Synthesis and Evaluation of [^{18}F]1-Amino-3-fluorocyclobutane-1-carboxylic Acid to Image Brain Tumors

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We have developed a new tumor-avid amino acid, 1-amino-3-fluorocyclobutane-1-carboxylic acid (FACBC), labeled with ^{18}F for nuclear medicine imaging. **Methods:** [^{18}F]FACBC was prepared with high specific activity (no carrier added [NCA]) and was evaluated for its potential in tumor localization. A comparative study was performed for [^{18}F]FACBC and [^{18}F]2-fluorodeoxyglucose (FDG) in which the uptake of each agent in 9L gliosarcoma (implanted intracerebrally in Fisher 344 rats) was measured. In addition, the first human PET study of [^{18}F]FACBC was performed on a patient with residual glioblastoma multiforme. Quantitative brain images of the patient were obtained by using a Siemens 921 47-slice PET imaging system. **Results:** In the rat brain, the initial level of radioactivity accumulation after injection of [^{18}F]FACBC was low (0.11 percentage injected dose per gram [%ID/g]) at 5 min and increased slightly to 0.26 %ID/g at 60 min. The tumor uptake exhibited a maximum at 60 min (1.72 %ID/g), resulting in a tumor-to-brain ratio increase of 5.58 at 5 min to 6.61 at 60 min. In the patient, the uptake of [^{18}F]FACBC in the tumor exhibited a maximum concentration of 146 nCi/mL at 35 min after injection. The uptake of radioactivity in the normal brain tissue was low, 21 nCi/mL at 15 min after injection, and gradually increased to 29 nCi/mL at 60 min after injection. The ratio of tumor to normal tissue was 6 at 20 min after injection. The [^{18}F]FACBC PET scan showed intense uptake in the left frontal region of the brain. **Conclusion:** The amino acid FACBC can be radiofluorinated for clinical use. [^{18}F]FACBC is a potential PET tracer for tumor imaging.

Key Words: neoplasm; PET; amino acid; radiofluorine

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The development of noninvasive methodologies to identify both qualitative and quantitative differences between normal (or benign) tissue and neoplastic tissue is crucial to the understanding of cancer and to the improvement of cancer treatment. The two diagnostic techniques most widely used for anatomical imaging of tumors are CT and MRI. Clinical trials using these techniques have demonstrated that a sensitivity and specificity of >90% can be achieved in brain tumors with diameters ≥2 cm (*1*). Disruption of the blood-brain barrier accounts for the high sensitivity of these imaging techniques. These modalities have been found to be less valuable as diagnostic methods in differentiating benign growths from malignant tumors or recurrent tumors after therapy (*1,2*).

One technique that is more sensitive and accurate than CT or MRI is a measure of the physiological process associated with the utilization of nutrients in tumors using PET (*3–8*). In conjunction with the appropriate biologic substrate, labeled with a short-lived isotope, this metabolic imaging technique can provide a means of staging tumors and differentiating between radiation-induced tissue necrosis and tumor recurrence (*8,9*).

The metabolically active amino acid L-methyl-^{11}C-methionine is being used clinically to distinguish malignant tissue from normal tissue or benign growths in the brain (*10,11*). Methionine, a naturally occurring amino acid, has a high brain uptake index (BUI; 38%) (*12*) and is rapidly cleared from plasma and incorporated into brain protein. L-Methyl-^{11}C-methionine has been found to be a more useful PET tracer than [^{18}F]2-fluorodeoxyglucose (FDG) for the delineation of cerebral glioma and its uptake tends to correlate with cell proliferative activity (*13*). In clinical studies, mean tumor-to-normal tissue ratios for ^{11}C-labeled methionine range from 1.57 (low grade) to 2.36 (high grade) and can be as high as 3.99 (for glioblastoma) (*13*). The major limitation of L-methyl-^{11}C-methionine is that the half-life of ^{11}C is only 20 min; consequently, this imaging agent cannot be stored for a long time before use. The development of a positron-emitting amino acid analog that is not a metabolic substrate for normal brain, benign growths or infection but is a substrate for tumors would be an excellent diagnostic radiopharmaceutical for staging tumors and measuring the regression or recurrence of tumors after therapy.

We report the synthesis and biodistribution of ^{18}F-labeled 1-amino-3-fluorocyclobutane-1-carboxylic acid (FACBC), an unnatural amino acid. The uptake and retention of this agent was studied in Fisher rats implanted intracerebrally with 9L gliosarcoma cells. The rat 9L tumor is a well-characterized model for human intracerebral neoplasms that mimics the

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clinical situation of a rapidly growing, infiltrative glioblastoma multiforme (*14*). In a preliminary study, we report the detection of residual glioblastoma multiforme in a patient after surgery by using [^{18}F]FACBC and PET imaging.

MATERIALS AND METHODS

General

All chemicals and solvents were analytical grade and were used without additional purification. The [^{18}F]fluoride was produced at Emory University with a Siemens RDS 112 11-MeV negative-ion cyclotron (Knoxville, TN) by the ^{18}O(p,n)^{18}F reaction on 95% enriched [^{18}O]water. The configuration of triflate precursor 1 in Figure 1 was determined by x-ray crystallography. Chromatograms of the radiolabeled compounds were counted with a Bioscan System 200 (Washington, DC) on either 250-μm silica gel AL SIL G/UV plates (Whatman Ltd., Kent, UK) or 0.25-mm RP Chiralplates (Macherey-Nagel; Alltech Co., Deerfield, IL).

Chemistry

A 1-mL aliquot of solution containing 125 mg Kryptofix, 25 mg potassium carbonate, 0.5 mL water and 12 mL acetonitrile were added to a Wheaton 5-mL reaction vial containing ~500 mCi (20-μ amp, 60-min bombardment) in 350 mg of [^{18}O]water.

The solution was heated at 118°C and the solvent was evaporated with the aid of an argon gas flow. Remaining moisture was removed by adding 2 mL dry acetonitrile to the vial followed by evaporation using argon flow. This process was repeated three more times to ensure dryness of the fluoride. A solution of 8 mg 1-*tert*-butyl carbamate-3-trifluromethanesulfonoxy-1-cyclobutane-1-carboxylic acid methyl ester (compound **1** in Fig. 1) in 500 μL dry acetonitrile was added to the vial and the fluorination (no carrier added [NCA]) reaction was performed at 85°C for 5 min. The mixture was diluted with 1 mL methylene chloride, and unreacted $^{18}F^-$ was removed by passage through a silica gel Sep-Pak (Waters, Milford, MA). The Sep-Pak was rinsed with 6 mL methylene chloride, and the combined eluant was evaporated using an argon flow that gave the ^{18}F-labeled intermediate (68 mCi) in 18% end of bombardment (EOB) yield. Deprotection was achieved by using 1 mL 4 N HCl at 120°C for 15 min. The aqueous solution containing [^{18}F]FACBC was passed through a 12 × 1.5-cm column of an ion-retardation resin (AG 11A8, 50–100 mesh) in series with an alumina Sep-Pak (wet), a C_{18} Sep-Pak and a 0.22-μ Millipore sterile filter using sterile water. The eluant containing compound **2** was collected in a sterile vial and was made isotonic by adding 0.96 mL of a 22.4% solution of sodium chloride. The [^{18}F]FACBC solution was tested for pyrogens and sterility before use in humans. The synthesis was completed in 60 min after EOB, with an overall radiochemical yield of 42 mCi (12% EOB). Radio thin-layer chromatography (TLC) showed 99% radiochemical purity (Chiralplates, 20:5:5 acetonitrile/water/methanol; radiofrequency [Rf] = 0.63).

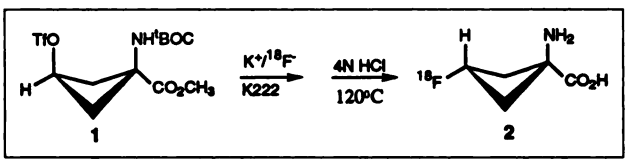

FIGURE 1. Synthesis of [^{18}F]FACBC.

Tumor Induction and Animal Preparation

All animal experiments were approved by the Emory University Institutional Animal Care and Use Committee. Rat 9L gliosarcoma cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 50 U/mL penicillin and 50 mg/mL streptomycin. Immediately before implantation, the cells were removed from their flasks with trypsin, were washed and were resuspended in serum-free DMEM at a concentration of 1×10^7 cell/mL.

Each rat was anesthetized with ketamine (50 mg/kg intraperitoneally) and xylazine (10 mg/kg intraperitoneally) and was placed in a stereotactic head holder (David Kopf Instruments, Tujunga, CA). The scalp was shaved, prepared and draped. A midline incision was made and a small burr hole was cut 3 mm lateral (right) and 1 mm anterior to the bregma. With a Hamilton syringe, 4 μL of the 9L tumor suspension (4×10^4 cells) was injected 5 mm deep to the outer table. The injection was administered over a period of 2 min and then withdrawn over 1 min to minimize the backflow of cells in the needle track. The burr hole was closed with bone wax and the subgaleal space was run with 4-O silk sutures. The animals were allowed to recover in the indirect heat of a warm lamp before being returned to their original colony. Within 17–21 d, intracranial 9L tumors were established in the rats that produced the clinical signs of weight loss and apathy. The tumor masses were easily detected by the naked eye after dissection; mean diameter was 4 ± 0.5 mm.

Animal Tissue Distribution Experiments

The distribution of radioactivity was determined in tissues of male Fisher 344 rats (175–225 g) after intravenous administration of the radiofluorinated compound. The animals were allowed food and water ad libitum before the course of the experiment. The rats were anesthetized with an intramuscular injection of 0.1 mL/100 g body weight of a 50:50 mixture of ketamine (50 mg/mL) and xylazine (20 mg/mL). Either radiolabeled [^{18}F]FACBC or [^{18}F]FDG (80–100 μCi) was injected directly into the femoral vein of each rat, under anesthesia. Animals were killed at 5 and 60 min after injection, four rats at each time point. Blood samples were obtained by cardiac puncture. Syringes were weighed before and after injection to determine the volume delivered. The activity per unit volume was obtained from standards. Eleven different tissues were excised, weighed and counted. These tissues included blood, heart, muscle, lung, kidney, spleen, liver, testis, bone, whole brain and tumor. The excised tissues were blotted, weighed and counted with a Packard Cobra II Auto-Gamma Counter (Packard Instrument Co., Downers Grove, IL). The raw counts were decay-corrected. All results are expressed as the percentage injected dose per gram (%ID/g; means ± SE) and tumor-to-brain tissue ratios. Biodistribution results were evaluated by one-way analysis of variance (ANOVA).

Toxicity Studies

Sprague-Dawley rats (n = 18) were anesthetized with a 50:50 mixture of ketamine/xylazine. All injections were administered intramuscularly through the femoral vein. The rats were divided into three groups of six rats each. Group A was injected with diluent only (normal saline); group B was injected with 9.5 μg/kg body weight of "cold" FACBC, corresponding to approximately 1×10^3 excess of what a normal patient would receive; group B was injected with 9.5 mg/kg body weight of "cold" FACBC, approximately 1×10^6 excess of what a normal patient would receive. The rats were monitored for 4 wk.

Human PET Imaging

A 51-y-old man known to have a malignant brain tumor volunteered to participate in this study. The study was approved by the Emory University Human Investigation Committee, and informed consent was obtained. The T1-weighted MR image with gadolinium enhancement clearly showed a left frontal tumor with extension in the midline (Fig. 2, top row). The tumor was histologically confirmed as glioblastoma multiforme.

The patient underwent surgery for debulking of the tumor before [^{18}F]FACBC and [^{18}F]FDG PET scans. Brain imaging was conducted using a Siemens 921 47-slice PET imaging system (CTI Inc., Knoxville, TN). The patient was placed in the tomographic gantry for attenuation correction of emission data (10-min transmission scan) and then received 6.0 mCi [^{18}F]FACBC injected intravenously over 2 min. Dynamic emission data were acquired using 12 consecutive 5-min frames and then were reconstructed with a ramp filter to the Nyquist frequency and were smoothed with a three-dimensional Hanning filter with a cutoff of 1.5 cycles/cm. The resulting resolution was 9 mm in all directions.

The [^{18}F]FDG PET image sets were acquired 45 min after intravenous administration of 10 mCi [^{18}F]FDG. For [^{18}F]FDG, a 25-min emission scan was performed to obtain 47 transaxial images 3.375 mm apart. Images were then reconstructed with a Shepp-Logan filter (0.35 × Nyquist frequency), giving a resolution of 7.8 mm full width at half maximum (FWHM) in the transaxial plane and 6.2 mm FWHM in the axial direction.

Human Dosimetry

Tissue distribution data (%/g) obtained from normal Fisher rats after injection of [^{18}F]FACBC were converted to human values of %organ using the %-kg/g method of Kirschner et al. (*15*). The data were then fit to single-component exponential curves using SAAM software (*16*). Areas under the curves were expressed as residence times (*17*) and entered into the MIRDOSE 3.1 program (*18*) to calculate the dose estimates. Data for total body clearance were used to calculate the residence times for the urinary bladder, assuming that all excretion was through the urinary pathway and using the dynamic bladder model of Cloutier et al. (*19*).

RESULTS

Chemistry

Radiofluorination (NCA) of 1-*tert*-butyl carbamate-3-trifluoromethane sulfonoxy-1-cyclobutane-1-carboxylic acid methyl ester (**1**) and subsequent hydrolysis and purification steps were performed using a remote-manual system that was developed in our laboratory. The synthesis was completed in 60 min with an overall radiochemical yield of 42 mCi (12% EOB) from 500 mCi [^{18}F]fluoride. The synthesis of [^{18}F]FACBC resembles that of [^{18}F]FDG; therefore, an automatic FDG-synthesis unit can be adapted for [^{18}F]FACBC production.

Biodistribution Studies in Tumor-Bearing Rats

Table 1 shows the biodistribution of [^{18}F]FACBC at 5 and 60 min after intravenous administration in rats with intracranial 9L gliosarcoma implants. A significant main effect of organs ($P < 0.001$) at 5 and 60 min on [^{18}F]FACBC accumulation was determined by ANOVA. At 5 min, accumulation of [^{18}F]FACBC in the spleen was greater than in all other organs (2.7-fold higher than in blood) ($P < 0.001$). Accumulation of [^{18}F]FACBC in lungs, liver and kidneys was greater than in blood, brain and tumor ($P < 0.02$). The initial level of accumulation of radioactivity in the brain after injection of [^{18}F]FACBC was low (0.11 %ID/g) at 5 min and increased slightly to 0.26 %ID/g. The agent, however, showed marked accumulation in the brain tumor. At 60 min, uptake of radioactivity in the tumor was 1.72 %ID/g, resulting in an increase in the tumor-to-brain ratio from 5.58 at 5 min to 6.61 at 60 min. Sufficient clearance of radioactivity had occurred within 60 min in all

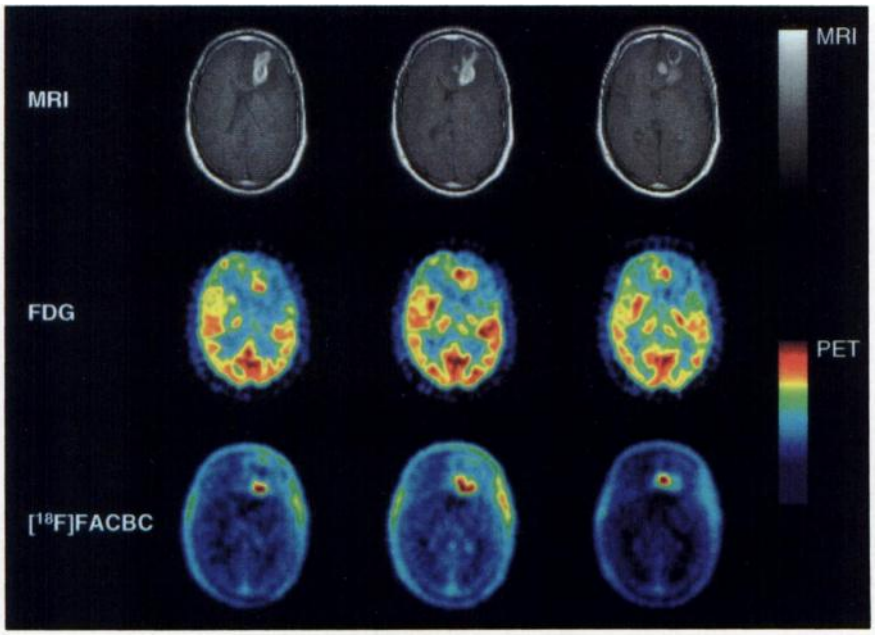

FIGURE 2. Patient with glioblastoma multiforme. T1-weighted transaxial MRI scans with gadolinium enhancement taken before surgery show lesion throughout left frontal lobe with extension over midline (top row). FDG PET images taken 8 wk after surgery (middle row). PET images using [^{18}F]FACBC taken 1 wk after surgery show residual tumor (bottom row).

TABLE 1
Distribution of Radioactivity in Tissues of Tumor-Bearing Fisher Rats After Intravenous Administration of [^{18}F]FACBC

Tissue	Accumulation (%ID/g)	
	5 min	60 min
Blood	0.58 ± 0.08	0.32 ± 0.05
Heart	0.70 ± 0.11	0.56 ± 0.11
Muscle	0.27 ± 0.06	0.41 ± 0.07
Lung	1.13 ± 0.15	0.64 ± 0.08
Kidney	1.08 ± 0.05	0.60 ± 0.08
Spleen	1.55 ± 0.25	0.68 ± 0.09
Liver	1.10 ± 0.22	1.70 ± 0.48
Testis	0.25 ± 0.04	0.28 ± 0.03
Bone	0.52 ± 0.09	0.38 ± 0.07
Brain	0.11 ± 0.03	0.26 ± 0.03
Tumor	0.61 ± 0.10	1.72 ± 0.64

FACBC = 1-amino-3-fluorocyclobutane-1-carboxylic acid; %ID = percentage injected dose.
Values are means ± SD for four rats.

other organs except for the liver ($P < 0.001$) relative to the tumor. Accumulation of radioactivity in the tumor was greater than in the kidney ($P < 0.05$), blood ($P < 0.01$), spleen ($P < 0.01$) and brain ($P < 0.001$). The bone with marrow radioactivity showed no increase (0.52 %ID/g at 5 min, 0.38 %ID/g at 60 min), demonstrating the expected stability of the 3-fluorocyclobutyl moiety to significant in vivo defluorination.

We compared the tumor uptake of [^{18}F]FACBC with [^{18}F]FDG in a separate group of male Fisher rats with 9L gliosarcoma implants at 5 min and 60 min after intravenous administration of [^{18}F]FDG (Table 2). Five minutes after injection, [^{18}F]FDG accumulation in the kidneys, liver, heart ($P < 0.001$), brain and tumor was greater than in blood ($P < 0.001$). The initial level of accumulation of radioactivity in the brain tumor after injection of [^{18}F]FDG was good (1.29 %ID/g). [^{18}F]FDG uptake, however, decreased in the brain tumor to 1.05 %ID/g at 60 min. The decrease of radioactivity in the tumor at 60 min in conjunction with initial high brain uptake and retention resulted in a low tumor-to-brain ratio of 0.84 at 60 min (Table 3).

TABLE 2
Distribution of Radioactivity in Tissues of Tumor-Bearing Fisher Rats After Intravenous Administration of [^{18}F]FDG

Tissue	Accumulation (%ID/g)	
	5 min	60 min
Blood	1.18 ± 0.32	0.37 ± 0.10
Heart	0.91 ± 0.54	0.91 ± 0.41
Muscle	0.24 ± 0.06	0.20 ± 0.05
Lung	0.96 ± 0.26	0.60 ± 0.13
Kidney	1.66 ± 0.27	0.37 ± 0.25
Spleen	0.70 ± 0.24	0.88 ± 0.28
Liver	1.20 ± 0.22	0.45 ± 0.11
Testis	0.41 ± 0.14	0.68 ± 0.25
Bone	0.42 ± 0.14	0.36 ± 0.08
Brain	1.21 ± 0.33	1.29 ± 0.46
Tumor	1.30 ± 0.31	1.05 ± 0.29

%ID = percentage injected dose.
Values are means ± SD of three to four rats.

PET Brain Imaging of [^{18}F]FACBC in Humans

PET images using [^{18}F]FACBC and [^{18}F]FDG taken 1 wk and 8 wk, respectively, after the patient had undergone surgery for debulking of the tumor are compared in Figure 2. The results of a dynamic imaging study of [^{18}F]FACBC show intense uptake in the frontal region of the hemisphere—in the area of the tumor—well defined by MRI. The regional distribution of radioactivity (in nCi/mL; no partial volume correction was applied) is shown in Figure 3. The tumor (T) exhibited the highest uptake of radioactivity with prolonged retention. The uptake in the tumor exhibited a maximum concentration of 146 nCi/mL at 35 min after injection. The uptake of radioactivity in the normal brain tissue (Bkgd) was low, 21 nCi/mL at 5 min after injection, and gradually rose to 29 nCi/mL at 60 min after injection. The uptake of radioactivity in the sagittal sinus (S) was 88 nCi/mL at 5 min after injection and gradually decreased to 50 nCi/mL at 60 min after injection. The T/Bkgd and T/S ratios were 6 and 2.6, respectively, at 20 min after injection. After imaging, the tumor was resected and was histologically verified as residual glioblastoma.

Radiation Dosimetry of [^{18}F]FACBC

Radiation dose estimates for ^{18}F-labeled FACBC were calculated using phantoms of Cristy and Eckerman (Report ORNL/TM-8381/V1 and V7). Bone and marrow models of Eckerman (*20*) were used. The mean tissue cumulated activities and radiation dose estimates of [^{18}F]FACBC for humans are given in Table 4. The tissue distribution data obtained from [^{18}F]FACBC in normal Fisher rats were used. Absorbed dose (rad/mCi) from [^{18}F]FACBC to the urinary bladder wall, kidneys, liver, bone surfaces and red marrow were calculated to be 0.14, 0.14, 0.09, 0.05 and 0.052, respectively. The highest radiation dose estimates are for the pancreas (0.21 rad/mCi), kidneys (0.14 rad/mCi) and the urinary bladder wall (0.14 rad/mCi). The total body dose for [^{18}F]FACBC was 0.046 rad/mCi.

Toxicity

The results of the study are shown in Table 5. The initial deaths within 24 h of injection could be attributed to an

TABLE 3
Ratio of Tumor-to-Brain Accumulation in Rats

Tracer	Tumor-to-brain ratio	
	5 min	60 min
[^{18}F]FACBC	5.6	6.6
[^{18}F]FDG	1.05	0.84

FACBC = 1-amino-3-fluorocyclobutane-1-carboxylic acid.

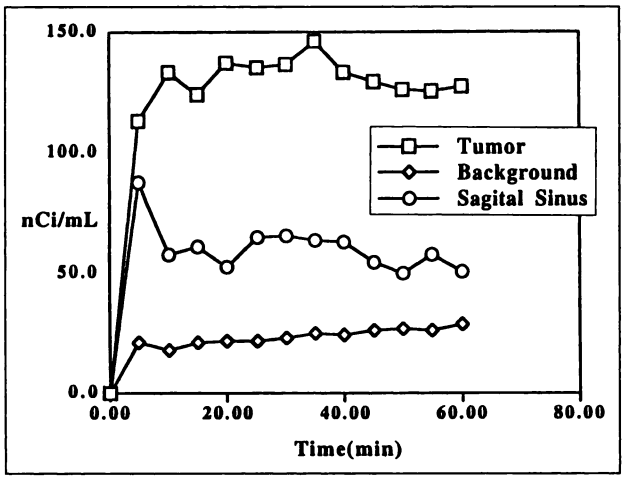

FIGURE 3. Time-activity curves for tumor, background and venous sinus in patient's brain after receiving 6.0 mCi [^{18}F]FACBC. Serial images were acquired for total time of 60 min (12 scans).

inability to recover from anesthesia. After the initial 24 h had passed, there was no change in status in any of the groups of rats. All rats appeared healthy up to doses of 9.5 mg/kg body weight. Based on our estimates of specific activity (1.5 Ci/mmol), the normal dose of FACBC that would be administered is 9.5 ng/kg body weight. The level of 9.5 mg/kg body weight or 1×10^6 times the expected dose did not demonstrate any acutely toxic effects.

DISCUSSION

This study shows that [^{18}F]FACBC can be prepared routinely for clinical use. The no-carrier-added radiofluorination with K-^{18}F and subsequent hydrolysis procedures are similar to those used for the production of [^{18}F]FDG; hence, an automated FDG-synthesis unit could be used to prepare [^{18}F]FACBC with only minor modifications. The radiation dose estimates calculated in this study suggest that use of [^{18}F]FACBC will result in acceptable radiation dose levels in humans, given normal amounts of administered activity (5–10 mCi). Naturally, however, definitive radiation dose estimates will be established only through kinetic studies in humans.

Over the past few years, a host of unnatural nonmetabolized amino acids labeled with ^{11}C have been evaluated to assess the effect of the amino acid structure on tumor-to-nontumor concentration ratios. The most selective reported compounds are [^{11}C]α-aminocyclobutane carboxylic acid (ACBC) (*21–23*), [^{11}C]α-aminocyclopentane carboxylic acid (ACPC) (*21*) and [^{11}C]α-aminoisobutyric acid (AIB) (*24–27*). Washburn et al. (*21*) showed that the alicylic α-amino acid, [^{11}C]ACBC, was the most selective substrate for metastatic lesions by several tumor types in animals. In a clinical study, [^{11}C]ACBC showed intense uptake in a grade 2 astrocytoma with little observable uptake in normal brain tissue (*28*). Unfortunately, ^{11}C is a short-lived radioactive element with a 20-min half-life, which makes the preparation of [^{11}C]ACBC impractical in a hospital-based radiopharmacy. The fluorine-18 isotope is the most attractive radioactive element for tagging radiopharmaceuticals for hospital use because it has a longer half-life of 110 min. This half-life allows sufficient time for synthesis, quality control and multidosing from a single-batch production. In this regard, we developed [^{18}F]FACBC, which potentially has widespread use as a diagnostic imaging agent for visualizing malignant tumors.

Evaluation of [^{18}F]FACBC showed marked uptake of

TABLE 4
Human Radiation Dose Estimates for [^{18}F]FACBC*

Tissue	Dose mGy/MBq	Dose rad/mCi	Tissue	Dose mGy/MBq	Dose rad/mCi
Adrenal	1.6×10^{-2}	5.8×10^{-2}	Ovary	1.5×10^{-2}	5.7×10^{-2}
Brain	6.3×10^{-3}	2.3×10^{-2}	Pancreas	5.6×10^{-2}	2.1×10^{-1}
Breast	1.0×10^{-2}	3.8×10^{-2}	Red marrow	1.4×10^{-2}	5.2×10^{-2}
Gallbladder wall	1.7×10^{-2}	6.3×10^{-2}	Bone surface	1.3×10^{-2}	5.0×10^{-2}
Lower large intestine wall	1.5×10^{-2}	5.5×10^{-2}	Skin	9.6×10^{-3}	3.5×10^{-2}
Small intestine	1.5×10^{-2}	5.6×10^{-2}	Spleen	1.6×10^{-2}	6.0×10^{-2}
Stomach	1.5×10^{-2}	5.5×10^{-2}	Testis	1.0×10^{-2}	3.7×10^{-2}
Upper large intestine wall	1.5×10^{-2}	5.5×10^{-2}	Thymus	1.3×10^{-2}	4.6×10^{-2}
Heart wall	1.4×10^{-2}	5.1×10^{-2}	Thyroid	1.2×10^{-2}	4.5×10^{-2}
Kidney	3.8×10^{-2}	1.4×10^{-1}	Urinary bladder wall	3.8×10^{-2}	1.4×10^{-1}
Liver	2.4×10^{-2}	9.0×10^{-2}	Uterus	1.6×10^{-2}	6.0×10^{-2}
Lung	1.3×10^{-2}	4.8×10^{-2}	Total body	1.2×10^{-2}	4.6×10^{-2}
Muscle	1.2×10^{-2}	4.5×10^{-2}	Effective dose equivalent	2.0×10^{-2} mSv/MBq	7.3×10^{-2} rem/mCi

*Tissue distribution data were obtained from normal Fisher rats injected with ^{18}F-labeled 1-amino-3-fluorocyclobutane-1-carboxylic acid ([^{18}F]FACBC).

TABLE 5
Acute Toxicity Data for FACBC in Sprague-Dawley Rats

Dose	Time					
	<24 h	24 h	1 wk	2 wk	3 wk	4 wk
(Control)	1 death	NC	NC	NC	NC	NC
9.5 μg/kg	2 deaths	NC	NC	NC	NC	NC
9.5 mg/kg	No deaths	NC	NC	NC	NC	NC

Values are for six rats.
FACBC = 1-amino-3-fluorocyclobutane-1-carboxylic acid; NC = no change.

radioactivity in the brains of rats with intracerebral 9L gliosarcoma implants. The high tumor-to-brain ratio of 6.6 at 60 min for [^{18}F]FACBC compared to the nearly 1:1 ratio of [^{18}F]FDG in the same tumor rat model demonstrates the superiority of this ^{18}F-labeled amino acid over [^{18}F]FDG. The higher ratio for [^{18}F]FACBC is due to the lower uptake of radioactivity in normal brain tissue. This is in sharp contrast to [^{18}F]FDG with its high uptake in normal brain tissue, which can obscure tumor delineation depending on the glucose metabolic rate of the tumor (*29*). At 60 min after injection of radioactivity, blood clearance reached 0.32 %ID/g, giving a tumor-to-blood ratio of 5.4. A washout of radioactivity was also seen for lung, kidney, spleen and heart. At this time point, the majority of the radioactivity was found in the tumor (1.72 %ID/g) and the liver (1.70 %ID/g). Bone uptake at 5 min was 0.52 %ID/g and then decreased to 0.38 %ID/g after 1 h (the same as in blood). The slow clearance suggests that [^{18}F]FACBC is clearing from bone marrow and that although this percentage ID per gram value is small at 60 min, it is difficult to access the amount of defluorination that has occur based solely on bone uptake.

The [^{18}F]2-FDG tumor-to-brain uptake ratio of 1:1 in the rat experiments is due to normal brain uptake of [^{18}F]FDG and perhaps to secondary effects on regional brain metabolism. Gliomas tend to excite a pronounced local edematous response that alters the membrane permeability of normal brain, thus facilitating the diffusion of macromolecules into adjacent normal and neoplastic tissue. This effect can be seen in [^{18}F]2-FDG PET images that show accumulation of activity over a larger area than the actual brain tumor (*30*).

In the human study, we observed that [^{18}F]FACBC uptake in normal brain tissue was low (29 nCi/mL at 60 min), as expected based on our animal data and reported results obtained from the ^{11}C analog (*21*). The uptake in the tumor exhibited a maximum concentration of 146 nCi/mL. Interestingly, the tumor-to-normal brain tissue ratio of 6 is similar to that found in our animal model. The pattern of the time-activity curve (Fig. 3) shows that no significant changes seem to occur after 10 min after injection, so the in vivo imaging of the labeled amino acid distribution in the head and brain can be measured soon after the injection of [^{18}F]FACBC. This result is in contrast to [^{18}F]FDG, which requires 40–60 min after injection before the emission scan can be started.

A comparison of the T1-weighted MR image of the patient after surgery (not shown) with that of the [^{18}F]FACBC PET scan, also after surgery, clearly shows the distinctions between these two modalities and how PET complements MRI. The T1-weighted transverse scan shows a large area of T1 signal throughout the left frontal lobe of the patient, representing tumor, tissue necrosis and proteinaceous fluid resulting from the surgery. [^{18}F]FACBC accumulation, however, is localized in the frontal region of the left hemisphere in the area of the remaining malignant tumor. There is some increased uptake of [^{18}F]FACBC at the site of craniotomy that is probably related to amino acid utilization in healing tissue.

The mode of FACBC transport through the blood-brain barrier and subsequent passage through the cell membrane remains unelucidated in this study. In the unnatural neutral amino acids series, FACBC falls in between AIB (*24*), known to use the Na-dependent alanine transport, or A-system, and cycloleucine, which uses the Na-independent equilibrative leucine transport, or L-system (*31*). [^{18}F]FACBC uptake in the rat brain is the lowest of all the organs, which suggests that FACBC is selectively excluded from the brain by the blood-brain barrier. The low brain uptake also suggests that the compound is not metabolized or incorporated into protein.

The high tumor accumulation is presumably due to the pathological permeability of tumor capillaries and increased tumor cell activity of the amino acid transport (*32,33*). The blood-brain barrier was damaged in our animal experiment during gliosarcoma cell implantation and in the patient because of previous surgery for debulking of the tumor. Just how [^{18}F]FACBC tumor uptake is influenced by a breakdown of the blood-brain barrier is not addressed in this investigation. However, uptake of activity by capillary leakage as the dominant mechanism is not supported by other studies. It has been shown that there is transport selectivity taking place among different amino acids in tumors. In a clinical study by Hubner et al. (*34*), in which the accumulation of two amino acids (DL-valine and DL-tryptophan) were compared in the same patients with cerebral tumors, a distinct difference in amino acid uptake was observed. A more extensive evaluation of [^{18}F]FACBC in patients with brain tumors of different stages of malignancy is needed. In such a study, uptake kinetics for [^{18}F]FACBC could be measured and compared with glucose metabolic rates as measured by [^{18}F]FDG. The transport of [^{18}F]FACBC into malignant gliomas also could be assessed relative to the damage of the blood-brain barrier by a comparison with that of ^{68}Ga-ethylenediaminetetraacetic acid (EDTA).

[^{18}F]FACBC is an analog of ACBC in which a fluorine atom replaces a hydrogen atom. Unsubstituted ^{11}C-labeled ACBC has not been evaluated in our tumor rat model and therefore the effect of fluorine substitution on tumor uptake is unclear; however, it can be inferred from reported studies involving [^{11}C]1-amino-2-cyclopentane-1-carboxylic acid

and its derivatives that ring substituents can lower the percentage ID per gram in the tumor as well as lower uptake in other organs (e.g., liver and kidneys) (*21*). But at the same time, these structural changes can increase the tumor-to-brain ratio (*2*). Just how much [^{18}F]FACBC tumor accumulation is influenced by this change in the molecule relative to that of ACBC can only be answered by a direct comparison in our tumor model.

In contrast to naturally occurring amino acids, which can participate in a wide variety of biochemical pathways in vivo, ACBC is not metabolized after transport into cells. It is expected that [^{18}F]FACBC behaves similarly and that it will remain intact after transport through the plasma membrane. Thus, the distribution of activity in the body represents the distribution of [^{18}F]FACBC.

^{11}C-labeled methionine (Met) is one of the most frequently used PET agents for the evaluation of amino acid metabolism of brain tumors (*11,35–37*). In an effort to overcome the disadvantages of the short half-life of ^{11}C-Met, several [^{18}F]fluoro- and [^{123}I]iodo-amino acids have been developed and evaluated for their ability to diagnose and classify brain tumors. These agents include L-2-[^{18}F]fluorotyrosine (Tyr) (*33,38*), [^{18}F]fluorophenylalanine (Phe) (*39,40*) and [^{123}I]iodine-α-methyl-L-tyrosine (^{123}IMT) (*41–43*). These analogs are based on natural amino acids that are transported by the same system as for large neutral amino acids. Moreover, the natural amino acids exhibit high BUIs and high influx constants, and their concentrations in brain tissue are in the range of 0.04–0.07 μmol/g (*44*). Accumulation of ^{18}F-Tyr, ^{18}F-Phe and ^{123}IMT in brain tumors is associated with increased transport rather than with protein synthesis and uptake is believed to be independent of blood-brain barrier disruption. Tumor uptake is rapid, and tumor-to-normal brain tissue ratios (T/Bkgd) are typically in the range of 1.3–2.8:1. T/Bkgd for high-grade glioma is higher than for low-grade glioma; however, it is reported that differentiation between low- and high-grade glioma is difficult because it is based solely on tracer accumulation (*38–43*). We are inclined to believe that an amino acid that is a substrate for active transport but has a greater sensitivity for tumors than normal brain tissue would be better suited for tumor grading. [^{18}F]FACBC should offer greater tumor delineation because it has a higher T/Bkgd than other imaging agents. The higher ratio of [^{18}F]FACBC also may allow sharper distinction between high- and low-grade gliomas. Another advantage of [^{18}F]FACBC is its mode of production. Like FDG, [^{18}F]FACBC synthesis lends itself easily to automation. ^{18}F-Try and ^{18}F-Phe are most often prepared by electrophilic radiofluorination and consequently are of lower specific activity and usually are produced in low radiochemical yields, which can limit their widespread use (*5*).

There are two potential applications of [^{18}F]FACBC PET: monitoring recurrent tumors after therapy and detecting small low-grade brain tumors. Currently, [^{18}F]FDG is the principal PET imaging agent for grading primary brain tumors and for determining the prognoses of patients with these lesions (*29,45–49*). A correlation between the rate of glycolysis and histologic grade of cerebral gliomas in patients with tumors was found by DiChiro et al. (*29*). However, variability in the rates of glucose utilization within similar tumors with the same pathological grade have also been reported (*50*). Furthermore, quantification of tumor metabolism by [^{18}F]FDG in the same patient at different times can vary as much as 20% or 30% (*7*). Studies with [^{18}F]FDG have shown that it cannot always distinguish low-grade gliomas from normal tissue or from vascular lesions (*51*). Because malignant cells have an abnormally high rate of amino acid metabolism, the measurement of amino acid transport may represent a more sensitive and specific method of tumor detection (*7,8*). Using methyl-^{11}C-Met, Lilja et al. (*3*) were able to show that this ^{11}C-labeled amino acid was a more sensitive PET agent for delineation of low-grade astrocytomas than [^{18}F]FDG. In a comparative PET study of [^{18}F]FDG and L-methyl-^{11}C-Met, each appears equally effective in detecting large malignant tumors, but it was concluded that [^{18}F]FDG and L-methyl-^{11}C-Met both had a limited diagnostic sensitivity for small (<1.5 cm) tumors (*11*). Because [^{18}F]FACBC is not a metabolic substrate for normal brain, it is possible that [^{18}F]FACBC may be a more sensitive PET agent than L-methyl-^{11}C-Met for the detection of small low-grade brain tumors.

CONCLUSION

This study demonstrates that there is marked uptake of [^{18}F]FACBC in the brain of rats with 9L gliosarcoma. Moreover, PET imaging revealed high tumor accumulation of [^{18}F]FACBC in a patient with residual glioblastoma multiforme. The significant tumor-to-brain ratios of 6.6 at 60 min in animals and 6.0 in the patient strongly suggest that [^{18}F]FACBC is a potentially valuable imaging agent for the diagnosis of metastatic disease in humans by PET. Additional clinical studies of [^{18}F]FACBC as a tumor imaging agent are in progress at our institution.

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