ORIGINAL ARTICLE

Non-invasive estimation of ^{10}B-4-borono-L-phenylalanine-derived boron concentration in tumors by PET using 4-borono-2-^{18}F-fluoro-phenylalanine

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In boron neutron capture therapy (BNCT), ^{10}B-4-borono-L-phenylalanine (BPA) is commonly used as a ^{10}B carrier. PET using 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA PET) has been performed to estimate boron concentration and predict the therapeutic effects of BNCT; however, the association between tumor uptake of ^{18}F-FBPA and boron concentration in tumors remains unclear. The present study investigated the transport mechanism of ^{18}F-FBPA and BPA, and evaluated the utility of ^{18}F-FBPA PET in predicting boron concentration in tumors. The transporter assay revealed that 2-aminobicyclo-(2.2.1)-heptane-2-carboxylic acid, an inhibitor of the L-type amino acid transporter, significantly inhibited ^{18}F-FBPA and ^{14}C-4-borono-L-phenylalanine (^{14}C-BPA) uptake in FaDu and LN-229 human cancer cells. ^{18}F-FBPA uptake strongly correlated with ^{14}C-BPA uptake in 7 human tumor cell lines ($r = .93$; $P < .01$). PET experiments demonstrated that tumor uptake of ^{18}F-FBPA was independent of the administration method, and uptake of ^{18}F-FBPA by bolus injection correlated well with BPA uptake by continuous intravenous infusion. The results of this study revealed that evaluating tumor uptake of ^{18}F-FBPA by PET was useful for estimating ^{10}B concentration in tumors.

KEYWORDS
^{10}B-4-borono-L-phenylalanine, 4-borono-2-^{18}F-fluoro-phenylalanine, boron neutron capture therapy, L type amino acid transporter, PET

1 | INTRODUCTION

Boron neutron capture therapy (BNCT) is a cancer therapy based on the nuclear capture and fission reactions of ^{10}B and low energy thermal neutrons, which produces high energy α particles and recoiling lithium. This unique targeted radiotherapy is expected to contribute to the eradication of intractable tumors such as glioblastoma multiforme.

Accumulation of ^{10}B in tumors at a concentration of >15 ppm and a tumor-to-normal tissue (T/N) ratio >3-5 are required for successful BNCT.[1,2] Therefore, an effective ^{10}B carrier is required; currently, ^{10}B-4-borono-L-phenylalanine (BPA) is widely used for this

purpose. The boron concentration in tumors depends on tumor characteristics, including the expression level of transporters and tumor vascularity; thus, the pharmacokinetics of BPA should be determined in individual patients. To estimate BPA accumulation and the T/N ratios, PET using 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA PET) has been performed.[3-7] Previously, we reported that ^{18}F-FBPA was predominantly transported by L-type amino acid transporter (system L).[8] In rat 9L gliosarcoma cells, 2-aminobicyclo-(2.2.1)-heptane-2-carboxylic acid (BCH) inhibited BPA uptake, which suggested that system L played a role in this phenomenon.[9] However, the transport and uptake of ^{18}F-FBPA have not been compared with those of BPA in detail.

In addition, there are 2 differences between ^{18}F-FBPA in PET and BPA in BNCT. First, the administration dose of BPA is 250-500 mg/kg compared with tracer doses of ^{18}F-FBPA in PET.[10,11] Second, BPA is administrated via continuous intravenous infusion (CIV), whereas ^{18}F-FBPA is administrated via bolus injection. Owing to the difficulty of excising tumor tissue samples from patients with glioblastoma for evaluation of boron concentration in tumor tissues, it remains unclear whether tumor uptake of ^{18}F-FBPA is associated with that of BPA.

The present study verified whether the transport mechanism of ^{18}F-FBPA is identical to that of BPA. Subsequently, we determined the tumor uptake of ^{18}F-FBPA and BPA in human tumor xenograft models. The present study also investigated the utility of ^{18}F-FBPA PET for estimation of BPA-derived boron concentrations in tumors.

2 | MATERIALS AND METHODS

2.1 | Cell culture and animal model

In this study, the LN-18, LN-229, U-87 MG, U-118 MG and U-251 MG human glioblastoma cell lines, A-253 epidermoid carcinoma cell line and FaDu squamous cell carcinoma cell line were used. A-253, FaDu, LN-18, LN-229, U-87 MG and U-118 MG were obtained from American Type Culture Collection (Manassas, VA, USA); and U-251 MG was supplied by the European Collection of Authenticated Cell Cultures (Salisbury, UK). All cells were cultured according to the suppliers' protocols and were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C. Six human tumor xenograft models were used in the biodistribution and small animal PET/computed tomography (CT) studies. Female BALB/c nude mice and female SCID mice (4-5 weeks old) were obtained from CLEA Japan (Tokyo, Japan). Prior to the experiments, the mice were acclimatized for ≥1 week. The 6 tumor cell lines (A253, FaDu, LN-229, U-87 MG, U-118 MG, U-251 MG; 5-10 × 10^{6} cells) suspended at a 1:1 ratio of culture media/Matrigel (Corning, Bedford, MA, USA) were subcutaneously injected into the shoulders of the mice. The protocol was approved by the Committee for Ethics of Animal Experimentation of the National Cancer Center, Tokyo, Japan. Animal experiments were performed in accordance with the Guidelines for the Care and Use of Experimental Animals established by the Committee.

2.2 | In vitro uptake of 4-borono-2-^{18}F-fluoro-phenylalanine and ^{14}C-4-borono-L-phenylalanine

In vitro uptake analyses were performed as previously described, with minor modifications.[2] ^{18}F-FBPA was synthesized by direct electrophilic radiofluorination of L-p-boronophenylalanine using ^{18}F-acetyl hypofluorite as previously described.[2] ^{14}C-4-borono-L-phenylalanine (^{14}C-BPA) was purchased from NEMOTO SCIENCE (Tokyo, Japan). The sodium-containing assay buffer comprised PBS (Na^{+}-PBS) supplemented with 137 mmol/L NaCl, 2.7 mmol/L KCl, 8 mmol/L Na_2HPO_4, 1.5 mmol/L KH_2PO_4, 5.6 mmol/L D-glucose, 0.9 mmol/L $CaCl_2$ and 0.5 mmol/L $MgCl_2$. In the sodium-free assay buffer (Na^{+}-free PBS), NaCl and Na_2HPO_4 were replaced with choline chloride and K_2HPO_4, respectively. Following removal of the culture medium, the cells were pre-incubated with 500 μL assay buffer for 10 minutes at 37°C. The cells were subsequently incubated with 500 μL assay buffer supplemented with ^{18}F-FBPA (185 KBq) or ^{14}C-BPA (18.5 kBq) for 5 minutes at 37°C. Then the cells were washed twice with ice-cold assay buffer and the cell numbers were counted. Following dissolution in 0.1 N NaOH, the radioactivity was determined using a gamma counter (AccuFLEX γ7001; Aloka, Tokyo, Japan) or a liquid scintillation counter (Tri-Carb 3110TR; PerkinElmer, Waltham, MA, USA). The transport systems of ^{18}F-FBPA and ^{14}C-BPA were characterized by an inhibition assay using the following inhibitors: BCH (Sigma-Aldrich, Tokyo, Japan) for system L and 2-(methylamino)-isobutyric acid (MeAIB; Sigma-Aldrich) for system A. The contribution of Na^{+}-independent amino acids transporters to ^{18}F-FBPA and ^{14}C-BPA uptake was investigated using Na^{+}-free PBS.

2.3 | Western blot analysis

The expression levels of the 3 isoforms of system L (LAT-1, LAT-2, LAT-4) were analyzed. Cells were lysed in RIPA buffer (Thermo Fisher Scientific, Rockford, IL, USA) supplemented with a protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Protein concentration was determined using the BCA Protein Assay Reagent Kit (Thermo Fisher Scientific). Lysates were separated by 4%-20% SDS-PAGE and transferred onto a PVDF membrane (Bio-Rad Laboratories, Tokyo, Japan). Membranes were blocked for 30 minutes in Blocking One (Nacalai Tesque, Kyoto, Japan) and subsequently incubated with primary and secondary antibodies. Rabbit monoclonal anti-SLC7A5 (LAT1; clone ID: EPR3492(2); Abcam, Cambridge, UK), rabbit polyclonal anti-SLC7A8 (LAT2; Medical & Biological Laboratories, Nagoya, Japan), rabbit polyclonal anti-SLC43A2 (LAT4; Abcam) and rabbit monoclonal anti-GAPDH (clone ID: 14C10; Cell Signaling Technology, Tokyo, Japan) were the primary antibodies used. HRP-conjugated anti-rabbit IgG (GE Healthcare, Tokyo, Japan) was the secondary antibody used. The membranes were treated with ECL Prime Western Blotting Detection Reagent (GE Healthcare), and the protein bands were detected using the ChemiDoc Imaging System (Bio-Rad Laboratories).

2.4 | Biodistribution of 4-borono-2-^{18}F-fluoro-phenylalanine

Tumor-bearing mice were injected via the tail vein with 150 kBq/100 μL ^{18}F-FBPA. The mice were killed at 0.5 and 1 hour post-injection, after which the tissue samples were excised. Tissue samples were weighed, and radioactivity was determined using a γ-counter. Uptake in organs is expressed as the percentage of injected dose per gram of tissue (% ID/g).

2.5 | Small animal PET/computed tomography imaging

The mice were anesthetized by inhalation of 1.5%-2% isoflurane. The pharmacokinetics of bolus injection of ^{18}F-FBPA was compared with that of CIV of ^{18}F-FBPA with or without BPA (250 mg/kg body weight; Stella Pharma, Osaka, Jpan). For the bolus injection study, tumor-bearing mice were intravenously injected with 1.5-7.4 MBq/100 μL ^{18}F-FBPA. For the CIV study, 2.7-7.0 MBq/250 μL ^{18}F-FBPA with or without BPA was intravenously injected at a rate of 8.33 μL/min for 30 minutes using a syringe pump (Harvard Apparatus, Holliston, MA, USA). The BPA solution was prepared using a complex with fructose.

PET/CT images were obtained using a small animal PET/CT scanner (Inveon; Siemens Medical Solutions, Knoxville, TN, USA). A series of dynamic PET images were acquired for 60 minutes consisting of 15 frames (10 × 60 s, 5 × 600 s), starting from the initiation of ^{18}F-FBPA injection. CT imaging was performed prior to PET acquisition. The images were reconstructed using 3-D ordered subset expectation maximization (OSEM3D; 2 iterations and 2 MAP iterations), including scatter and attenuation correction with Inveon Acquisition Workplace software (Siemens Medical Solutions). Regions of interest (ROI) were manually drawn over the tumor, brain, heart, kidneys and liver. Tracer uptake was quantified as the mean standard uptake value (SUV$_{mean}$) of each ROI determined using ASIPro software (CTI Molecular Imaging/Siemens).

2.6 | Boron evaluation in tumor-bearing mice

A total of 250 μL BPA (250 mg/kg body weight) was injected via the tail vein at a rate of 8.33 μL/min for 30 minutes using the syringe pump. The mice were sacrificed 1 hour after initiation of BPA injection, and the blood, brain and tumor were excised. The tissue samples were digested with perchloric acid and hydrogen peroxide (2:1) at 75°C. The boron concentrations in each sample were evaluated by inductively coupled plasma-atomic emission spectrometry (SPS3500DD; SII Nanotechnology, Tokyo, Japan), and were normalized as ppm.

2.7 | Statistical analysis

Results are expressed as the mean ± SD. Statistical analysis was performed using Graph Pad Prism 5 (Graph Pad Software, San Diego, CA, USA). The Pearson coefficients were calculated for the correlations between ^{18}F-FBPA uptake and ^{14}C/^{10}B-BPA uptake. $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Transport assay of 4-borono-2-^{18}F-fluoro-phenylalanine and ^{14}C-4-borono-L-phenylalanine

The inhibition analysis revealed that BCH significantly inhibited the uptake of ^{18}F-FBPA and ^{14}C-BPA in FaDu and LN-229 cells (Figure 1). Sodium-independent BCH-specific uptake of ^{18}F-FBPA was 71.1% and 78.4% in LN-229 and FaDu cells, respectively, and that of ^{14}C-BPA was 68.1% and 81.3% of total uptake in LN-229 and FaDu cells, respectively. Conversely, sodium-dependent uptake of ^{18}F-FBPA and ^{14}C-BPA was 7.2%-17.9%, and MeAIB-specific uptake of ^{18}F-FBPA and ^{14}C-BPA was <17.7%.

3.2 | Uptake of 4-borono-2-^{18}F-fluoro-phenylalanine and ^{14}C-4-borono-L-phenylalanine and expression levels of amino acid transporters

The uptake analysis revealed that ^{18}F-FBPA was significantly associated with ^{14}C-BPA (Figure 2A). The ^{18}F-FBPA and ^{14}C-BPA uptake varied among tumor cell lines (^{18}F-FBPA: 1.2%-7.3% AD/10^5 cells; ^{14}C-BPA: −1.2%-6.3% AD/10^5 cells). The expression levels of LAT1, 2 and 4 were analyzed by western blotting (Figure 2B). LAT1 was highly expressed in A-253, FaDu and LN-229 cells, with high uptake of ^{18}F-FBPA and ^{14}C-BPA. LAT2 expression was observed in U-87 MG, U-118 MG and U-251 MG cells. Expression of LAT4 was only found in U-87 MG and U-118 MG cells.

3.3 | Biodistribution of 4-borono-2-^{18}F-fluoro-phenylalanine in tumor-bearing mice

The biodistribution analysis indicated high uptake of ^{18}F-FBPA in the kidney (21.79% ± 2.02% ID/g at 30 minutes and 12.72% ± 1.19% ID/g at 60 minutes) and pancreas (40.46% ± 4.31% ID/g and 29.46% ± 6.73% ID/g at 60 minutes; Figure 3A). Tumor uptake, and the tumor-to-blood (T/Bl), tumor-to-brain (T/Br) and tumor-to-muscle (T/M) ratios of ^{18}F-FBPA were investigated in 6 human tumor-bearing mice models (Figure 3B-E). High accumulation levels of ^{18}F-FBPA were demonstrated in A-253 (9.9% ID/g at 30 and 60 minutes) and FaDu (8.5% and 10.9% ID/g at 30 and 60 minutes, respectively), which revealed the high expression level of LAT1. There was no apparent tumor clearance of ^{18}F-FBPA 30-60 minutes after injection. In these mice models, ^{18}F-FBPA demonstrated high T/Bl, T/Br and T/M ratios.

3.4 | Pharmacokinetics of 4-borono-2-^{18}F-fluoro-phenylalanine by bolus injection, continuous intravenous infusion, and continuous intravenous infusion with ^{10}B-4-borono-L-phenylalanine

The pharmacokinetics of ^{18}F-FBPA by bolus injection and CIV with or without co-injection of BPA were compared in LN-229-bearing mice model (Figure 4). In all of the injection protocols, the
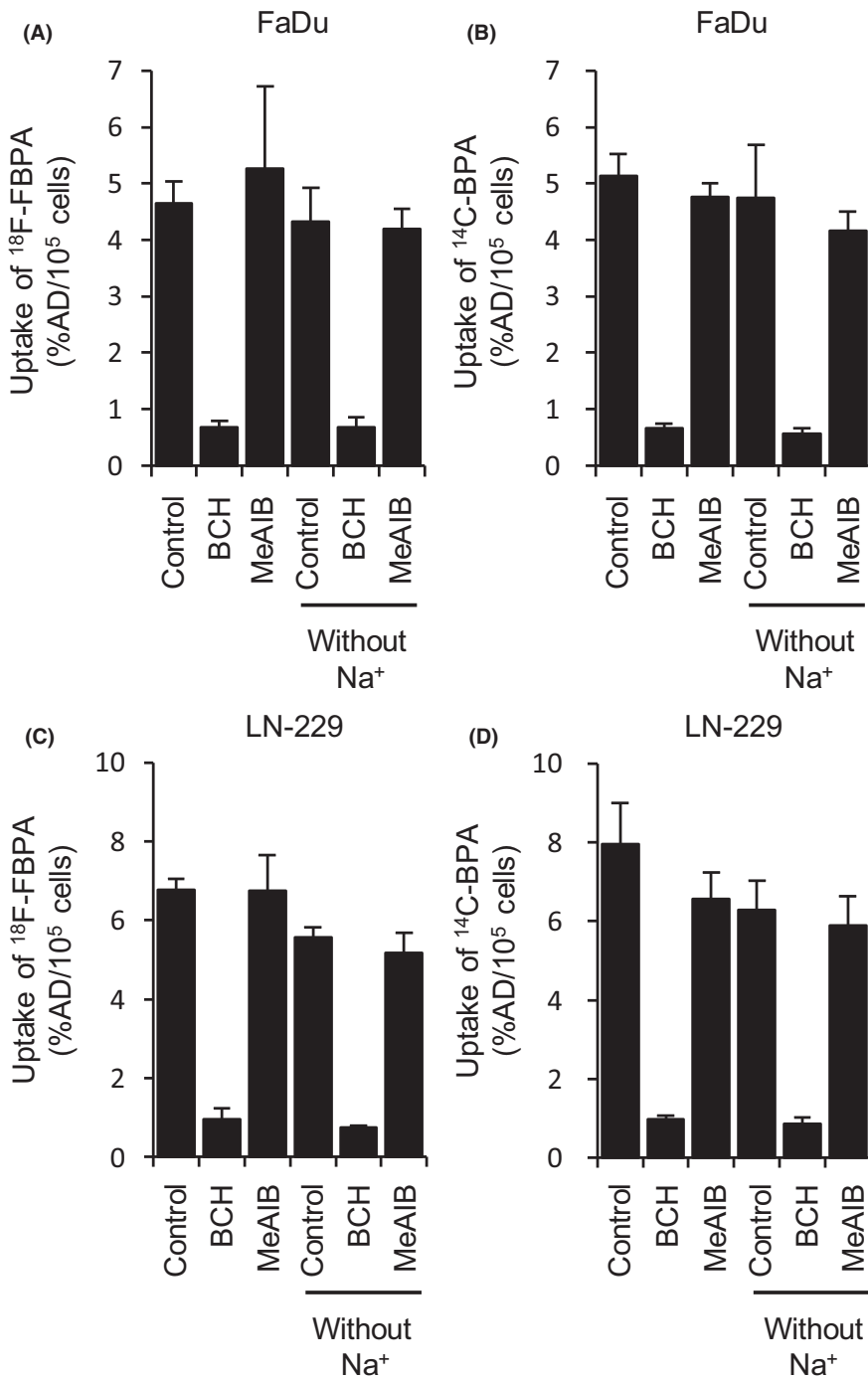
FIGURE 1 Effects of 2-aminobicyclo-(2.2.1)-heptane-2-carboxylic acid (BCH) and MeAIB on the uptake of ^{18}F-FBPA (A and C) and ^{14}C-BPA (B and D) in LN-229 and FaDu cells (n = 3-4). Cells were incubated in Na$^+$-PBS or Na$^+$-free PBS. Data are represented as the mean $\pm$ SD

kidney, liver and LN-229 were clearly visualized by PET (Figure 4A,C,E). Following bolus injection, initial high uptake was observed in the heart, kidney and liver 5 minutes after injection (Figure 4B). The radioactivity was rapidly cleared from the heart, kidney and liver during PET scanning. The high radioactivity in the kidney was sustained for 1 hour (1.97 at 55 minutes).

Continuous intravenous infusion resulted in the continuous uptake of ^{18}F-FBPA to tissues <25-35 minutes (Figure 4D). No significant difference in SUV at 55 minutes in the heart, kidney, liver,

brain and LN-229 between bolus injection and CIV were revealed (1.03 vs 1.13 in the heart; 1.97 vs 1.91 in the kidney; 1.47 vs 1.54 in the liver, 0.49 vs 0.33 in the brain, and 1.26 vs 1.22 in the LN-229).

The CIV with BPA showed similar time-activity curves with the CIV (Figure 4F). ^{18}F-FBPA uptake in the heart, kidney and liver continuously increased up to 25-35 minutes; however, the kidney showed significant high uptake with co-injection of BPA (4.92 at 55 minutes). SUV were 1.19 in the heart, 1.57 in the liver, 0.24 in the brain and 1.12 in LN-229.
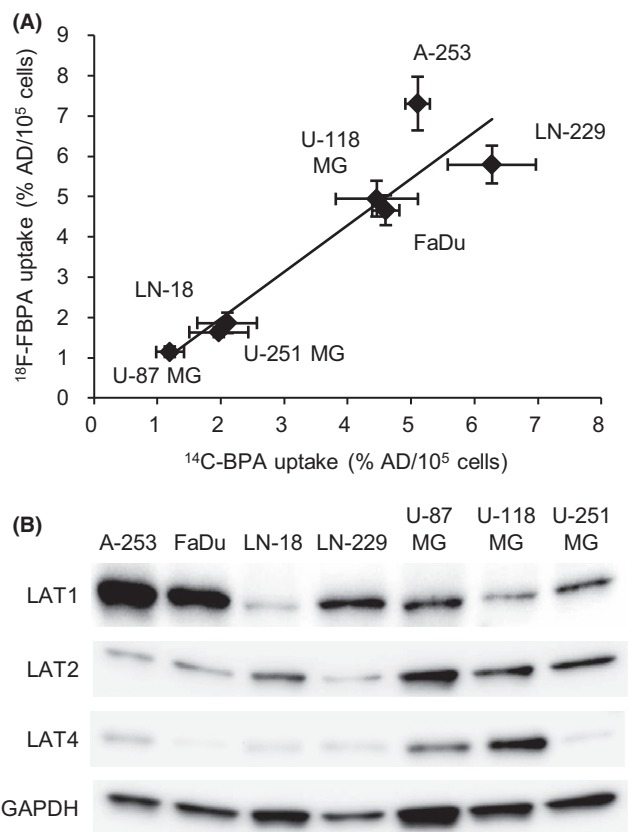
FIGURE 2 A, Correlation between ^{14}C-4-borono-L-phenylalanine (^{14}C-BPA) and 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA) uptake levels in 7 tumor cell lines (r = .93, P < .01). Data are represented as the mean ± SD. B, Expression levels of amino acid transporters (LAT1, LAT2 and LAT4) in 7 tumor cell lines. %AD/10^5 cells, percentage of the administered dose per 10^5 cells

3.5 | Comparison of tumor uptake of 4-borono-2-^{18}F-fluoro-phenylalanine between bolus injection and continuous intravenous infusion

Tumor uptake reached a plateau <25 minutes following bolus injection (Figure 5A). Only A-253 demonstrated continuous uptake during PET scanning. The SUV of tumors by bolus injection were 0.79-1.99 at 55 minutes. CIV progressively increased tumor uptake during PET scanning (Figure 5B). The SUV of tumors by CIV were 1.05-1.63 at 55 minutes. Based on the tumor uptake of ^{18}F-FBPA at 55 minutes, a correlation analysis between the both administration protocols was conducted. A significant association was revealed between tumor uptake of ^{18}F-FBPA by bolus injection and by CIV (r = .92, P < .01; Figure 5C).

3.6 | Association between ^{10}B-4-borono-L-phenylalanine and 4-borono-2-^{18}F-fluoro-phenylalanine uptakes in tumor tissues

Biodistribution analysis of BPA was performed to evaluate the boron concentration in the blood, brain and tumors. Boron concentration in tumors ranged from 7.83 to 16.54 ppm (Figure 6). Boron
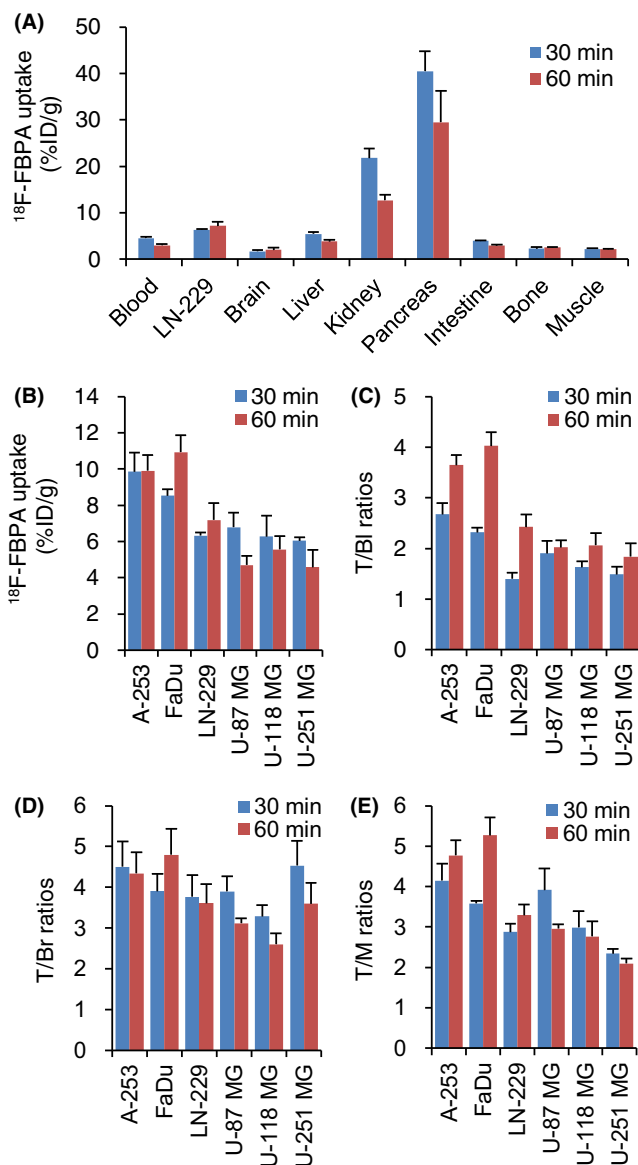

FIGURE 3 Biodistribution of 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA) in tumor-bearing mice at 30 and 60 minutes after the injection. A, ^{18}F-FBPA uptake in LN-229 and normal tissues, B, 6 tumor uptakes of ^{18}F-FBPA, C, T/Bl ratios, D, T/Br ratios and E, T/M ratios. ^{18}F-FBPA was injected via the tail vein by the bolus injection. Data are represented as mean ± SD; n = 3-4. T/Bl, tumor-to-blood; T/Br, tumor-to-brain; T/M, tumor-to-muscle

concentration in the brain was low (1.58-3.36 ppm). Boron concentration in tumors was associated with ^{18}F-FBPA uptake regardless of the administration method (Figure 7).

4 | DISCUSSION

The selection of patients with high BPA uptake in tumors or high T/N tissue ratios of boron concentration is essential for successful BNCT. The pharmacokinetics of BPA and the expression of amino acid transporters vary among individuals; therefore, non-invasive
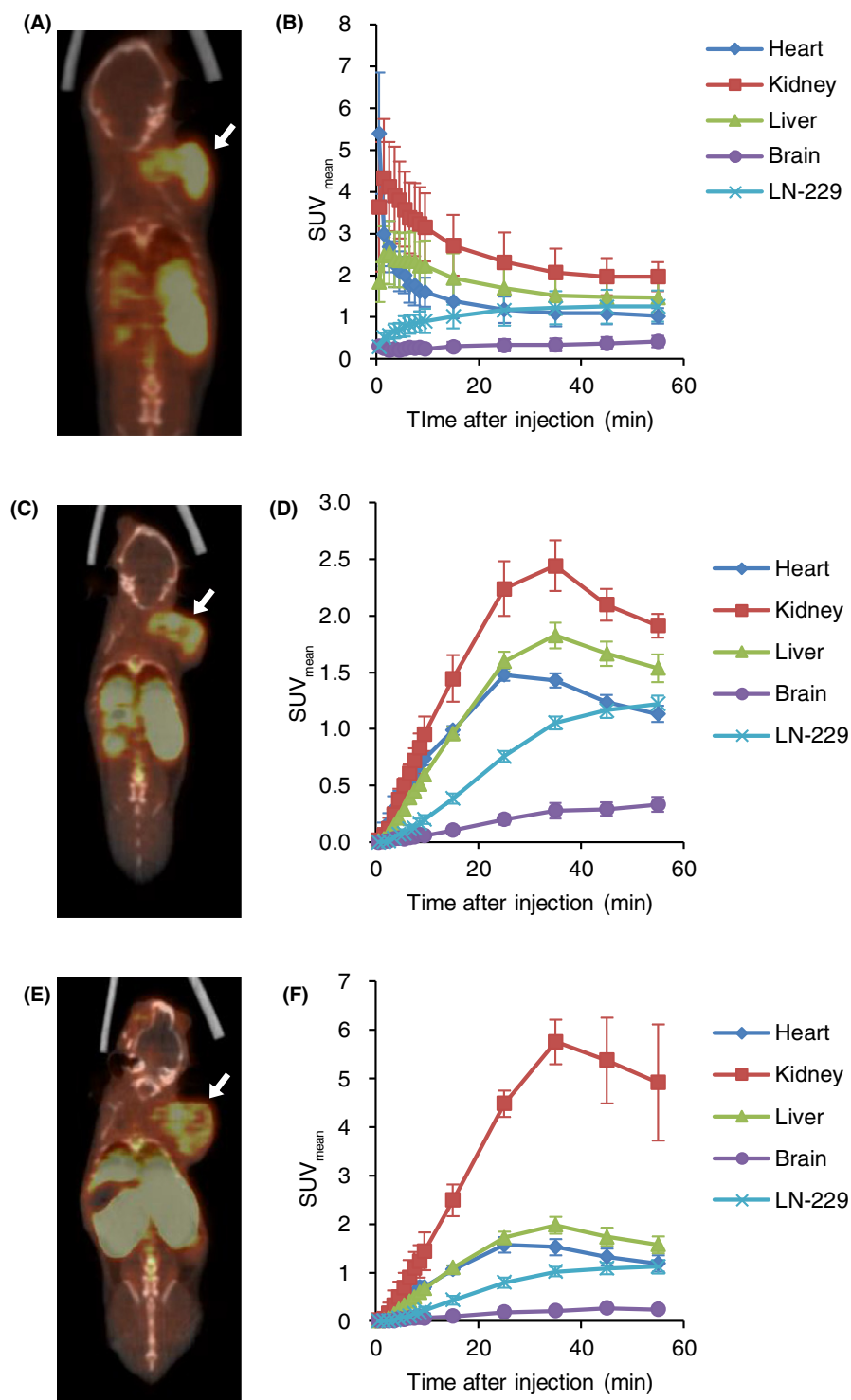
FIGURE 4 Comparison of the pharmacokinetics of 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA) by different injection protocols. ^{18}F-FBPA was injected by bolus injection (A and B), and continuous intravenous infusion (C and D). ^{18}F-FBPA was also co-injected with ^{10}B-4-borono-L-phenylalanine (BPA) (250 mg/kg) by continuous intravenous infusion (E and F). Representative PET/computed tomography (CT) images at 50-60 minutes of the same LN-229-bearing SCID mouse (A,C,E). White arrows indicate LN-229 tumors. Time-activity curves (n = 3-4) (B,D, F)

methods are needed to estimate BPA uptake in tumors and the surrounding normal tissues. To estimate BPA uptake in tumors, ^{18}F-FBPA PET has been performed in clinical studies. An FBPA PET study of healthy volunteers found that boron concentration from ^{18}F-FBPA uptake in the brain was comparable to that shown in previous reports.[12] However, it has remained unclear if tumor uptake of ^{18}F-FBPA is associated with boron concentration in tumors. To the best of our knowledge, this is the first study to investigate the

transport mechanisms of BPA and ^{18}F-FBPA and to compare their tumor uptakes based on clinical protocol.

To investigate the similarity between ^{18}F-FBPA and BPA, this study compared the transport mechanisms of ^{18}F-FBPA and ^{14}C-BPA. In the inhibition analysis, BCH significantly inhibited the uptake of ^{18}F-FBPA and ^{14}C-BPA in LN-229 and FaDu cells, which suggested that system L predominantly contributed to their transport. Our previous results showed that system L was mainly involved in
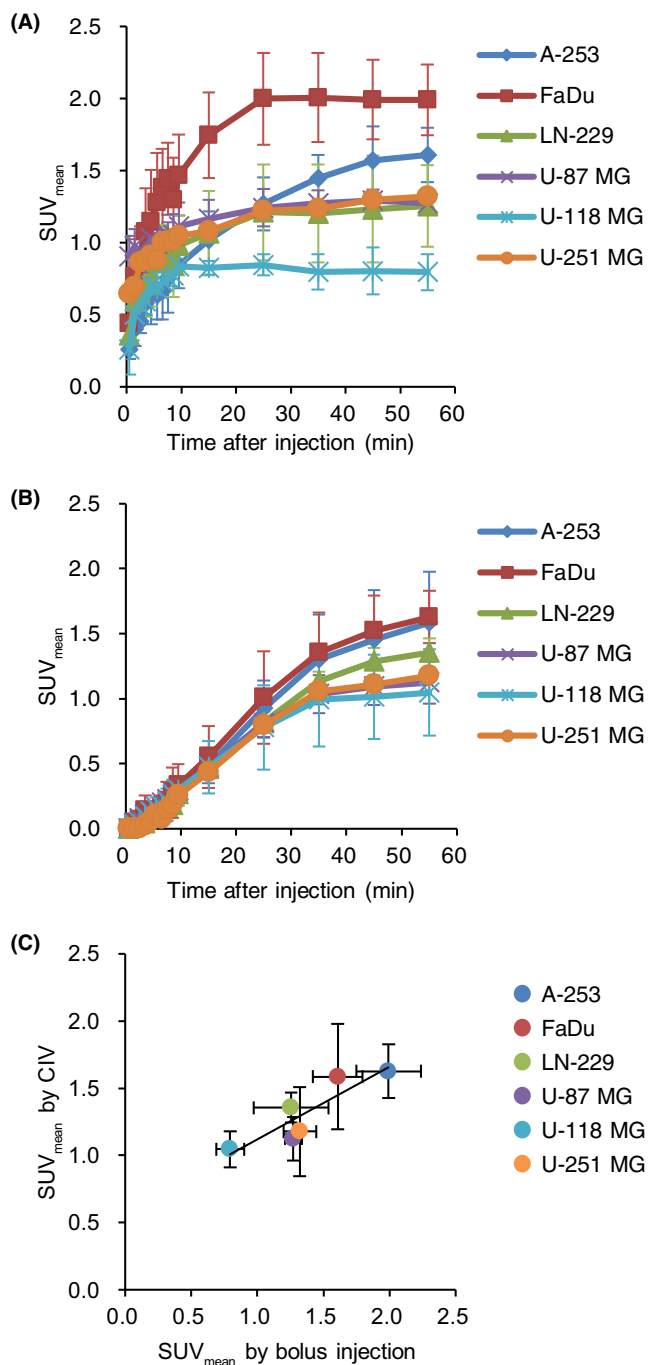
FIGURE 5 A, Time-activity curves of 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA) in tumors by bolus injection of ^{18}F-FBPA (n = 3-4/group). B, Time-radioactivity curves in tumors by continuous intravenous infusion (CIV) of ^{18}F-FBPA (n = 3-4/group). C, Correlation of tumor uptakes (50-60 minutes) by bolus injection and CIV (r = .92, P < .01)
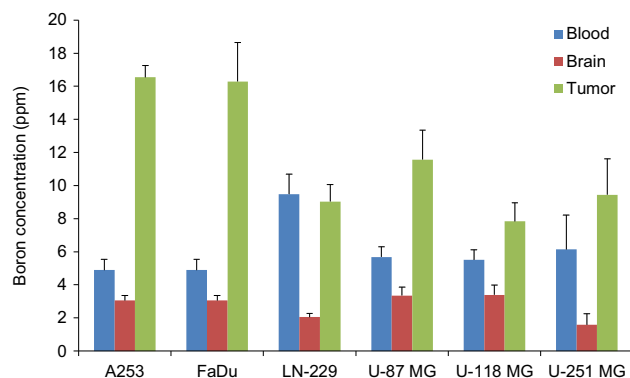

FIGURE 6 Boron concentrations in blood, brain, and tumors at 60 minutes. [10]B-BPA-fructose was injected via the tail vein using the syringe pump (8.33 μL/min for 30 minutes). Boron concentration in tissues was measured by ICP-AES. Data are represented as the mean ± SD, n = 3-4

cell lines analyzed. In addition, this supports the hypothesis that ^{18}F-FBPA PET may be useful for estimating BPA concentration in tumor tissues.

LAT1, LAT2, LAT3 and LAT4 isoforms of system L have previously been identified.[14-17] Western blot analysis in this study suggests that LAT1 may predominantly contribute to ^{18}F-FBPA and ^{14}C-BPA uptake. LAT1 was abundantly expressed in A-253, FaDu and LN-229 cells, with high uptake of ^{18}F-FBPA and ^{14}C-BPA. Detta et al[13] demonstrate that LAT1 is involved in the transport of BPA. Wongthai et al[18] reveal that BPA is transported by LAT2 and LAT1, but not LAT3 or LAT4. Although this study did not provide evidence for the involvement of LAT2 and LAT4 in the uptake of ^{18}F-FBPA and ^{14}C-BPA, inhibition analysis using short interfering RNA would directly elucidate the role that system L isoforms serve in their uptake.

To estimate BPA accumulation in tumors, ^{18}F-FBPA PET is widely used in clinical settings.[19,20] Because the administration protocols and the doses are different from those of BPA, the association between ^{18}F-FBPA and BPA has been a subject of debate. BPA (250-500 mg/kg) is administered via 2-hours intravenous infusion in BNCT, whereas ^{18}F-FBPA is administered at a tracer dose (pmol-μmol) via intravenous bolus injection in PET.[5,10,11] Therefore, we compared ^{18}F-FBPA uptake via bolus injection, CIV, and CIV with co-injection of BPA. PET analysis revealed no difference in their distribution patterns 50-60 minutes after injection, with the exception of the kidney. CIV of ^{18}F-FBPA with co-injection of BPA significantly enhanced uptake in the kidney. The CIV of BPA temporarily increases BPA concentration in the blood. In addition, amino acid tracers including ^{18}F-FBPA show physiologically high uptake in the kidney and are excreted in urine.[21,22] Therefore, to recover the enhanced blood concentration of amino acids to physiological levels, ^{18}F-FBPA would need to be markedly excreted together with BPA.

Unexpectedly, the co-injection of BPA did not inhibit ^{18}F-FBPA uptake in tumors. The BPA concentration in the blood would not be sufficient for the inhibition of ^{18}F-FBPA. Previously, we reported that more than 100 μmol/L BPA significantly inhibited ^{18}F-FBPA

the tumor uptake of ^{18}F-FBPA in 3 glioblastoma cell lines (74.5%-81.1% of total uptake), supporting the results of this study.[8] Previous studies have also indicated that BPA is transported by system L.[9,13] This similarity in transport mechanisms resulted in an association between the uptake of ^{18}F-FBPA and ^{14}C-BPA in the 7 tumor
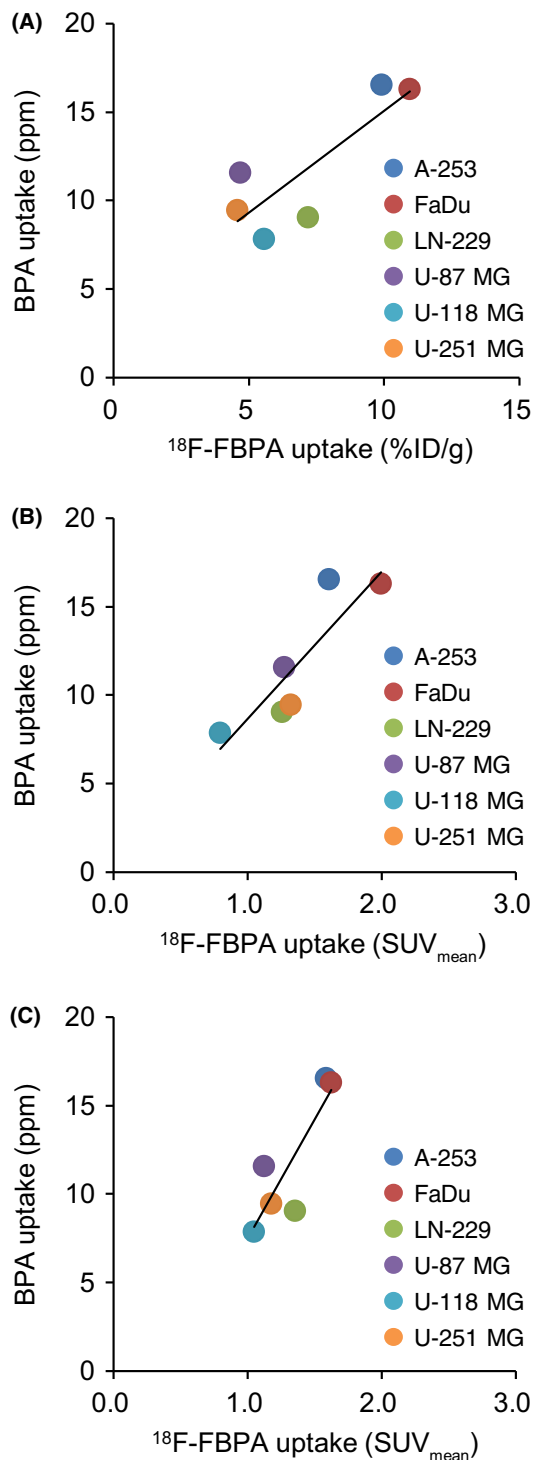
FIGURE 7 A, Correlation between [10]B-4-borono-L-phenylalanine (BPA) uptake and 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-FBPA) uptake 60 minutes after injection in the biodistribution analysis ($r = .83$, $P < .05$). B, Association between BPA and ^{18}F-FBPA uptakes 50-60 minutes after intravenous bolus injection obtained in the PET analysis ($r = .88$, $P < .05$). C, Correlation between BPA and ^{18}F-FBPA uptakes 50-60 minutes after continuous intravenous infusion (CIV) obtained in the PET analysis ($r = .86$, $P < .05$)

uptake in tumor cells.[8] In this study, ^{18}F-FBPA was co-injected with 110 mmol/L BPA at a rate of 8.33 μL/min for 30 minutes. This slow injection decreased the blood concentration of BPA to insufficient levels to inhibit ^{18}F-FBPA uptake.

In addition to the similarity in biodistribution in normal tissues, the tumor uptake of ^{18}F-FBPA via bolus injection significantly correlated with that via CIV 50-60 minutes after injection. These results indicate that bolus injection of ^{18}F-FBPA may be useful for estimating the biodistribution of BPA via CIV.

The accumulation of ^{18}F-FBPA in tumors administered by these 2 methods was not identical. The use of CIV resulted in lower uptake, particularly in FaDu cells with high LAT1 expression levels. This may have been induced by the lower input by CIV. The bolus injection rapidly increased the radioactivity in blood; subsequently, radioactivity in the tumors peaked soon after the injection. Conversely, CIV maintained radioactivity in the blood at a lower level via infusion. In addition, the rapid renal excretion of ^{18}F-FBPA may have also suppressed the increase in radioactivity in the blood, and the low radioactivity in blood would induce a decrease in tumor uptake via CIV of ^{18}F-FBPA.

An advantage of CIV in animal studies is that it can be used to reproduce human pharmacokinetics of BPA. In particular, BPA concentration in the blood may affect its tissue distribution. Boron concentration in the blood continued to increase during infusion in a previous clinical study.[10] In our PET analysis, CIV of ^{18}F-FBPA also showed a continuous increase of uptake in the heart, suggesting an increase in the blood concentration of ^{18}F-FBPA. In previous animal studies, BPA has been intraperitoneally administered due to the ease of the experimental procedure.[23-27] However, the pattern of increase in the blood remains unknown, and it is difficult to control blood concentration by intraperitoneal injection.

Our animal studies demonstrated that ^{18}F-FBPA uptake was significantly associated with boron concentration in 6 tumors, despite the fact that the administered dose of ^{18}F-FBPA is different from that of BPA. Hanaoka et al[28] recently revealed an association between BPA and ^{18}F-FBPA uptakes by bolus injection in RGC-6 rat glioma and normal tissues. Wang et al also[29] demonstrated that tumor uptake of BPA or ^{18}F-FBPA reached maximum levels at 1 hour following bolus injection, which indicated their similar pharmacokinetics. Conversely, Watanabe et al[30] compared the pharmacokinetics of ^{19}F-FBPA and BPA by subcutaneous injection of 500 mg/kg. The boron concentrations in tumor and normal tissues were the same at 0.5, 1, 2, 3 and 4 hours after injection. These results suggest that a therapeutic dose of BPA and ^{18}F-FBPA accumulated in tumor cells in a system L-dependent manner. BPA accumulation in tumor stroma and cellular uptake by passive diffusion would be negligible.

Recently, a practical calculation method was developed to estimate the absolute boron concentration in tissues after BPA administration using ^{18}F-FBPA PET. Watabe et al[31] report that boron concentrations calculated based on SUV were equal to the measured

boron concentration after BPA administration in a rat model. Our results, which showed that the SUV of tumors measured by PET was well correlated with BPA uptake, are in accordance with that study.

In addition to selection of the BNCT candidates, determination of irradiation timing of neutrons is required for BNCT. Neutrons should be irradiated when there is high boron concentration in tumors or high T/N ratios, to provide curable dosimetry to tumors without exposure to the surrounding normal tissues. The dynamic PET analysis of this study compared the bolus injection with CIV and revealed that the pharmacokinetics of ^{18}F-FBPA were distinctly different. Therefore, the pharmacokinetics of ^{18}F-FBPA by bolus injection is required to compare with those by the same protocol as BPA administration in the clinical study. The further analysis of pharmacokinetics would lead to the determination of irradiation timing by conventional ^{18}F-FBPA PET.

In conclusion, this study demonstrated that tumor uptake of ^{18}F-FBPA was strongly associated with boron concentration in tumors in human tumor xenograft models. Therefore, ^{18}F-FBPA PET may be applied to estimate boron concentration and T/N ratios in BNCT. ^{18}F-FBPA PET may provide information for selection of patients and prediction of the therapeutic effects; however, further clinical investigation is required to compare tumor uptake of ^{18}F-FBPA with the therapeutic effects of BNCT.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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