Uptake of the BPA into glioblastoma multiforme correlates with tumor cellularity

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Introduction

Boron neutron capture therapy (BNCT) is based on the nuclear reaction ^{10}B (n,α) ^{7}Li which produces short-range high-LET alpha and ^{7}Li particles. BNCT depends upon compounds that selectively deliver sufficient amounts of ^{10}B to the tumor. Previous studies in this laboratory have shown that in rats bearing intracerebral gliosarcomas, BNCT, using p-boronophenylalanine-fructose (BPA-F) as the delivery agent, can yield a high percentage of long-term tumor control with little or no damage to the surrounding normal brain [1]. During irradiation, boron levels in these gliosarcomas were > $80\mu g$ $^{10}B/g$ and the tumor: blood and tumor: brain boron concentration ratios were greater than 3:1.

Under an FDA-sanctioned Investigational New Drug protocol, biodistribution studies of BPA-F in patients with glioblastoma multiforme (GBM) were begun in 1994. In the first subjects examined, we observed that boron concentrations in tumor samples removed during surgical debulking varied among patients and within multiple samples from individual patients. Examination of representative histologic sections prepared from specimens analyzed for boron strongly suggested that there was a correlation between the degree of tumor cellularity and boron concentration. We now report results from fourteen subjects in which this correlation was quantified microscopically.

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Methods

Each of the fourteen subjects was scheduled to undergo craniotomy and surgical debulking of their GBM and gave informed consent for their participation in the biodistribution study. BPA, solubilized as a 0.14M solution by complexity with a 10% molar excess of fructose (BPA-F), was infused intravenously over 2 hours at doses ranging from 100 to 290 mg BPA/kg body weight. Craniotomy began at the end of infusion and tissue specimens were obtained between 0.5 and 1.5 hours after the end of infusion as shown in Figure 1. A total of 104 specimens were analyzed for boron by direct current plasma-atomic emission spectroscopy [2]. From each specimen, a 5 μm-thick hematoxylin/eosin-stained section was prepared from a contiguous formalin-fixed tissue sample of similar macroscopic appearance.

Quantitative morphologic assessment of the ratio of nuclear volume to total tissue volume (i.e., index of tissue cellularity) was done according to the method of Chalkley [3]. Briefly, the number of points in a reticle that lie over viable appearing nuclei divided by the total number of points in the reticle approximates the fraction of tissue volume occupied by nuclei. Square microscopic fields (glass reticle) scattered at random throughout the entire tissue area on a slide were selected for analysis so that the ratio of the estimated tissue area to the number of fields examined in that area would remain about constant for all subjects. Thus, the possibility of preferential sampling within the various specimens from a patient was lessened. When one microscopic slide represented a tissue that had been divided into more than one piece to facilitate boron analysis, the average ^{10}B concentration associated with that tissue was deemed to be the tissue-weighted average of the multiple ^{10}B concentrations.

Blood samples for boron analysis were taken during infusion and for several hours after the end of infusion.

Results and Discussion

The maximum blood boron concentration observed at the end of infusion was approximately a linear function of the amount of BPA administered. As shown in Figure 1, the average blood boron concentration at the end of infusion of 250 mg BPA/kg (the dose used for BNCT) was ~ 22 μg/ml. Clearance of boron from the blood followed biexponential kinetics with an initial half-time of 0.53 hrs. and the second phase half-time of 6.15 hrs. During BNCT the average blood boron concentration is ~ 12 μg/ml.

Average boron concentrations (normalized to an infusion dose of 250 mg BPA/kg) in surgically removed tumor samples varied from 3.2 to 64.5 μg ^{10}B/g. Histologic examination of the representative sections showed considerable variation in the proportion of necrotic tissue. As shown in Figure 2, quantification of the nuclear volume in the 104 sections from 14 patients showed a linear relationship between cellularity and normalized boron concentration. Of the 14 patients, 3 received a dose of 250 mg BPA/kg (i.e., dose used in BNCT), 10 patients received doses less than 250 mg BPA/kg and 1 received a dose greater than 250 mg/kg.

The correlation coefficient of this calculated regression line is r = 0.84. The highest nuclear volume fraction (i.e., maximum cellularity) observed was 0.65 which corresponds to a tissue boron concentration of ~ 49 μg ^{10}B/g. The intercept, where the

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nuclear volume fraction is nominally zero, corresponds to a tissue boron concentration of ~ 9 μg ^{10}B/g and is an estimate of the average extracellular tumor ^{10}B concentration in these 14 subjects.

Since the ^{10}B concentration is the same in nucleus and cytoplasm, as was observed by ion-spallation mass-spectrometric microscopy, [Duane Smith, personal communication and reference 4], the average ^{10}B concentration in a maximally cellular zone of GBM tissue must be the weighted average of boron concentrations in its intracellular and extracellular components, the two weighting factors being their relative volumes. It has been observed that cell/tissue volume fractions in the most densely cellular zones of GBM are as high as 90-95% i.e., that minimal extracellular volumes are in the 5-10% range [P.C. Warnke; personal communications to J-O. Gebbers]. It follows algebraically that the average intracellular ^{10}B concentration in viable, densely cellular zones of GBM tumor tissue 0.5 to 1.5 hours after the end of a two-hour intravenous infusion of 250 mg BPA/kg body weight must be about 52 μg ^{10}B/g. To date, no microassays of boron in individual tumor cells or in small groups of tumor cells from glioblastoma patients so infused have been reported. This boron concentration (52 μg/g) corresponds to a tumor-to-blood ratio greater than 4.0. In patients receiving BNCT, radiation doses to the tumor are presently calculated using a tumor-to-blood ratio of 3.5 The results of this study therefore suggest that these calculated physical tumor absorbed doses may be somewhat conservative.

The quasilinear relationship between the amount of BPA administered and the GBM tissue ^{10}B concentrations appears to hold for doses between 100 and 290 mg BPA/kg body weight. Our current protocol permits dose escalation up to 370 mg BPA/kg. Assuming quasilinearity is maintained at the higher dose, the blood and tumor boron concentrations at the time of BNCT would be > 20 and > 80 μg ^{10}B/g, respectively, following infusion of 370 mg BPA/kg. This would decrease reactor irradiation time and thereby increase the therapeutic gain Gy as much as 20%.

Acknowledgement:

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Figure Legends

Figure 1

Blood ^{10}B concentrations during and after a 2 hour infusion of *p*-boronophenylalanine-fructose (BPA-F) at a dose of 250 mg BPA/kg body weight. Data from 3 subjects who were infused with BPA-F prior to craniotomy and 8 patients who were infused with BPA-F prior to BNCT are combined. The time intervals of tumor sampling or BNCT are indicated on the graph.

Figure 2

Tissue ^{10}B concentration vs. nuclear volume fraction in samples from fourteen GBM patients. The symbols representing data from patients infused with different doses of BPA were: O-100 mg/kg (1 patient); □-130 mg/kg (3 patients); ∆-170 mg/kg (3 patients); ∇-210 mg/kg (3 patients); ♦-250 mg/kg (3 patients); and ●-290 mg/kg (1 patient). The dotted curves above and below the regression line indicate the 95% confidence interval.