



# Pharmacokinetics of BPA in Gliomas with Ultrasound Induced Blood-Brain Barrier Disruption as Measured by Microdialysis

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## Abstract

The blood-brain barrier (BBB) can be transiently disrupted by focused ultrasound (FUS) in the presence of microbubbles for targeted drug delivery. Previous studies have illustrated the pharmacokinetics of drug delivery across the BBB after sonication using indirect visualization techniques. In this study, we investigated the *in vivo* extracellular kinetics of boronophenylalanine-fructose (BPA-f) in glioma-bearing rats with FUS-induced BBB disruption by microdialysis. After simultaneous intravenous administration of BPA and FUS exposure, the boron concentration in the treated brains was quantified by inductively coupled plasma mass spectroscopy. With FUS, the mean peak concentration of BPA-f in the glioma dialysate was 3.6 times greater than without FUS, and the area under the concentration-time curve was 2.1 times greater. This study demonstrates that intracerebral microdialysis can be used to assess local BBB transport profiles of drugs in a sonicated site. Applying microdialysis to the study of metabolism and pharmacokinetics is useful for obtaining selective information within a specific brain site after FUS-induced BBB disruption.

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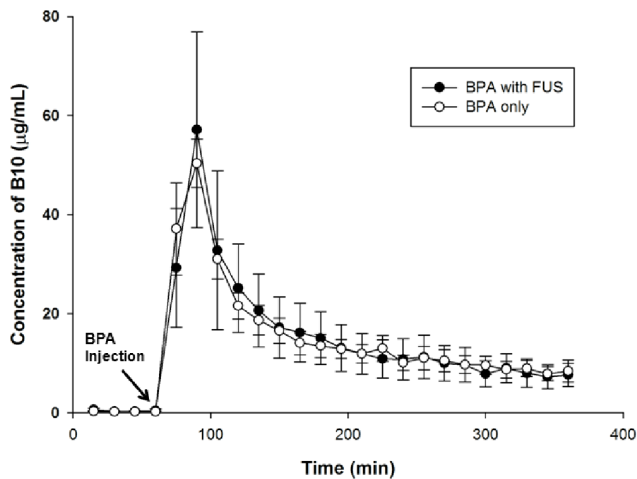
## Introduction

The blood-brain barrier (BBB) is a highly specialized endothelial structure occurring along the brain capillaries [1]. The transport of many drugs from the blood into the brain tissue is limited by the BBB. Several methods have been developed to circumvent this barrier in order to enhance drug delivery into the brain, such as the chemical modification of drugs, the osmotic opening of tight junctions, and the direct injection of the therapeutic agent or agents into the targeted brain area [2]. Recent studies have shown that focused ultrasound (FUS) can enhance the delivery of chemotherapeutic drugs into brain tumors and improve antitumor effects due to FUS-induced BBB disruption [3,4]. As such, image-guided FUS technology may provide a novel strategy for targeted drug delivery in brain tumor treatment [5]. However, free drug concentrations cannot be measured if there is interference from signals produced by metabolites.

Patients with malignant gliomas have poor prognoses after radiation therapy and chemotherapy. Recently, several clinical studies have reported encouraging results in the treatment of patients with malignant brain tumors by boron neutron capture therapy (BNCT) [6–8]. For successful BNCT, sufficient neutrons and a selective accumulation of boron-10 (<sup>10</sup>B) must be delivered to the tumor site [9,10]. The major challenge of boron delivery has

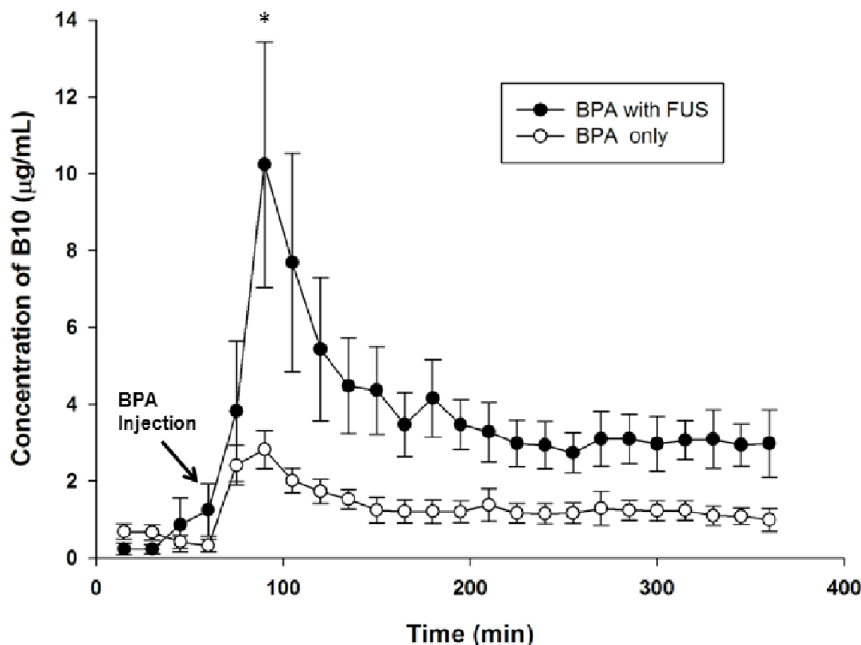
been achieving sufficiently accurate tumor targeting to deliver therapeutic levels of boron to the tumor with minimal risk of toxicity to normal tissues. Therefore, chemical synthetic techniques and hyperosmotic BBB opening methods have been used to deliver the boron concentration into the brain tumor tissue for enhanced treatment efficacy [11–13]. However, these techniques produce dose-limiting side effects because they result in the boron concentration being increased throughout the whole brain.

The influx and efflux transport processes across the BBB are considered a key factor influencing the brain extracellular fluid (ECF) concentration of certain drugs. Intracerebral microdialysis is a tool to monitor the concentrations of free drugs and endogenous compounds at specific sites within the brain [14,15]. Drug concentrations in brain dialysate reflect concentrations in brain ECF. Using intracerebral microdialysis, simultaneous measurements of the brain ECF concentration and the plasma concentration can produce useful information for evaluating the pharmacokinetics of drugs that have crossed the BBB. In a previous study, microdialysis was used to continuously assess the concentration of boronophenylalanine (BPA) in the ECF of the brain tumor tissue in patients undergoing BNCT for glioblastoma treatment [16]. The data showed that the uptake of BPA in the ECF of the tumor tissue was clearly higher than in the ECF of normal brains.



**Figure 1. The boron concentration versus time profiles in the dialysate of the plasma after BPA administration at 500 mg/kg in glioma-bearing rats with or without FUS exposure.** The concentration of boron measured in each 15-min sample of plasma represents the average concentration of that interval. Data are expressed as mean  $\pm$  SEM,  $n=6$ . doi:10.1371/journal.pone.0100104.g001

Previous studies have demonstrated that BPA-fructose (BPA-f) administration assisted by FUS can increase the accumulation of boron in brain tumors and elevate the tumor-to-normal-brain boron ratio [17,18]. The purpose of this study was to evaluate the concentration-time profile of boron in brain tumors with FUS exposure after intravenous administration of BPA-f. Furthermore, the pharmacokinetics of boron in sonicated brain tumors were compared with those in non-sonicated brain tumors.



**Figure 2. Representative data for boron concentration versus time profiles in tumor ECF after BPA administration at 500 mg/kg in glioma-bearing rats with or without FUS exposure.** The concentration of boron measured in each 15-min sample of tumor ECF represents the average concentration of that interval. Data are expressed as mean  $\pm$  SEM,  $n=5$ . (\* $p<0.05$ ). doi:10.1371/journal.pone.0100104.g002

## Results

According to retrodialysis experiments, the *in vivo* relative recoveries were determined to be  $48\pm 7\%$  ( $n=3$ ) for the plasma vascular probe and  $30\pm 5\%$  ( $n=4$ ) for the brain tumor probe. The concentrations of BPA measured in the physiological samples were corrected for the relative recovery of the probe used.

The concentration versus time curves for boron in plasma before and after intravenous injection both with and without FUS exposure were almost identical (Fig. 1). The mean peak values were similar to those of the concentration profiles, in which the highest boron accumulation in the brain tumor ECF occurred at 30 min after drug injection and declined rapidly during the elimination phase.

The concentration versus time curves for boron in the brain tumor with and without FUS exposure after BPA (500 mg/kg) administration are shown in Fig. 2. The concentrations of boron in brain tumor dialysates were determined from the calibration curve. No significant difference in boron concentrations in the sonicated tumors and control tumors was found before intravenous bolus injection of BPA-f. Compared to the control tumors, a significant accumulation of boron can be seen in the sonicated tumors for a period of 5 h after BPA-f injection. The concentrations of boron in both types of tumors reached a peak at 30 min after drug administration and then declined rapidly. Furthermore, the mean peak value of boron in the ECF of the sonicated tumors was  $\sim 3.63$ -fold higher than in control tumor ECF.

Tables 1 and 2 show the pharmacokinetic parameters of boron with or without sonication in plasma and tumor ECF, respectively. Table 1 shows that no significant differences were found for the four parameters in plasma after FUS-induced BBB disruption as compared to the parameters for the BPA-alone group. Table 2 indicates that the maximum tumor ECF concentration ( $C_{tmax}$ ), MRT, and area under the concentration versus time curve (AUC) values clearly increased after FUS exposure as compared with

**Table 1.** Pharmacokinetic Parameters of BPA in Plasma after Intravenous Injection.

Parameters	BPA 500 mg/kg only	BPA 500 mg/kg with FUS
$C_{p_{max}}$ ( $\mu\text{g/mL}$ )	53.0 $\pm$ 6.6	70.8 $\pm$ 12.0
MRT (min)	208 $\pm$ 29	209 $\pm$ 46
AUC (min $\mu\text{g/mL}$ )	6412 $\pm$ 1030	7210 $\pm$ 1430
AUC/dose	12.8 $\pm$ 2.1	14.4 $\pm$ 2.9

$C_{p_{max}}$ : maximum plasma concentration ( $n=6$  for each group).

MRT: mean residence time.

AUC: area under the concentration.

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those values for the BPA administration only group. The increases in MRT (from 313 to 503) and AUC/dose (from 1.8 to 3.8) show that the retention time of BPA in the tumor was extended with FUS exposure.

## Discussion

BPA has been applied as a potential boron carrier in clinical trials with BNCT. BPA conjugated with fructose has been proven to enhance drug accumulation in the tumor due to increased solubility [19]. Non-invasive methods used for the *in vivo* determination of the local pharmacokinetics of a drug within the brain have been revolutionized by the development of positron emission tomography (PET) and nuclear magnetic resonance spectroscopy. In a previous study, PET was proposed to assess the biodistribution of  $^{18}\text{F}$ -labeled BPA-f [20].  $^{18}\text{F}$ -FBPA-f showed specific tumor uptake in F98 glioma-bearing rats, indicating that PET can be used as a probe for BPA-f in BNCT [21]. Nevertheless, the key limitation of PET scanning is the instability of the isotope, and no free drug concentrations can be obtained. Furthermore, the spatial resolution of non-invasive PET imaging techniques is limited.

Since gliomas are highly infiltrative tumors, it actually would be highly desirable to have boron delivery to infiltrating tumor cells [22]. The barrier function of the BBB can become altered after FUS exposure. Thus, the further study as to whether FUS can enhance delivery to these infiltrating tumor cells in the F98 glioma model are needed to be investigated. Many studies have investigated the pharmacokinetics of BBB permeability in brains after FUS-induced BBB disruption using dynamic contrast-enhanced (DCE)-MRI [23–25]. In addition,  $^{99\text{mTc}}$ -DTPA micro-SPECT/CT shows that FUS not only increases the permeability of the BBB significantly at the sonicated site, but also elevates the lesion-to-normal brain ratio significantly in the focal region [26]. However, non-invasive imaging can provide

neither chemical information nor information on the metabolites. Although intracerebral microdialysis has the disadvantage of being invasive, microdialysis sampling coupled to an appropriate analytical model can be used to study pharmacokinetics and metabolism in the target site. This technique has a temporal and spatial resolution suitable for evaluating neuropharmacokinetics in the brain ECF following drug administration with FUS exposure. Thus, such data may facilitate knowledge of the temporal features of pharmacological change after FUS-induced BBB disruption.

To our knowledge, this is the first *in vivo* study evaluating the local BBB transport profile in tumor ECF during FUS exposure. The BBB functionality is not significantly influenced by microdialysis probe implantation when experimental procedures are well-controlled. The results (Fig. 2) showed that FUS exposure increased the peak tumor ECF uptake value (10.23 $\pm$ 3.19  $\mu\text{g/mL}$ ) while resulting in the same peak uptake time (at 30 min after BPA injection) compared with the control tumor (2.82 $\pm$ 0.49  $\mu\text{g/mL}$ ). After FUS-induced BBB disruption, the boron concentration in tumor ECF followed the concentration in blood (Fig. 1), suggesting that neutron irradiation should be delivered as closely as possible to the time when the peak blood level is reached. In terms of the pharmacokinetic parameters (Table 2), the increases of  $C_{t_{max}}$  (from 3.8 to 6.8), MRT (from 313 to 503), and AUC/dose (from 1.8 to 3.8) imply that the absorption of BPA in the brain tumor was enhanced by FUS exposure. The major strength of intracerebral microdialysis lies in the fact that it not only measures the drug concentrations, but also monitors metabolite concentrations selectively within the targeted brain site. Thus, further investigations exploring the metabolism of BPA-f in the brain should be feasible.

This study demonstrated that the microdialysis technique is useful for the assessment of pharmacokinetics during FUS-induced BBB disruption while also not causing any severe side effects. The uptake of boron in the sonicated tumor ECF was clearly higher than that in the control tumor ECF, reaching a maximum of 3.63

**Table 2.** Pharmacokinetic Parameters of BPA in Tumor ECF after Intravenous Injection.

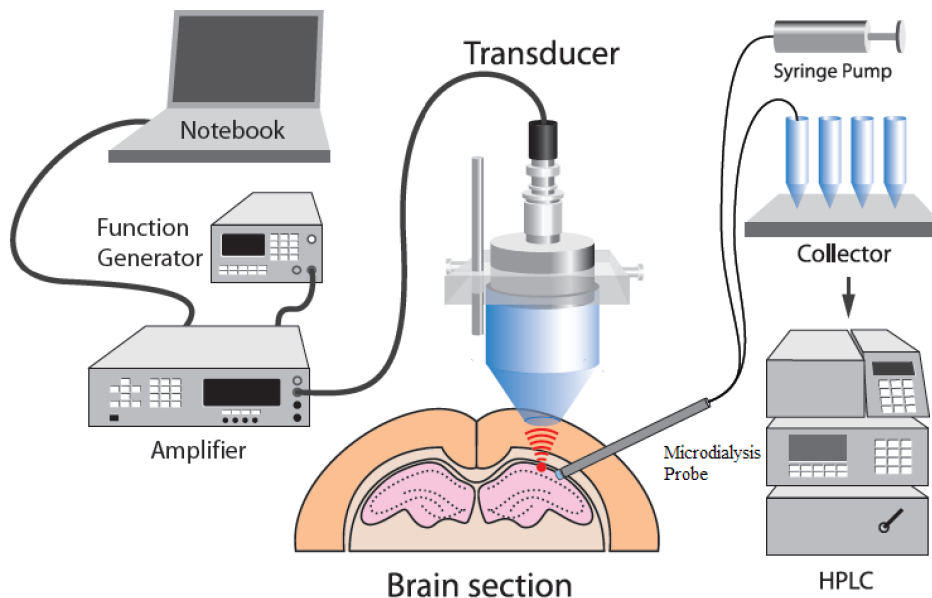
Parameters	BPA 500 mg/kg only	BPA 500 mg/kg with FUS
$C_{t_{max}}$ ( $\mu\text{g/mL}$ )	3.8 $\pm$ 0.5	6.8 $\pm$ 1.9
MRT (min)	313 $\pm$ 87	503 $\pm$ 192
AUC (min $\mu\text{g/mL}$ )	888 $\pm$ 225	1887 $\pm$ 600
AUC/dose	1.8 $\pm$ 0.5	3.8 $\pm$ 1.2

$C_{t_{max}}$ : the maximum tumor ECF concentration ( $n=5$  for each group).

MRT: mean residence time.

AUC: area under the concentration.

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**Figure 3. Schematic diagram illustrating the experimental setup for FUS and microdialysis.**  
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times higher. The peak boron concentration in sonicated tumor ECF was reached at 30 min after BPA-f injection and at the same time as in blood. The boron concentration versus time profile in blood could thus be a good indicator of the optimal time for neutron irradiation when FUS exposure is applied in a brain tumor during BNCT.

## Materials and Methods

### Glioma Brain Tumors

Male Fischer 344 rats (11–13 wk, 250–280 g) were anesthetized with an intraperitoneal injection of pentobarbital at a dose of 40 mg/kg of body weight. Then,  $1 \times 10^5$  F98 rat glioma cells (a generous gift from Dr. Rolf F. Barth, Ohio State University) in 10  $\mu$ L Hanks' balanced salt solution without  $Mg^{2+}$  and  $Ca^{2+}$  were stereotactically injected into the right hemisphere (5.0 mm posterior and 3.0 mm lateral to the bregma) of each rat's brain at a depth of 5.0 mm from the brain surface. Finally, the hole in the skull was sealed with bone wax, and the wound was flushed with iodinated alcohol. The F98 rat glioma has been described in previous study [27]. All *in vivo* experiments were performed according to ARRIVE guidelines and were approved by the Animal Care and Use Committee of the National Yang-Ming University.

### Focused Ultrasound Exposure

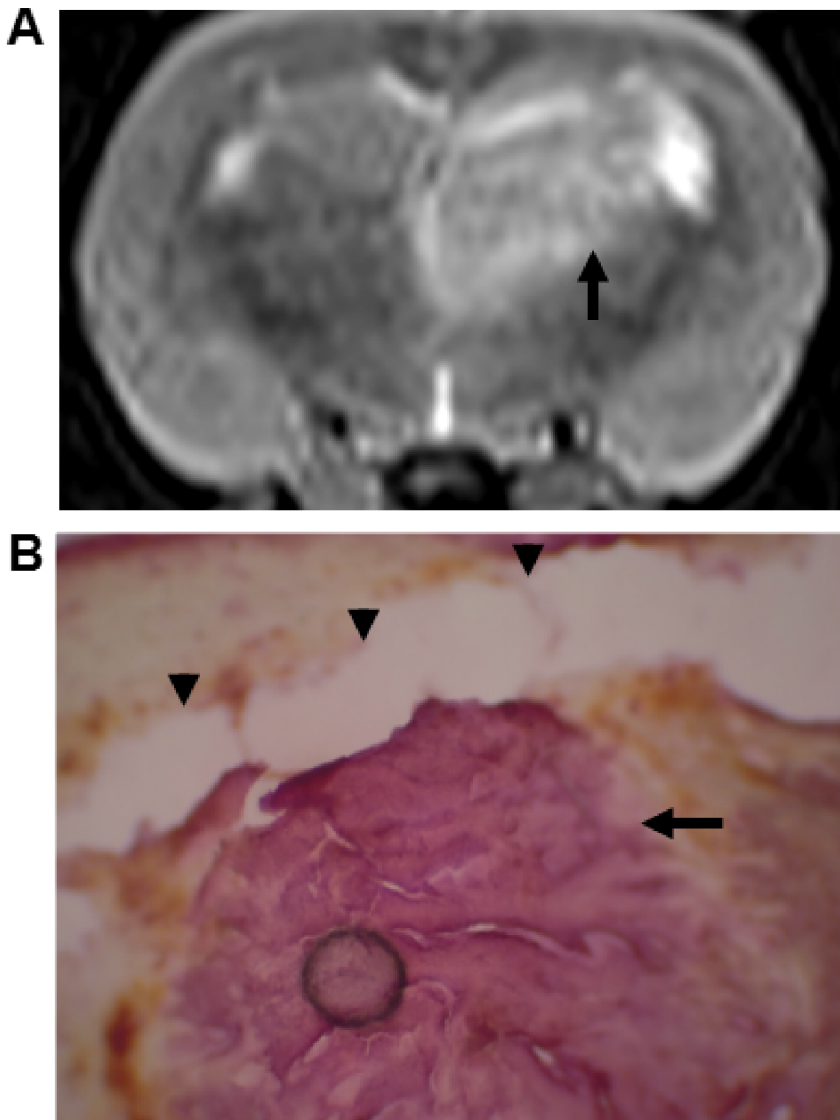
FUS was produced by a 1 MHz single-element focused transducer (A392S, Panametrics, Waltham, MA, USA) with a diameter of 38 mm and a radius of curvature of 63.5 mm. The half-maximum of the pressure amplitude of the focal zone had a diameter and length of 3 mm and 26 mm, respectively. The transducer was mounted on a removable cone filled with degassed water, and its tip was sealed with a polyurethane membrane. The FUS system setup was the same as described previously [28]. Animals were anesthetized intraperitoneally with urethane (1.2 g/kg) prior to the experiments. Ultrasound contrast agent (UCA, SonoVue, Bracco International, Amsterdam, the Netherlands) was injected into the femoral vein of the rats approximately 15 s before

each sonication. The transducer was applied with a burst length of 50 ms at a 5% duty cycle and a repetition frequency of 1 Hz. The sonication time was 60 s at an acoustic power of 2.86 W and a UCA dose of 300  $\mu$ L/kg. Ultrasound exposure was delivered to the tumor site on day 10 after tumor cell implantation.

### Microdialysis

To evaluate the variability of BPA concentration within the brain tumor, a commercially available microdialysis probe (MAB 6, Stockholm, Sweden) was applied to sample the unbound BPA in the rat brain. Simultaneously, another microdialysis probe (MAB 7, Stockholm, Sweden) was implanted into the jugular vein toward the rat's right atrium. For brain ECF sampling, the rat was mounted on a stereotaxic frame. An incision was made in the scalp, and a small hole was drilled on the right side of the skull for implantation of the rigid microdialysis probe in the tumor. After implantation of the microdialysis probes, the probes were perfused with Ringer's solution (147 mM sodium chloride, 4 mM potassium chloride, 2.2 mM calcium chloride) by a microinjection pump (CMA 402, Stockholm, Sweden) at a flow rate of 2  $\mu$ L/min. Dialysate samples were collected using a microfraction collector (MAB 85, Stockholm, Sweden). The entire experimental system is shown in Fig. 3.

Tumor progression was monitored by means of T2-weighted MR images (TRIO 3-T MRI, Siemens MAGNETOM, Germany) obtained on day 10 after tumor implantation (Fig. 4A). The maximum tumor size was about 0.125 cm<sup>3</sup>. Moreover, the track of the probe within the tumor was verified by hematoxylin and eosin (H&E) staining (Thermo-Scientific, Waltham, MA, USA) (Fig. 4B) following microdialysis experiment. At the time of sacrifice, all rats were deeply anesthetized under isoflurane anesthesia (5%). After probe placement, 2 h were allowed to elapse so that probe and tumor ECF could equilibrate [29,30]. Subsequently, 24 sets of microdialysis samplings of tumor ECF and plasma were performed every 15 min for 6 h before and after intravenous bolus injection of BPA-fr (Taiwan Biotech Co., LTD., Taoyuan, Taiwan) at a dose of 500 mg/kg. These 24 sets of microdialysis samplings consisted of the four sets of samplings taken prior to BPA-fr



**Figure 4. Tumor growth was monitored by MRI and histology.** (A) Representative sample of tumor (arrow) by T2-weighted MR images on day 10 after implantation. (B) Hematoxylin-eosin-stained section shows the tunnel (arrow heads) punctured by the probe within the tumor tissue (arrow). doi:10.1371/journal.pone.0100104.g004

injection. Boron concentration in the tumor ECF and plasma were assayed by inductively coupled plasma mass spectroscopy (ICP-MS) and calibrated with the standard curve derived from the measurement of boric acid standard.

#### Recovery of the Microdialysate

To determine the unbound BPA concentration in the tumor ECF and plasma from the microdialysis data, the concentration of BPA in the microdialysis samples must be adjusted. A retrodialysis technique was used for the experiment of in vivo recovery [31]. One hour after probe implantation, Ringer's solution containing BPA (350  $\mu\text{g}/\text{mL}$ ) was perfused through the probes and separately into the rat's blood and brain at a constant rate of 2  $\mu\text{L}/\text{min}$ . Furthermore, the dialysate ( $C_{\text{dial}}$ ) and perfusate ( $C_{\text{perf}}$ ) concentrations of BPA were measured by ICP-MS. The relative in vivo recovery of BPA across the dialysis membrane was calculated using the following equation:  $R_{\text{dial}} = 1 - C_{\text{dial}}/C_{\text{perf}}$ , where  $R_{\text{dial}}$  is the in vivo BPA recovery.

#### Pharmacokinetics

The pharmacokinetic parameters of boron in the plasma and tumor ECF were calculated for each set of sampled data using noncompartmental methods in the WinNonlin Version 5.2.1 software program (Pharsight, Montreal, Quebec, Canada). The area under the concentration versus the time curve extrapolated to infinity ( $\text{AUC}_{0-\infty}$ ) was calculated using log-trapezoidal methods. Maximum observed concentration ( $C_{\text{max}}$ ) and time to maximum concentration values were obtained directly from concentration versus time profiles. Mean residence time (MRT) extrapolated to infinity was calculated as  $\text{AUMC}_{0-\infty}/\text{AUC}_{0-\infty} - (\text{infusion time})/2$ , where  $\text{AUMC}_{0-\infty}$  is the area under the first moment curve extrapolated to infinity.

#### Histological Observation

Two glioma-bearing rats were prepared for histological examination. The rats were perfused with saline and 10% neutral buffered formalin. The brains were removed, embedded in



paraffin, and then serially sectioned into 30- $\mu\text{m}$ -thick slices. The slices were stained with H&E to visualize their general cellular structure. Photomicrographs of 5  $\mu\text{m}$ -thicknesses of the H&E stained tissues were obtained using a Mirax Scan digital microscope slide scanner (Carl Zeiss, Mirax 3D Histech) with a Plan-Apochromatic 20/0.8 objective lens.

### Statistical Analysis

All data are shown as mean  $\pm$  SEM. Data analysis was performed using an unpaired Student *t* test. Statistical significance was noted if  $p \leq 0.05$ .

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### Author Contributions

Conceived and designed the experiments: FYY YLL. Performed the experiments: FYY YLL YCL LWC YLH. Analyzed the data: FYY YLL LWC. Contributed reagents/materials/analysis tools: FYY FIC YWHL. Wrote the paper: FYY.