Published in final edited form as:

Eur J Nucl Med Mol Imaging. 2010 November ; 37(11): 2048–2059. doi:10.1007/s00259-010-1517-y.

Biodistribution and dosimetry of ^{18}F -EF5 in cancer patients with preliminary comparison of ^{18}F -EF5 uptake versus EF5 binding in human glioblastoma

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Abstract

Purpose—The primary purpose of this study was to assess the biodistribution and radiation dose resulting from administration of ^{18}F -EF5, a lipophilic 2-nitroimidazole hypoxia marker in ten cancer

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Conflicts of interest EF5 and its labeled counterpart (^{18}F -EF5) are patented (CJK and AVK and others not part of this manuscript, co-inventors) with patents owned by several universities. These patents have been licensed to Varian Biosynergy but this work was entirely supported by grants as indicated in the acknowledgement.

patients. For three of these patients (with glioblastoma) unlabeled EF5 was additionally administered to allow the comparative assessment of ^{18}F -EF5 tumor uptake with EF5 binding, the latter measured in tumor biopsies by fluorescent anti-EF5 monoclonal antibodies.

Methods— ^{18}F -EF5 was synthesized by electrophilic addition of $^{18}\text{F}_2$ gas, made by deuteron bombardment of a neon/fluorine mixture in a high-pressure gas target, to an allyl precursor in trifluoroacetic acid at 0° then purified and administered by intravenous bolus. Three whole-body images were collected for each of ten patients using an Allegro (Philips) scanner. Gamma counts were determined in blood, drawn during each image, and urine, pooled as a single sample. PET images were analyzed to determine radiotracer uptake in several tissues and the resulting radiation dose calculated using OLINDA software and standard phantom. For three patients, 21 mg/kg unlabeled EF5 was administered after the PET scans, and tissue samples obtained the next day at surgery to determine EF5 binding using immunohistochemistry techniques (IHC).

Results—EF5 distributes evenly throughout soft tissue within minutes of injection. Its concentration in blood over the typical time frame of the study (~ 3.5 h) was nearly constant, consistent with a previously determined EF5 plasma half-life of ~ 13 h. Elimination was primarily via urine and bile. Radiation exposure from labeled EF5 is similar to other ^{18}F -labeled imaging agents (e.g., FDG and FMISO). In a de novo glioblastoma multiforme patient, focal uptake of ^{18}F -EF5 was confirmed by IHC.

Conclusion—These results confirm predictions of biodistribution and safety based on EF5's characteristics (high biological stability, high lipophilicity). EF5 is a novel hypoxia marker with unique pharmacological characteristics allowing both noninvasive and invasive measurements.

Keywords

Hypoxia; PET; ^{18}F -EF5; Molecular imaging; Brain; Glioma; EF5; Immunohistochemistry

Introduction

The importance of hypoxia in tumors has been persuasively demonstrated to change their biological and molecular characteristics and their response to several treatment modalities (reviewed in [1–4]). Hypoxic tumor cells are particularly refractory to radiation therapy due to lack of oxidation of radiation-induced free radicals on critical target molecules such as DNA [5]. Since hypoxia is known to vary widely between human tumors with otherwise similar characteristics [6–9], its accurate measurement plays a critical role in the optimization of individualized treatment using anti-hypoxia therapies.

Several noninvasive methods have been proposed to image hypoxia: magnetic resonance imaging (MRI), phosphorescence quenching, various measures of blood flow and oxygenation, glucose uptake, etc. However, the ones most closely linked to actual tissue $p\text{O}_2$ (rather than vascular oxygen supply) include isotopically labeled 2-nitroimidazoles and metal chelates, as detected by positron emission tomography (PET) or single photon emission computed tomography (SPECT). Cu(II)-diacetyl-bis(N^4 -methylthiosemicarbazone) (Cu-ATSM) exemplifies the most promising of the metal chelates, and clinical studies with this compound have shown a correlation with tumor grade and early response in cancer of the uterine cervix [10]. ^{18}F -labeled fluoromisonidazole [FMISO; 1-(2-nitro-1- H -imidazol-1-yl)-3-fluoro-2-propan-2-ol] is the prototype for drugs of the 2-nitroimidazole class [11] and has been studied extensively in animals and humans [12]. FMISO uptake has also shown predictive value for patient outcome [13,14], and a recent study in head and neck cancer has demonstrated both predictive and prognostic capability [15].

Ballinger has reviewed several perceived problems with hypoxia imaging drugs [16], and these problems have spawned the development of several new agents (see [12] for review) including a separate group of nucleoside-conjugated 2-nitroimidazoles labeled with various isotopes of iodine [17]. More recently a fluorinated analog (FAZA) has been tested in both animal and human studies [18,19]. One reason for this continuing development is the relatively poor correlation statistic (r^2) for response prediction in clinical studies. Thus, although the significance (p value < 0.05) of correlation for outcome versus drug uptake has been established in some studies, scatter in the data would often prevent optimization of a specific treatment for an individual patient. This problem is amplified in preclinical studies since there is only one study in an animal model exemplifying the therapeutic goal of predicting response in individual animal tumors [20]. The scarcity of such data may be associated with the relatively uniform characteristics and responses of most rodent tumor models. Alternatively, factors related to drug characteristics that are as yet poorly understood may be involved.

In light of these and other considerations, 2-(2-nitro-1H-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5) was specifically designed as a hypoxia marker, its structure incorporating several favorable characteristics derived in biochemical and pharmacological studies [21]. EF5's structure was based upon the known biological stability of etanidazole [22] coupled with the strength of C-F bonds that were added to a modified side chain to allow various detection modalities [23]. EF5 is relatively lipophilic (octanol-water partition coefficient 5.7¹ versus 0.4 for FMISO), whereas almost all other hypoxia markers under current development are more hydrophilic than FMISO. The rationale behind our reversal in drug development was that it would be better to emphasize uniform biodistribution (a characteristic of moderately lipophilic drugs) rather than rapid renal elimination (a characteristic of hydrophilic drugs) [24]. Neurotoxicity of lipophilic 2-nitroimidazoles [25], a possible side effect of use at high, therapeutic drug concentrations (e.g., hypoxic cell radio-sensitization or killing), has not been found at the relatively low doses used for immunohistochemical (IHC) analysis (21 mg/kg) and therefore would certainly not be a factor for labeled drug (1,000-fold lower concentrations) [21].

EF5 was first used as an imaging agent for tissue-based assays, using antibodies to detect drug adducts bound to cellular macromolecules [26] in biopsy or whole tumor specimens. These antibodies were the first monoclonals produced for such a purpose and they were conjugated to Cy3 or Cy5 allowing a single antibody detection system. The "Cy dyes" (carboxymethylindocyanine) have very stable characteristics as described by Southwick et al. [27]. The resulting biopsy-based IHC assay system has undergone human clinical trials in several disease sites and has shown correlation with outcome in brain tumors, sarcomas, and head and neck cancer [28–30].

The PET version of EF5 is the identical drug but with one of its five fluorines made radioactive (¹⁸F-EF5) and its synthesis was first demonstrated in 2001 [31–33]. The first clinical data for ¹⁸F-EF5 have been reported by Komar et al. in head and neck cancer [34]. In this report we assess the biodistribution and radiation dose associated with use of ¹⁸F-EF5 in humans. Our results suggest an extremely rapid and even biodistribution, confirming our expectations for a stable, lipophilic drug. An initial use for patients with glioblastoma indicates EF5's ability to equilibrate in normal brain and to bind to the hypoxic portions of brain tumors, as confirmed by a biopsy-based IHC assay.

¹EF5's partition coefficient was originally determined to be 4 by outside contractors and this value was reported in an initial pharmacology paper [21]. The correct value is 5.7 (at room temperature) and ~6.25 at 37°.

Materials and methods

Patients

The Institutional Review Board (University of Pennsylvania Clinical Trials) and Scientific Monitoring Committee (University of Pennsylvania Abramson Cancer Center) and the Cancer Therapeutics Evaluation Program (National Cancer Institute) approved this study. All patients signed an approved consent form and privacy form (Health Insurance Portability and Accountability Act) for the study. Patients of all ethnic and gender groups were eligible for accrual. Two subgroups of patients were included: (1) for ^{18}F -EF5 biodistribution only ($n=7$) a diagnosis of cancer was required but active disease did not have to be present and (2) for the PET/IHC comparison ($n=3$) radiographic evidence of a brain mass that was likely to be a tumor was required. For all patients, ^{18}F -EF5 was administered for PET scanning and blood and urine analysis. For the PET/IHC group, nonradioactive EF5 was administered after the PET scans, and on the following day surgery was performed and tissue collected. When possible, the specific location of tissue collection was noted so that it could be compared with the PET image. These three patients also had a gadolinium-enhanced T1 MRI image prior to surgery.

^{18}F -EF5 synthesis and administration

Synthesis of ^{18}F -EF5 was performed as previously published [31]. Each subject was administered an intravenous bolus of 90–280 MBq of ^{18}F -EF5 followed immediately by PET imaging.

PET imaging acquisition and analysis

Whole-body biodistribution PET studies were performed on an Allegro PET scanner (Philips Medical Systems). This instrument consists of a gadolinium oxyorthosilicate (GSO) crystal-based PET camera with an internal ^{137}Cs transmission source. It has an axial and transverse field of view of 18 and 57.6 cm, respectively. The corresponding resolutions are 5.6 mm axial and 5.5 mm transverse [35]. Each patient received three serial PET scans, with the first scan beginning within 15 min of radiopharmaceutical injection. Patients were scanned with their arms at their sides and the axial extent of the scan covered the entire body. The emission scans were acquired for between 60 and 110 s per bed position and took between 20 and 40 min. A standard transmission scan was acquired after the emission scan, taking approximately 20 min. After each transmission scan, the patient was required to get up from the imaging table to void and was then repositioned and the next serial PET scan started. Blood was collected at each whole-body image at a time chosen with respect to patient convenience. The total time to completion of study was approximately 210 min. The standard Philips 3-D row action maximum likelihood algorithm (RAMLA) for image reconstruction was used. It included corrections for radioactive decay, attenuation, scatter, detector nonuniformities, and detector dead time.

PET dosimetry analysis

Volumes of interest (VOIs) were drawn around nine organs (brain, heart, lungs, liver, gallbladder, kidneys, muscle, marrow, and urinary bladder) in the axial planes of all three PET image data sets. In the three patients with brain masses, analyses of the uptake of the tumor and adjacent normal tissue were performed (see below). The reconstructed counts were recorded for each VOI which were then multiplied by an acquisition-specific calibration factor to obtain the activity in each VOI. The muscle and marrow VOI were drawn in the quadriceps and femur, respectively, and were extrapolated to estimate uptake in the entire organ. Any activity unaccounted for by the VOI estimates was assumed to be uniformly distributed throughout the rest of the body. Urinary excretion (see the “Results” section) was not used

directly for calculation of overall dosimetry. Instead, a voiding bladder model was used for this purpose.

Time-activity curves were generated for the VOI data in the nine organs of interest and mono-exponential functions were iteratively fit to the curves using a least-squares regression algorithm. The curves were then numerically integrated to infinity to determine the number of disintegrations in the respective organs. The number of disintegrations in the urinary bladder was estimated using the voiding bladder model in the OLINDA/EXM software package (Vanderbilt University, Nashville, TN, USA) [36,37]. The biological half-life for the voiding bladder model was determined from the whole-body time-activity curves; the voiding interval employed was 2.4 h. Renal excretion was assumed to account for 100% of the total body excretion. Gallbladder activity was estimated from the VOIs and was assumed to decay fully in the gallbladder.

For each patient, the OLINDA software package was used to estimate the absorbed radiation doses in 25 organs, using standard male and standard female phantoms. The effective dose equivalent (EDE) and effective dose (ED) were calculated according to current definitions. Then, the EDE and the ED were averaged for all ten patients.

Assessment of blood and urine

Blood, plasma, and urine were diluted 1+1 with iced 10% trichloroacetic acid (TCA) using a Gilson Pipetman with polypropylene tips. Volumes were verified by mass using densities for blood, plasma, urine, and 10% TCA of 1.06, 1.03, 1.00, and 1.05, respectively. Acidified samples were left on ice for 10 min, then centrifuged at 10,000 g. Clear supernatants were analyzed by liquid scintillation counter (LSC) and high-performance liquid chromatography (HPLC). As reported previously there was no EF5 binding to the blood or plasma precipitates [21], but serum binding of ^{18}F -EF5 under physiological conditions was not directly assessed. The counting efficiency for the LSC was determined to be 85%. HPLC was performed on JASCO equipment with isocratic mobile phase of 40% methanol in 0.1 M ammonium acetate (pH 4.5). The column was an Alltech Alltima with detection by serial UV and radioactivity (IN/US BetaRAM II) [21]. Values from the LSC were decay-corrected and calculated as MBq/l. To correct for the different patient masses and injected doses, blood activity per cc was normalized by the patient mass to provide standard uptake values (SUV_{mass}) and plasma was assessed per the volume of distribution (V_d) also normalized by patient mass (l/kg).

Image interpretation (brain masses)

The ^{18}F -EF5 PET brain images were evaluated subjectively by an experienced nuclear medicine physician (CD) in correlation with the MRI images in three patients. In addition, regions of interest were drawn in multiple planes on the summed images guided by gadolinium-enhanced T1 MRI images to create VOIs. Data collected included the tumor volume and SUV (mean and SD) for tumor, normal brain, and muscle.

Unlabeled EF5 administration

The National Cancer Institute, Division of Cancer Treatment supplied sterile vials of EF5 (3 mg/ml) dissolved in an aqueous solution of 5% dextrose and 2.4% ethanol. This EF5 solution was administered as previously described [38]. Blood samples for EF5 levels were obtained 1 h after the completion of drug administration and at the time of surgery for calculation of drug exposure (area under the curve, AUC) [21].

Immunohistochemical analysis of EF5—The methods for preparation of tissues for IHC, quantitative analysis of EF5 binding, and conversion into oxygen maps have been previously described [29,30,38,39].

Patient follow-up—The three patients who received both ^{18}F -EF5 and unlabeled EF5 were followed for recurrence and survival by their neurosurgeon (KJ) at 3 months and then 6-month intervals. Times to progression and death were based on the date of EF5 administration.

Results

Biodistribution and dose estimates

Patient characteristics are documented in Table 1. As indicated in the “Materials and methods” section, only the first three subjects underwent surgical resection for brain masses at the time of the study. For all patients, the fractional urinary excretion averaged $13\pm 4\%$ over the roughly 200-min time frame of each study. Blood SUVs, calculated by gamma count, averaged 0.72 ± 0.21 . Importantly, the SUVs for the initial blood sample were essentially in equilibrium with whole-body distribution at all times sampled (8–230 min) except for a single sample collected 2 min following injection (Fig. 1). The average volume of distribution for labeled EF5 was 0.76 ± 0.16 l/kg. Minor changes to blood SUV or V_d over the time frame of the study were consistent with urinary excretion (see Fig. 1). HPLC analysis of blood values indicated that virtually all circulating radioactive drug was authentic EF5, but a significant fraction of more polar/hydrophilic metabolites was observed in urine (Fig. 2, see the “Discussion” section).

Radiation doses for 24 organs plus whole body were calculated (Table 2). The male phantom and the source organ radiation doses were used because the estimated absorbed doses were the largest. Only the urinary bladder was estimated to receive more than 0.050 mGy/MBq (0.19 rad/mCi); its dose was estimated to be 0.17 mGy/MBq (0.62 rad/mCi). The average values (adult man) for the EDE and ED were 0.029 mSv/MBq (0.107 rem/mCi) and 0.023 mSv/MBq (0.086 rem/mCi), respectively (Table 2). It was clear from the time dependence of drug equilibration that brain had the overall slowest rate of approach to equilibrium concentration. Thus, for the two patients who were imaged in the direction head to foot (patients 4 and 8) the temporal variation in SUVs were plotted for several organs (brain, muscle, heart, liver and bladder, Fig. 3). As with the blood SUV data, soft tissues had nearly constant drug exposure over the total imaging time.

PET and immunohistochemical analyses of hypoxia in brain masses

Three of the ten patients studied had brain masses, two with newly diagnosed glioblastoma multiforme (GBM) and one with previously treated esophageal carcinoma with a solitary brain metastasis (Table 1). Tissue specimens were collected by the surgeon (KJ) guided by the ^{18}F -EF5 images when possible. In patient 1, the tumor was removed en bloc and was oriented and labeled in the operating room from four sites identified by sutures (dorsal, cranioventral, caudal and ventricle-adjacent). In pathology, the tissue adjacent to the sutures used for identifying these sites was resected and saved for EF5 binding analysis. The lesion in patient 2 was grossly necrotic and was removed in fragments. In patient 3, the surgeon was able to resect the superficial portion of the tumor en bloc and orient it for specimen collection as described for patient 1, but the deep portion was removed in small fragments. The demographics, tumor pathological analysis, PET findings, EF5 IHC findings, and outcome for these three patients are shown in Table 3.

EF5 uptake and binding in glioma patients

Whole-body PET and combined MRI, PET, and IHC data for patient 1 are illustrated in Figs. 4 and 5. The whole-body PET images reinforce the extreme uniformity of biodistribution for this agent (Fig. 4). Although the whole-body scanner was not optimal for brain scans, it is readily apparent that the regions of highest ^{18}F -EF5 uptake did not coincide with the entire tumor, as delineated by the MRI image (Fig. 5). There was no increasing PET signal in the rostral region of the tumor and IHC examination of this tissue revealed very low EF5 binding.

Using the terminology defined previously, the CF95 (95% of pixels less than or equal to this value) indicated physiologically oxic tumor tissue [29,40]. Conversely, the caudal region of the tumor was ^{18}F -EF5 positive and biopsy from this region showed very high EF5 binding, indicating severe hypoxia.

The MRI image for patient 2 had indicated a change that could have been interpreted as tumor recurrence. There was no ^{18}F -EF5 uptake observed in the PET scan and no EF5 binding in the tissue. At surgery, pathological examination of the tissue exclusively indicated necrosis (data not shown).

In the tissues removed from the second patient with glioblastoma (patient 3), high EF5 binding confirmed the presence of ^{18}F -EF5 uptake in the PET scan. The spatial variations in the PET image and difficulty in removing the tumor as a single piece prevented the specific identification of low-binding versus high-binding regions based on the PET image (data not shown).

Discussion

The data presented herein show that the radiation exposure from ^{18}F -EF5 is clinically acceptable. Several advantageous properties of this drug include: (1) that EF5's biodistribution is extremely rapid and uniform, (2) that EF5 is highly stable in humans (consistent with earlier animal studies), and (3) that uptake of ^{18}F -EF5 (measured by PET) can identify regions of high EF5 binding (determined by IHC analysis).

- 1 Property 1 can be exploited to determine tissue perfusion deficits. Subnormal perfusion causes poor therapeutic drug access and can itself be associated with hypoxia [40]. Thus, if a single imaging agent could monitor two important tissue properties (perfusion and hypoxia) a substantial saving in imaging costs and patient inconvenience could be achieved. Several prior reports have suggested the possibility that noninvasive hypoxia imaging agents could be used to monitor tissue perfusion (reviewed in [12]). However, the limited time-activity data available in the literature would suggest that hydrophilic drugs have complex equilibration characteristics even in normal tissue [14,41]. This, coupled with a more rapid excretion, causes a highly variable background of circulating activity that often changes manyfold during the typical imaging time employed [42]. Since metabolism is directly related to drug concentration, such changes can make interpretation of uptake very complex. Based on the blood and other organ SUVs reported herein, EF5's biodistribution contrasts sharply with that for hydrophilic drugs. In fact, a reasonable model for EF5's biodistribution is that it takes place uniformly within minutes of injection and then remains constant except for the small fraction of urinary and biliary excretion. This behavior is unique for imaging agents and is supported by some limited time-activity data reported by Komar and colleagues who used ^{18}F -EF5 to image patients with head and neck cancer—see Fig. 2 of reference [34].

The uniform distribution of ^{18}F -EF5 in normal brain (Fig. 3) contrasts sharply with imaging agents like FAZA that are much more hydrophilic [43]. Although this reference showed an example of FAZA uptake in a GBM tumor, the magnitude of the uptake was similar to that in the scalp, and biodistribution of this drug was extremely low in normal brain tissue. Many hydrophilic compounds can access brain tumors only if the blood-brain barrier is compromised [24]. Thus, tumor uptake will depend on variables in addition to the level of oxygen and such complexity makes inter-patient comparisons problematic.

- 2 With respect to property 2 (see above) EF5's stability in patients is indicated by the dominant circulating form, namely unaltered drug (Fig. 2). This confirms our former

data in rat and mouse models as well as the results reported by Komar et al. [32, 34]. It is important to note that the signal to noise ratio of the HPLC data is too small to detect minor metabolites. Indeed, since EF5 has formerly been shown to be completely stable in blood and urine [21], the readily visible metabolites found in urine (Fig. 2) are likely concentrated there from other tissues by the blood through normal kidney function. The distribution of these metabolites is observed in several minor HPLC peaks more polar/hydrophilic than the parent drug. These peaks are found at similar positions to those found using model bioreduction systems [44] and can be identified as having no UV absorbance at 325 nm, the characteristic absorption maximum for 2-nitroimidazole compounds. Unfortunately, the concentrations found herein (micromolar range) are too low to confirm the lack of absorbance at 325 nm due to a high background of other UV-absorbing, acid-soluble urinary contents (data not shown). If, as we have claimed, EF5 metabolism is limited to hypoxia-dependent bioreduction, a reasonable question to be asked is why there should be any EF5 metabolites in urine? The answer to this question involves our former demonstration that metabolism of EF5 can be measured at all oxygen levels throughout the physiological and pathological range [45]. Although the rate of this metabolism decreases by more than 100-fold as the pO_2 rises from 0 to 100 mmHg, there should be a minor rate of bioreductive metabolism by all tissues and particularly by normal tissues with more hypoxic components (e.g., liver, gut, skin; [46]). This should be true for any 2-nitroimidazole, but the expected low rates of bioreduction in relatively oxidic tissue are often overshadowed by nonspecific metabolism [45,47]. Thus, confirmation in humans of EF5's anticipated properties [48] will allow specific tests of detection strategies to be made. Foremost among these has been our suggestion that even small changes in drug uptake might be measurable if the background of unmetabolized drug remains nearly constant, as has been found here [48].

A largely untested factor that could affect the biodistribution and hence metabolism of imaging agents is that of drug concentration. At very low drug concentrations, variations in noncovalent binding by serum or other tissues could in principle dramatically change drug access to target tissues. Since EF5 was originally developed as a hypoxia marker with detection of bound adducts using quantitative IHC, earlier studies have employed drug administration at a level of 21 mg/kg. The present studies employ a roughly 1,000-fold smaller drug amount for labeled EF5. Despite this substantial decrease, the volume of distribution determined herein was very close to that determined at the higher drug concentration (0.76 ± 0.16 versus 0.63 ± 0.03 l/kg). Similarly, the drug half-life (visualized in the blood SUV plots, Fig. 1) is consistent with the formerly determined average of 13.2 ± 6.4 h although it was not possible to accurately determine this value for the short blood collection times used in the present study [21]. Data will soon be available for another 100-fold reduction in drug concentration since the synthesis method developed in Turku by Solin and colleagues achieves much higher specific activity $^{18}F_2$ gas [49]. We are additionally planning studies that would allow the administration of unlabeled EF5 before the PET imaging studies (rather than the reverse used for patients 1–3 herein). A recent study demonstrating the possible importance of drug concentration is provided by EF5's sister drug, EF3. In rodents, we have found these compounds to act nearly identically at the relatively high concentrations (~ 100 μM whole-body) used for IHC. However, a recent pharmacokinetic study in humans, using ^{18}F -EF3-specific activities roughly 200,000-fold higher than employed here, indicated dramatic changes (roughly fivefold) in blood activity between 6 min and 1 h [50]. This change was quite similar to the changes observed for FMISO, also performed at high specific activity [47]. This is an area that requires careful future consideration.

The EDE and ED are measures of the stochastic risk associated with exposure to low levels of ionizing radiation. Biodistribution data of ^{18}F -EF5 obtained herein demonstrate that the EDE is 0.029 mSv/MBq (0.107 rem/mCi) which is approximately equal to that of the current estimate for ^{18}F -FDG (0.030 mSv/MBq or 0.11 rem/mCi). Calculated values for the individual organ dosimetry are also comparable to FDG, indicating that EF5 can safely be used for routine clinical studies where it may be diagnostically valuable. In order to provide the most conservative estimates, the dose calculations were made using the assumption that no activity was excreted. This assumption produces only a small positive error in estimated dose, due to the low actual rate of drug excretion. Although EF5's observed bladder values are high with respect to other tissues, they do not saturate the scanner as can more rapidly excreted drugs. This should allow uptake to be assessed in organs close to the bladder (e.g., cervix, prostate, gut). A new finding not detected in the former pharmacokinetic studies was the hepatobiliary excretion—obvious from the whole-body images. This causes a slight underestimate of small bowel absorbed dose but should not significantly affect the overall dosimetry since the gallbladder activity represents <1% of the injected dose.

The dosimetry data were obtained from three serial scans obtained over approximately 210 min that allow data collection for all tissues. Because of the very rapid biodistribution kinetics of EF5, it will be clearly beneficial to obtain future information for individual organs using dynamic scanning methods. This would allow detailed comparisons with other perfusion markers.

Although there were only three patients with comparison between ^{18}F -EF5 images and EF5 immunohistochemistry, some preliminary observations can be made. In both de novo GBMs, high regions of both ^{18}F -EF5 uptake (PET) and EF5 binding (IHC) were observed. Although we do not have an independent assessment of hypoxia for these patients, the only variable known to increase EF5 binding is hypoxia [23]. The present data support our previous study showing the existence of substantial EF5 binding in high-grade glial tumors [40].

In contrast, the patient with radionecrosis had minimal PET and IHC signal, consistent with the requirement of viable tissue for drug reduction. If confirmed, this type of information could be used to minimize the need for surgery in such situations. Noninvasive assays for necrosis and/or cell death represent an active area of investigation [51,52].

The understanding that tumor hypoxia is important to patient outcome has been known for decades and all recent studies point to the need for identification of the substantial variations in hypoxia that occur between otherwise similar patients. Similarly, recent advances in spatially variant therapy (e.g., intensity-modulated radiotherapy, proton and heavy ion therapy) can be combined with imaging data to intensify therapy to the most resistant tumor subvolumes. Our data and those from other groups have also shown that tumor hypoxia may be related to activation of signaling pathways that lead to lowered radiation sensitivity [53]. Drugs that target these pathways are currently being tested in the clinic. Hypoxia imaging could prove useful as a means to select patients for such therapies and to assess early response to such interventions.

Currently, PET hypoxia imaging is dominated by either ^{18}F -labeled 2-nitroimidazole compounds or copper-labeled (^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu) ATSM (for recent reviews see [12]). There are now several clinical trials that have established value in noninvasive hypoxia-based imaging in order to estimate patient outcome [14,15,42]. Although in this report we have established several possible advantageous properties of EF5, the value of our approach, emphasizing uniform biodistribution and a nearly time-independent background of radioactivity, remains to be proven in direct comparative trials. Certainly, the high lipophilicity of EF5 will assist in the assessment of brain lesions and its rapid distribution should allow an unambiguous simultaneous assessment of perfusion in any tissue. On the negative side, it must

also be mentioned that synthesis of ^{18}F -EF5 by fluorine gas is somewhat more challenging [31,49,54] than the more commonly employed nucleophilic displacement reactions used for labeling FDG and several other compounds. We anticipate that the simple analysis afforded by EF5's pharmacokinetic and metabolic properties will more than make up for the additional synthesis effort. Additionally, EF5 is the only current hypoxia imaging agent that allows independent determination of tissue hypoxia on the much finer spatial scale afforded by IHC analysis.

Acknowledgments

This work was supported by grants from the NIH/NCI RO1-75284, RO1-87645

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Fig. 1.

Blood SUVs calculated from gamma counts: the three individual values for eight of the ten patients are plotted with a common symbol (+). The *dotted line* represents the expected change associated with a 13-h plasma half-life (determined in [21]). Individual values from two patients are shown: the *solid circles* represent the sample containing the earliest time point for blood collection after drug injection (2 min) and the *solid triangles* indicate data from the patient with highest urinary drug excretion (20%)

Fig. 2.

HPLC tracing of three serial blood samples, a standard (visible only on the UV channel, not shown) and urine sample from patient 1. The main urine peak (3,600 s) is actually well off scale (max. 310). Urine metabolites are observed in the range 3,200–3,400 s. The main peaks (650, 1,400, 2,150 and 3,600 s) coincide precisely with the retention of authentic EF5, determined by spiking individual samples with authentic EF5 (data not shown)

Fig. 3. Individual organ SUVs plotted versus time for patients imaged in head to toe orientation (patients 4 and 8). Symbols correspond to brain (*closed squares*), muscle (*open circles*), heart (*closed triangles*), liver (*open diamonds*), and bladder (+)

Fig. 4. Whole-body PET images for patient 1 at average times of 45 and 165 min. The brain lesion's SUVs increase with time. Excretion is predominantly via kidney/bladder with a smaller component via gallbladder/bowel

Fig. 5. Gadolinium-enhanced MRI image of tumor from patient 1 (*upper left*) and color-coded PET image (longest time, *lower left*) from patient 1. The rostral portion of the tumor (*red arrow*) has little ^{18}F -EF5 uptake and tissue obtained from this region has no EF5 binding, determined by IHC (*upper right*). Conversely, the caudal portion of the tumor (*green arrow*) has high ^{18}F -EF5 uptake and tissue obtained from this region has high EF5 binding, determined by IHC (*lower right*)

Table 1
Patient characteristics and, from gamma counts, urinary excretion and blood SUV

Pt #	Study	A/S/R/M	Tumor	Clinical status	% Ex (urine)	SUV ^a (Bld)
1	BD & IHC	63/M/CC/73.6	GBM	ND	13.5	0.61
2	BD & IHC	63/M/CC/95.9	EsoMet	PT/Rec	13.4	0.91
3	BD & IHC	65/M/CC/96.4	GBM	ND	15.8	0.69
4	BD	61/M/CC/87.0	NSCLC	PT/RS	5.1	0.80 ^b
5	BD	44/F/CC/70.5	NSCLC	ND	20.4	0.54
6	BD	48/F/CC/74.5	mmM/E/U	ND	16.2	0.62
7	BD	64/M/AA/81.8	NSCLC	ND	11.3	0.73
8	BD	59/F/AA/80.4	ODG	OT	14.4	0.74
9	BD	58/M/CC/78.2	UP	PT	11.3	0.49
10	BD	62/F/CC/53.2	NSCLC	PT	12.9	1.23

A/S/R/M age/sex/race (CC Caucasian, AA African American)/Patient Mass (Kg), % Ex % excretion, Bld blood, BD biodistribution, IHC immunohistochemistry, GBM glioblastoma multiforme, EsoMet esophageal metastasis, NSCLC non-small cell lung cancer, mM/E/U malignant mixed Mullerian cancer (endometrium/uterus), ODG oligodendroglioma, UP unknown primary, ND newly diagnosed, PT/Rec prior therapy/recurrence, PT/RS prior therapy, restaged, PT/OT prior therapy/off treatment

^a Average of three samples (1 per scan)

^b This SUV should be closer to 0.6 since first time point taken at only 2 min post-injection

Table 2

Average absorbed dose estimates for all ten subjects

Target organ	Mean (mSv/MBq)	SD
Adrenals	1.4E-02	3.0E-03
Brain	1.2E-02	2.1E-03
Breasts	8.8E-03	1.1E-03
Gallbladder wall	5.5E-02	3.5E-02
LLI wall	1.7E-02	2.5E-03
Small intestine	1.5E-02	2.2E-03
Stomach wall	1.3E-02	1.4E-03
ULI wall	1.5E-02	1.9E-03
Heart wall	2.2E-02	2.9E-03
Kidneys	2.5E-02	3.8E-03
Liver	2.3E-02	2.9E-03
Lungs	1.7E-02	2.6E-03
Muscle	1.2E-02	2.2E-03
Ovaries	1.7E-02	2.5E-03
Pancreas	1.4E-02	1.9E-03
Red marrow	2.2E-02	3.2E-02
Osteogenic cells	2.1E-02	1.8E-02
Skin	8.0E-03	9.0E-04
Spleen	1.2E-02	1.5E-03
Testes	1.3E-02	1.0E-03
Thymus	1.1E-02	1.3E-03
Thyroid	1.1E-02	1.3E-03
Urinary bladder wall	1.7E-01	1.4E-03
Uterus	2.3E-02	1.9E-03
Total body	1.4E-02	2.3E-03
EDE	2.9E-02	5.6E-03
ED	2.3E-02	5.3E-03

LLI lower large intestine, *ULI* upper large intestine

Table 3

Patient outcome, IHC data, and (final) image SUV data

Outcome and IHC data				PET image data (final image)		
Patient #	Outcome	Histology	CRB	EF5 (% max)	ROI	SUV (mean±SD)
1	DOD (355)	GBM	100	65.2%	Whole lesion	1.0±0.3
					Rostral tumor	1.2±0.2
					Caudal tumor	0.7±0.1
					Normal brain	0.7±0.1
					Muscle	0.6±0.1
2	NED (1,360)	EsoMET	100	8.0%	Whole lesion	1.0±0.3
					Normal brain	0.9±0.3
					Muscle	NA
3	DOD (599)	GBM	267	20.6%	Whole lesion	0.9±0.3
					Normal brain	0.7±0.2
					Muscle	0.7±0.2

Number in parentheses after outcome is days following EF5 administration

CRB cube reference binding, ROI region of interest, DOD dead of disease, GBM glioblastoma multiforme, NED no evidence of disease, EsoMet esophageal metastasis, NA not enough local muscle tissue for assay