The Distribution Volume of ^{18}F -Albumin as a Potential Biomarker of Antiangiogenic Treatment Efficacy

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Abstract

Objective: ^{18}F -albumin, a vascular imaging agent, may have potential to assess tumor responses to anti-angiogenic therapies. In these studies tumor distribution volume of ^{18}F -albumin were first determined in various human tumor xenografts from biodistribution measurements and then one of the tumor type was used to evaluate changes in ^{18}F -albumin uptake in anti-angiogenic tumor model.

Method: ^{18}F -albumin was synthesized via conjugation of 6- ^{18}F fluoronicotinic acid-2,3,5,6-tetrafluorophenyl ester, [^{18}F]F-Py-TFP, with rat albumin. From the biodistribution of ^{18}F -albumin in various human tumor xenografts tumor distribution volumes (DVs; tumor_{%ID/g}:blood_{%ID/g}) were first determined at various time points. Then, the ability of ^{18}F -albumin to detect tumor angiogenic inhibition in one of these tumor types (U87MG) following treatment with sunitinib was evaluated by position emission tomography (PET) imaging at 0, 7, 14, and 21 days post treatment. Caliper measurements of tumor dimensions were also made at these same times. At Day 21, following imaging, biodistributions, autoradiography of tumor tissues and tumor blood vessel counts (CD31 IHC) were performed.

Results: ^{18}F -albumin retention in various tumors steadily increased over time with U87MG tumor exhibiting the highest uptake (DV) at all times. Significant decreases in ^{18}F -albumin DVs were observed one week post-treatment (−39%) vs. controls whereas tumor caliper volumes were not significantly decreased until days 14 and 21. At day 21 the significant decrease in DVs in the treatment group (−44%) paralleled biodistribution DV measurements and was consistent with autoradiography and CD31 IHC findings.

Conclusion: These data suggest that ^{18}F -albumin DVs obtained by imaging may serve as an early biomarker of the effectiveness of anti-angiogenic therapy and thus aid in patient management and treatment planning.

Keywords: angiogenesis, ^{18}F -albumin, PET, sunitinib

Introduction

Angiogenesis is a hallmark of cancer and plays a crucial role in supporting tumor growth and proliferation.^{1–3} However, the therapeutic efficacy of antiangiogenic drugs is often determined by changes in tumor volume/size, which can take weeks to months before differences in these measures are detectable by CT or MRI. Thus, there is a need for diagnostic imaging agents that can monitor tumor angiogenesis in real time to aid in patient treatment planning and management.^{4,5}

Various targeted position emission tomography (PET) imaging agents have been used to monitor angiogenesis.⁶ PET radiotracers labeled with ^{68}Ga ,⁷ ^{18}F ,⁸ and ^{64}Cu ⁹ targeted $\alpha_v\beta_3$ integrins, vascular endothelial growth factor receptors,¹⁰ and

matrix metalloproteinases.^{11,12} Although target-based imaging agents are specific, imaging efficiency depends upon target density, which can vary widely among tumor types. As a result, such targeted agents may not reliably reflect properties of the tumor vasculature and thereby fail to indicate the therapeutic effects of antiangiogenic agents.^{13,14}

An alternative to this targeted approach is to use a macromolecule whose accumulation does not vary with tumor type, but depends instead on the enhanced permeability and retention (EPR) effect that arises during growth of abnormal tumor blood vessels.^{15,16} One such molecule is albumin, an abundant plasma protein with a long serum half-life. This high-molecular-weight protein is highly retained with normal vasculature as required for any effective blood pool agent.^{17,18}

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In a previous publication, the authors described a new and efficient method for ¹⁸F labeling of albumin and proposed its use as a blood pool agent for PET.^{19,20} In the present work, the authors evaluate this albumin as a PET imaging agent to measure changes in tumor distribution volumes (DVs) in response to treatment with sunitinib,^{21,22} an antiangiogenic therapy.

Materials and Methods

Cell lines and reagents

Lung cancer (NCI-H460), colon cancer (DLD-1), gastric cancer (MKN-45), glioblastoma (U87MG), and prostate cancer (PC3) cells were used for this study. Cells were cultured in RPMI-1640 or DMEM at 37°C in a 5% CO₂:95% humidified air atmosphere supplemented with 10% FBS, 1% 2 mM L-glutamine, 1% pen/strep/amphotericin B, 0.1 mM NEAA, and 1 mM sodium pyruvate.

Radiosynthesis of ¹⁸F-albumin

All biodistribution and PET imaging studies were performed using ¹⁸F-rat serum albumin synthesized and fully characterized by Basuli et al.¹⁹ Briefly, ¹⁸F-albumin was synthesized through coupling of ¹⁸F- 2, 3, 5, 6-tetrafluorophenyl 6-fluornicotinate to albumin. The product was purified by a PD10 Minitrapp size exclusion column using phosphate buffer (pH 7.4) as an eluent. Product formation was confirmed by analytical HPLC (radiochemical purity >97%).

Mouse tumor models

Athymic female nude mice (NCr-nu/nu; NCI-Frederick, MD) were subcutaneously injected in the right thigh with 4–5 × 10⁶ cells of NCI-H460, DLD-1, U87MG, MKN-45, or PC3 in PBS:30% Matrigel. All animal studies were performed in accordance with IACUC-approved protocols.

In vivo biodistribution studies

Tumor-bearing mice were intravenously injected with ¹⁸F-albumin (0.37–0.74 MBq; 10–20 μCi). Postinjection, mice were euthanized (CO₂ inhalation) at different time points and a necropsy was performed. Blood/tissues were excised and the radioactive concentration was determined by γ counting (Perkin Elmer 2480 Wizard3). Radioactivity in the blood/tissue was expressed as a differential uptake ratio (DUR) normalized to a 20-g mouse:

$$\text{DUR} = \frac{[100 \times (\text{cpm}_{\text{tissue}}) \times (\text{body weight})]}{[(\text{Tissue weight}) \times (\text{cpm}_{\text{injected dose}}) \times (20 \text{ g}_{\text{normalized body weight}})]}$$

The DUR has units of %ID/g and represents the percentage of the injected dose resident in 1 g of tissue. Tissue DVs (DV; dimensionless) were defined for the biodistribution studies as follows:

$$\text{DV} = \text{DUR}_{\text{Tissue}} / \text{DUR}_{\text{blood}}$$

Statistical analysis of differences between the groups was performed using Student's *t*-test.

PET imaging studies

Tumor-bearing mice were anesthetized using isoflurane/O₂ (1.5%–3% v/v) and imaged (BioPET/CT; Sedecal, Madrid, Spain) at various time points post-¹⁸F-albumin injection (*i.v.*; 11.1–14.8 MBq; 300–400 μCi; anesthetized). ECG-gated cardiac imaging (1 bed position centered over the heart; 5-min duration) was followed by either a static image over the tumor (1 bed position; 5 min) or a whole-body image (2 bed positions; 10 min). The images were reconstructed with the three-dimensional ordered subset expectation maximum (3D-OSEM) algorithm.²³ A small 3D region of interest (ROI) was drawn in the center of the left ventricle of the heart at end diastole, as seen in the gated cardiac images, to determine ¹⁸F-albumin blood concentrations (expressed in units of MBq/mL of blood). 3D ROIs were also drawn encompassing the whole tumor, which represented the total radioactive content of the tumor. The total activity in the tumor (in MBq) was then divided by tumor volume (in mL) obtained by caliper measurements [(4/3) × *l* × *h* × *w* × π/3, where *l*, *h*, and *w* are the length, height, and width radii, respectively]. Tumor DVs by PET imaging were then calculated as follows:

$$\text{DV} = (\text{MBq/mL of whole tumor}) / (\text{MBq/mL of whole blood})$$

This definition of DV can be reconciled with the slightly different definition of DV used for biodistribution studies by multiplying this DV with the ratio of the density of blood to the density of tumor tissue. In the following work, the authors assume that these densities are equal and use PET and biodistribution DVs interchangeably. Note that in this formula, DVs are dimensionless and not in units of volume. Statistical analysis of differences between imaging groups was determined by Student's *t*-test.

In vivo treatment study

The U87MG tumor-bearing mice were randomly divided into control and treated groups. The treatment group received sunitinib (60 mg/kg, oral gavage) in vehicle (H₂O/HCl = 1:1.02, 0.5% polysorbate, 10% polyethylene glycol; pH adjusted to 3.3–3.7 using NaOH) with a 5-day on–2-

day off treatment cycle for 3 weeks.²² Mice in the therapy and control groups were intravenously injected with ¹⁸F-albumin (11.1–14.8 MBq; 300–400 μCi) and imaged (BioPET, 60 min postinjection) at baseline (day 0) and on days 7, 14, and 21 after initiation of therapy. On the same days as imaging, tumors were measured by caliper and changes in tumor volume (ΔV) were represented as the change from baseline:

$$\% \Delta V = ((\text{tumor volume}_{\text{day}} - \text{tumor volume}_{\text{baseline}}) / \text{tumor volume}_{\text{baseline}}) \times 100$$

At day 21 after ^{18}F -albumin imaging, tissue biodistribution and DVs were determined by γ counting, as described above (*in vivo* biodistribution studies). Treated and control tumors were then sectioned to determine blood vessel counts by CD31 immunohistochemistry (IHC) staining and to determine an effective DV (DV_E , as described in *Ex Vivo* autoradiography section) by *ex vivo* autoradiography.

Microvessel density measurements

U87MG xenograft tumors from control and treatment groups were excised and rapidly frozen with isopentane cooled with dry ice (-40°C to -50°C). IHC staining with CD31/PECAM-1 antibody was performed, as previously described.²⁴ Briefly, the acetone-fixed 20- μm -thick tumor slices were stained with CD31/PECAM-1 antibody. The number of stained vessels in the digitized images (Aperio Scanscope; Aperio, Vista, CA) were then counted (Analysis Toolbox; Aperio) and the results expressed as the number of

vessels per square millimeter of the section. Control and treatment vessel counts were compared with Student's *t*-test.

Ex vivo autoradiography

At the end of the study, excised U87MG tumors were sectioned (20- μm slices), air-dried, and placed on phosphor imaging plates (Fuji BAS-SR2025) overnight. The plates were then read with a Fuji FLA-5100 scanner to produce digitized autoradiographic images. ROIs were drawn on these images to just enclose the entire slice and the result expressed in total photostimulated luminescence (PSL) units using Image Gauge 4.0 (Fujifilm, Tokyo, Japan). A constant calibration factor (*C*, cpm/PSL) was then obtained as the slope of the best fit line from a plot of autoradiogram PSL values of a single section (6–12 sections) and counts per minutes (by γ counting) of a section adjacent to the one chosen for autoradiography. An effective $\text{DUR}_{\text{section}}$ (% injected dose/normalized to a 20g mouse/ mm^2) for each section was then determined by the following equation:

$$\text{DUR}_{\text{section}} = \frac{[100 \times (C) \times (\text{PSL}/\text{mm}^2_{\text{tumor section}}) \times (\text{body weight})]}{[(\text{cpm}_{\text{injected dose}}) \times (20\text{g}_{\text{normalized body weight}})]}$$

FIG. 1. (A) Biodistribution results in U87MG tumor-bearing mice after administration of ^{18}F -albumin (10–20 μCi). Biodistribution was performed at 15, 30, 60, and 120 min postinjection. Each bar represents the mean DUR (% ID/g) $\pm$ SD; ($n = 5$ per group); *represents a significant increase in DUR from 15 min ($p < 0.02$; Student's *t*-test). (B) DVs of ^{18}F -albumin in U87MG tumor-bearing mice at 15, 30, 60, and 120 min postinjection. Each bar represents $\text{DV}_{\text{mean}} \pm \text{SD}$ ($n = 5$ per group); *represents a significant increase in DV from 15 min ($p < 0.02$; Student's *t*-test). DUR , differential uptake ratio; DV, distribution volume; GI, gastrointestinal tract.

An effective distribution volume, DV_E , for each slice was obtained by dividing the DUR_{section} by the blood DUR (determined by γ counting) from the same mouse:

$$DV_E(\text{g}/\text{mm}^2) = DUR_{\text{section}}/DUR_{\text{blood}}$$

where DV_E is grams of blood per square mm of tumor tissue. Statistical analysis of the difference between groups was performed using Student's *t*-test.

Results

Biodistribution of ¹⁸F-albumin in different tumor types

Biodistribution of ¹⁸F-albumin was determined in U87MG, NCI-H460, MKN-45, PC3, and DLD-1 tumor xenografts at 15, 30, 60, and 120 min postinjection. Tissue DURs and DVs of ¹⁸F-albumin in mice bearing U87MG tumors are shown in Figure 1. The highest DUR of ¹⁸F-albumin was observed in blood at all times after injection (Fig. 1A and Supplementary Table S1). The blood residence half-time of ¹⁸F-albumin determined by single exponential fit to the blood results in Figure 1A was 3.6 h ($y = 42.35e^{-0.00325x}$, $R^2 = 0.94$). Heart, lungs, spleen, and kidneys showed the next highest ¹⁸F-albumin DURs, but much lower than blood at the same times. The femur DUR was low with little change over time, suggesting that ¹⁸F-albumin is stable *in vivo* with low defluorination, a result consistent with previous work confirming serum stability of ¹⁸F-albumin.¹⁹ With the exception of U87MG tumors, DURs of all other tissues/organs remained relatively constant or decreased slowly over time. Tumor DURs increased significantly with time from 4.2%ID/g at 15 min to 8.4%ID/g at 120 min, respectively ($p < 0.0001$; $n = 5$ per group).

U87MG tumor DVs (Fig. 1B) increased approximately threefold (from 0.1 to 0.29) between 15 and 120 min. Tissue DVs in the other organs, however, did not change or increased slowly with time, implying that ¹⁸F-albumin is retained in the vasculature of these tissues. Similar biodistribution results were obtained in nontarget tissues for other xenograft tumor types (data not shown).

In Figure 2, DURs and DVs of ¹⁸F-albumin in U87MG, NCI-H460, MKN-45, PC3, and DLD-1 tumor xenografts are compared at 15, 30, 60, and 120 min postinjection. All tumor types exhibited higher DURs of ¹⁸F-albumin at 120 min compared with 15 min postinjection of the tracer (Fig. 2A and Supplementary Table S2). U87MG tumors displayed the highest DURs at all time points (4.16, 4.35, 5.77, and 8.41 at 15, 30, 60, and 120 min, respectively).

U87MG tumor DVs (Fig. 2B) were higher than all other tumor types at 15, 30, 60, and 120 min. Tumor DVs for all tumor xenografts were increased at 120 min compared with 15 min. Relative CD31-IHC blood vessel counts are also shown for each tumor type (Fig. 2B). Blood vessel counts for the various tumor types at 120 min were lowest for DLD-1 and MKN-45 tumors, approximately twice that for NCI-H460 and PC3 tumors, and highest for U87MG tumors.

PET imaging of U87MG xenografts with/without sunitinib treatment

¹⁸F-albumin DVs were measured by PET imaging in U87MG tumor xenografts before and after treatment with sunitinib. In these studies, control and treated mice were

FIG. 2. (A) Comparison of ¹⁸F-albumin uptake (DUR) in colorectal adenocarcinoma (DLD-1), gastric adenocarcinoma (MKN-45), lung cancer (NCI-H460), prostate cancer (PC3), and glioblastoma (U87MG) tumor xenografts in mice at 15, 30, 60, and 120 min following injection of ¹⁸F-albumin (10–20 μCi). Each bar represents the $DUR_{\text{mean}} \pm SD$ ($n = 5$ for each group). (B) Comparison of ¹⁸F-albumin tumor DVs in different tumor types at 15, 30, 60, and 120 min and their corresponding vessel counts (CD31, IHC). Each bar represents the mean DV $\pm SD$ ($n = 5$ for each group). DUR, differential uptake ratio; DVs, distribution volumes.

imaged at baseline (day 0) and then at 7, 14, and 21 days post-treatment. Tumor volumes (by caliper) were also measured on this same schedule. Tumors from both groups were visible throughout the study period and both exhibited an increase in necrotic areas within the tumors (Fig. 3A).

Tumor DVs (Fig. 3B) determined from these images were decreased significantly in the sunitinib-treated tumors at 7 days (–39%), 14 days (–56%), and 21 days (–44%) post-treatment compared with control tumors ($p \leq 0.05$; control $n = 8$; treated $n = 9$). However, tumor volumes (by caliper) of treated tumors (Fig. 3C) were not significantly decreased compared with controls until day 14 (–29%) and day 21 (–33%; $p \leq 0.05$; control $n = 8$; treated $n = 9$ for both times).

Tumor DURs in the treatment and control groups were also obtained from the biodistribution study performed after the final imaging session at 3 weeks (Fig. 4A and Supplementary Table S3). Tumor DVs determined from this same study showed a 45% reduction in the treatment group versus

FIG. 3. (A) Representative PET images (10 min, 2 bed positions) at 60 min postinjection of 300–400 μCi of ^{18}F -albumin in anesthetized U87MG tumor-bearing mice (isoflurane/ O_2 [1.5%–3% v/v]) at baseline (0), 7, 14, and 21 days after sunitinib treatment. Control group mice were treated with saline, whereas mice in the treatment group received the antiangiogenic drug sunitinib. Tumors are indicated by arrows. (B) Tumor DVs of ^{18}F -albumin obtained by PET imaging in control and treatment groups. Each point represents the mean tumor DV $\pm$ SE (control $n=8$; treated $n=9$); *represents a significant difference in control versus treated DVs ($p \leq 0.05$; Student's t -test). (C) Comparison of changes in control and treated physical tumor volumes (caliper) over the course of therapy. Each point represents the change in mean tumor volume (Δ , % change from baseline) $\pm$ SE (control $n=8$; treated $n=9$); *represents a significant difference in the tumor volume of the treated group compared with the control group ($p \leq 0.05$; Student's t -test). DVs, distribution volumes; PET, position emission tomography.

the control group (Fig. 4B), a value similar to that obtained from the imaging study at the same time. Microvessel density as determined by CD31 IHC staining of these tumors revealed a significant decrease (-59%) in the vessel density of treated (51.2 vessels/ mm^2) tumor sections compared with controls (123.9 vessels/ mm^2) (Fig. 4C).

Ex vivo autoradiography sections obtained from these same tumors (Fig. 4D) also showed a decrease in effective slice DV (DV_E , -39% ; $p < 0.05$) of ^{18}F -albumin in treated versus control tumors. The same or adjacent sections of both treated and control tumors were found to be severely necrotic as graded by H&E staining (data not shown) and lack of activity in the center of the autoradiographic sections (Fig. 4D). This high degree of necrosis might be expected due to the large size ($>1\text{ g}$) of tumors at 3 weeks and their rapid growth. Despite this confounding factor, control tumors had increased DVs compared with treated tumors at 3 weeks.

Discussion

These results indicate that ^{18}F -albumin can be used to determine tumor DVs and detect responses to an antiangiogenic treatment. Significant reductions in ^{18}F -albumin DVs were detected at earlier times compared with physical tumor volumes in sunitinib-treated U87MG xenografts. In another comparable study with sunitinib treatment of U87MG mouse xenografts, early responses were detected using ^{18}F -fluorothymidine.^{25,26} Other studies have used

^{18}F -fluorodeoxy glucose,²⁶ ^{18}F -Arg-Gly-Asp,⁸ and ^{18}F -fluciclatide²⁷ to study angiogenesis, but these imaging agents are not ideal for early detection of antiangiogenic therapeutic responses. The uptake of these agents is either dependent on the density of specific targets in tumor blood vessels or on tumor metabolic activity. Tumor accumulation of ^{18}F -albumin, on the other hand, depends on the EPR effect, an effect directly related to vascular integrity and detectable in all of the tumor types studied here.¹⁶ Thus, changes in ^{18}F -albumin DVs measured with PET may more closely reflect changes in angiogenic processes during treatment.

Sunitinib treatment appears to delay excretion of ^{18}F -albumin from the blood pool, as evidenced by the elevated blood and large organ DURs shown in Figure 4A, which may explain the lack of a difference in tumor DURs between treated and control groups. However, when tumor activity is compared with blood activity, that is, by DV (Fig. 4B), a significant decrease (-44%) is evident between treated and control groups. DUR values represent an absolute value at a given time point that will increase or decrease as blood tracer concentration increases or decreases. DV values, on the other hand, remove this dependence and show a significant decrease.

In these studies, the ^{18}F -albumin tumor content is defined as a DV, which represents total tumor activity relative to blood activity at the same time. In normal tissues, in which blood vessels are intact, ^{18}F -albumin would be expected to be retained in the vasculature and tissue/organ DVs

FIG. 4. (A) ^{18}F -albumin biodistribution in control and treated U87MG tumor-bearing mice at 3 weeks postsunitinib treatment. Biodistribution was performed at 1 h postinjection of ^{18}F -albumin (300–400 μCi). Each bar represents the mean DUR (%ID/g) $\pm$ SD; control $n=8$; treated $n=9$. (B) Comparison of tissue DVs (tissue-to-blood ratios) in control and treated U87MG tumor-bearing mice. Each bar represents the mean DV $\pm$ SD; control $n=8$; treated $n=9$; *represents a significant difference in the control group versus the treated group ($p \leq 0.05$, Student's t -test). (C) Microvessel densities of tumors of control and treatment groups were obtained by CD31 IHC. Each bar represents mean tumor vessel density $\pm$ SD; control $n=8$; treated $n=9$; *represents a significant difference in the control group versus the treated group ($p \leq 0.05$, Student's t -test). (D) *Ex vivo* autoradiography analysis comparing ^{18}F -albumin uptake in treated and control tumors. Each bar represents the tumor relative effective DVs (DV_E) $\pm$ SD; control $n=8$; treated $n=9$; *represents a significant difference in the control group versus the treated group ($p \leq 0.05$, Student's t -test); below are representative autoradiograms of sections from control and treated tumors. DUR, differential uptake ratio; DVs, distribution volumes; GI, gastrointestinal tract.

would be expected to remain relatively constant and be representative of the physical blood volume of these tissues. In U87MG xenografts, ^{18}F -albumin DVs (Fig. 1B) were, in fact, relatively constant in all organs/tissues over time with the exception of tumors where a significant increase in U87MG tumor DVs was observed, suggesting leakage of ^{18}F -albumin out of the tumor vasculature into the tissue. These results are consistent with other literature reports in which tumor tissues are known to exhibit EPR effects: at early times, tumor DVs are proportional to actual tumor plasma volumes, whereas at later times, tumor content represents blood vessel plasma content plus the accumulation of ^{18}F -albumin in tumor tissue due to leakage.²⁸ Thus, ^{18}F -albumin tumor DV may serve as a surrogate measurement of tumor blood vessel patency and, indirectly, of tumor angiogenic activity. A clinical variant of this procedure might acquire the needed tracer blood concentration by imaging the heart or by drawing a physical blood sample at the time of tumor imaging.

A decline in the tumor DV of ^{18}F -albumin in U87MG tumors preceded physical changes in tumor volume after treatment with the antiangiogenic agent sunitinib. ^{18}F -albumin PET imaging of tumors may thus have clinical diagnostic and prognostic utility by detecting an early response to antiangiogenic therapies.

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Authorship Confirmation Statement

Listed below are the contributions of each author toward this article.

1. J.R.: Drafting of the manuscript and critical revision.
2. F.K.: Study conception and design, acquisition of data, and analysis and interpretation of data.
3. F.B.: ¹⁸F-albumin synthesis and critical revision.
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As a corresponding author of this article, E.M.J. confirms that all the coauthors have approved this article as submitted.

Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3

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