0.029*,**	0.882±0.119	1.032±0.155	0.888±0.104	-0.03
SUV _{N_iirrad}	1.170±0.121	1.232±0.323	1.098±0.187*	0.832±0.082*,**	0.922±0.239	1.328±0.265	0.49
SUV _{N_icontl}	1.223±0.242	1.561±0.647	1.467±0.177	1.199±0.152	1.210±0.215	1.724±0.300	0.38

* $p < 0.05$, paired t test; values are significantly different between the irradiated and the control tumor or SUV_Rs were significantly different from 1.0

** $p < 0.05$, unpaired t test; values are significantly different from the corresponding ones at the 0-Gy dose

Table 2

Values of rate constants obtained with kinetic analysis

Irradiation dose in Gy	0 (n=2)	2.5 (n=3)	5 (n=5)	10 (n=4)	20 (n=4)	4×2.5 (n=4)	Correlation coefficient w/ in vitro FLT uptake (r)
K_1	Irradiated	0.083±0.005	0.247±0.054	0.217±0.055	0.067±0.014	0.244±0.068	0.41
	Control	0.208±0.064	0.253±0.083	0.296±0.162	0.099±0.035	0.211±0.070	0.51
K_2	Irradiated	0.130±0.010	0.342±0.126	0.468±0.275	0.296±0.101	0.426±0.236	0.40
	Control	0.342±0.207	0.342±0.140	0.456±0.261	0.287±0.101	0.315±0.157	0.38
K_3	Irradiated	0.136±0.017	0.042±0.011	0.047±0.012 [*]	0.019±0.009 ^{**}	0.009±0.005 ^{*,**}	0.74
	Control	0.054±0.001	0.059±0.018	0.067±0.015	0.035±0.017	0.109±0.070	0.18
K_4	Irradiated	0.014±0.001	0.019±0.017	0.026±0.010	0.024±0.014	0.034±0.017	0.33
	Control	0.054±0.073	0.019±0.016	0.021±0.014	0.016±0.008	0.030±0.024	0.17
K_1	Irradiated	0.042±0.014	0.028±0.006	0.027±0.006 [*]	0.013±0.005 ^{**}	0.0065±0.003 ^{*,**}	0.89
	Control	0.030±0.008	0.038±0.007	0.037±0.008	0.022±0.008	0.052±0.026	0.61
K_1/k_2	Irradiated	0.641±0.05	0.77±0.16	0.65±0.08	0.76±0.11	0.632±0.170 [*]	-0.53
	Control	0.676±0.22	0.67±0.12	0.66±0.11	0.74±0.11	0.725±0.172	0.32

* $p<0.05$, paired t test; values are significantly different between the irradiated and the control

** $p<0.05$, unpaired t test; values are significantly different from the corresponding ones at the 0-Gy dose

Table 3

In vitro FLT uptake at various irradiation levels

Irradiation dose (Gy)	0 (n=3)	2.5 (n=3)	5 (n=3)	10 (n=3)	20 (n=3)
<i>In vitro</i> FLT uptake	68,945±3,385	65,737±1,516	61,556±3,794	53,493±1,669 [*]	42,674±2,686 [*]

^{*} *p*=0.05; value is significantly different from that of zero irradiation

Table 4

In vitro FLT uptake for fractionated irradiation

Irradiation dose (Gy)	0 (n=3)	2.5 (n=3)	10 (n=3)	4x2.5 (n=3)
<i>In vitro</i> FLT uptake	27,428±2,257	26,295±606	21,558±2,208*	26,244±1,549

* $p=0.05$; value is significantly different from that of zero irradiation

Table 5

Percent cells in different cell cycles after irradiation

Cell cycle phase	Control	Single dose (10 Gy)	Dose fractionation (4×2.5 Gy)
G2/M	10.42	37.25	28.11
G1	75.8	53.13	61.09
S	13.78	9.62	10.08