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The Therapeutic Ratio in BNCT: Assessment using the Rat 9L Gliosarcoma Brain Tumor and Spinal Cord Models

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1. INTRODUCTION

During any radiation therapy, the therapeutic tumor dose is limited by the tolerance of the surrounding normal tissue within the treatment volume. The short ranges of the products of the $^{10}B(n,\alpha)^{7}Li$ reaction produced during boron neutron capture therapy (BNCT) present an opportunity to increase the therapeutic ratio (tumor dose/normal tissue dose) to levels unprecedented in photon radiotherapy. The mixed radiation field produced during BNCT comprises radiations with different linear energy transfer (LET) and different relative biological effectiveness (RBE). The short ranges of the two high-LET products of the $^{10}B(n,\alpha)^{7}Li$ reaction make the microdistribution of the boron relative to target cell nuclei of particular importance. Due to the tissue specific distribution of different boron compounds, the term RBE is inappropriate in defining the biological effectiveness of the $^{10}B(n,\alpha)^{7}Li$ reaction. To distinguish these differences from true RBEs we have used the term "compound biological effectiveness" (CBE) factor. The latter can be defined as the product of the true, geometry-independent, RBE for these particles times a "boron localization factor", which will most likely be different for each particular boron compound. To express the total BNCT dose in a common unit, and to compare BNCT doses with the effects of conventional photon irradiation, multiplicative factors (RBEs and CBEs) are applied to the physical absorbed radiation doses from each high-LET component. The total effective BNCT dose is then expressed as the sum of RBE-corrected physical absorbed doses with the unit Gray-equivalent (Gy-Eq).

The radiobiology of BNCT has been evaluated using two very different boron compounds: the amino acid analog *p*-boronophenylalanine (BPA) and the sulfhydryl borane, $Na_2B_{12}H_{11}SH$ (BSH). Determination of the therapeutic ratio requires, for each compound, knowledge of boron concentrations in tumor, blood and normal brain as well as knowledge of the RBE of each of the BNCT radiation dose components for the tumor and the normal tissues, including the compound biological effectiveness (CBE) factor for the $^{10}B(n,\alpha)^{7}Li$ reaction. The RBEs of the beam components and the CBEs for BPA-based and BSH-based BNCT have been assessed in the rat brain tumor model[1], in the rat spinal cord model as a surrogate for the normal central nervous system[2], and in rat skin[3]. Our results indicate a therapeutic ratio for BNCT of the rat 9L gliosarcoma of over 5:1 for BPA and of $\approx$ 1.1:1 for BSH. We report here the results of BNCT irradiations of tumor bearing rats using either BPA or BSH. Due to the different therapeutic ratios between these two compounds, similar doses to the vascular endothelium produced markedly different results in terms of long-term survival.

2. METHODS

2.1 BNCT of the Rat Brain Tumor Model

The rat 9L gliosarcoma brain tumor model, the dosimetry of rat brain tumor irradiations using the Brookhaven Medical Research Reactor (BMRR) thermal neutron beam and the use of the soluble fructose complex of BPA (BPA-F) have been described[4]. Briefly, for rat brain tumor irradiations, the beam emerging from the BMRR thermal port was restricted to an 11.5 mm-diameter aperture by a 18 mm-thick collimator. Irradiation of rats bearing intracerebral 9L gliosarcomas was carried out 14 days after implantation when the tumors were $\approx$ 4 mm in diameter (40-50 mg).

Using the 9L rat gliosarcoma and an *in vivo/in vitro* colony forming assay after BNCT irradiations, we have determined the RBE of the high-LET beam components and the CBE for BPA and BSH[1]. In this report, the results of additional BNCT irradiations of tumor-bearing rats using BPA-F have been added to those already reported[4]. In addition, BNCT of rats bearing intracerebral 9L tumors was carried out using BSH as the capture agent.

2.2 Rat Spinal Cord Model

The response of the rat spinal cord to BNCT using either BPA or BSH as the neutron capture agent has been reported[2]. For the rat spinal cord irradiations, the thermal beam was restricted to a 15.5 mm diameter aperture by a 27 mm thick collimator. The large vertebral spine of thoracic vertebra 2 was used as the lower boundary of the irradiation field. In this region of the body the thickness of tissue between the skin surface and the center of the spinal cord was 1.0 $\pm$ 0.1 cm. Limb paralysis within 7 months was used as the endpoint.

2.3 Effect of BNCT on the Skin

The rat spinal cord irradiations also produced dose-response information for the skin. The 15.5 mm beam aperture was located on the mid-dorsal line of the neck. BNCT irradiation of rat skin produced a biphasic response. Moist desquamation was observed with a latency of $\approx$ 2 weeks; dermal necrosis was observed with a latency of $\geq$ 24 weeks[3]. The RBE for the high-LET beam components and the CBEs for BPA and BSH in the rat skin were derived[3].

3. RESULTS

3.1 Biodistribution

3.1.1 BPA

For BNCT, rats received a single i.p. injection of BPA-F that delivered 1200 mg of L-BPA/kg. At 4 hours post-injection, the boron concentrations in tumor, blood and normal brain were 89.6 $\pm$ 7.6, 27.7 $\pm$ 2.8 and 17.5 $\pm$ 1.5 μg ^{10}B/g, respectively[4]. For the spinal cord irradiations (and the concurrent skin irradiations) rats received two oral doses of BPA (2 x 750 mg L-BPA/kg) three hours apart. At 3 hours after the second oral dose the boron concentrations in brain and blood were 10.0 $\pm$ 0.5 μg ^{10}B/g and 12.0 $\pm$ 0.5 μg ^{10}B/g, respectively[5]. The boron concentration in the skin at the time of irradiation was 10.0 $\pm$ 0.5 μg ^{10}B/g.

3.1.2 BSH

For BNCT, rats received an i.p. injection (4 ml of 10 mg BSH/ml) of BSH and were irradiated

at 30 minutes post-injection. At the time of irradiation, the boron concentrations in tumor, blood and normal brain were 56 ± 6, 114 ± 5 and 2.9 ± 0.5, respectively. For the spinal cord (and concurrent skin) irradiations rats received 100 mg BSH/kg body weight by i.v. infusion via the femoral vein over a 10 minute period. Irradiations were carried out at 30 minutes after the end of the infusion. Blood samples were obtained from each rat immediately prior to irradiation and ranged from 98 to 146 μg ^{10}B/g. The ^{10}B concentration in the brain was 2.9 ± 0.5 μg ^{10}B/g. The boron content of skin was comparable to that found in the blood[2].

3.2 The Therapeutic Ratio for BNCT of the Rat 9L Gliosarcoma

Table 1 summarizes our previous reports on the RBEs and CBEs of the BNCT dose components in the 9L gliosarcoma, the spinal cord and in the skin. Using the data from the rat spinal cord as a surrogate for the brain, allows calculation of the therapeutic ratio (tumor dose/brain dose) for BNCT of the rat 9L gliosarcoma. The values of the CBE for BPA in both the tumor and in the spinal cord were higher than the corresponding values for BSH. The ratio, however, between the tumor CBE and the spinal cord CBE are similar for BPA and BSH: 2.9 and 2.4, respectively. Differences in the therapeutic ratio between BPA and BSH are, therefore, due primarily to differences in the boron concentration ratios between tumor and blood. (The CBE for spinal cord was calculated based on the BNCT radiation dose to the blood[2]). The RBEs and CBEs in Table 1, combined with a tumor/blood ^{10}B concentration ratio of over 3:1, produce a therapeutic ratio for BPA-based BNCT in excess of 5:1 (tumor dose/brain dose). For BSH, the favorable CBE ratio is negated by the unfavorable tumor/blood ^{10}B concentration ratio (0.5:1) producing a therapeutic ratio for BSH-based BNCT of only 1.1:1.

The CBE of BPA for moist desquamation is equal to the CBE measured in the tumor (Table 1). In addition, the RBE of the beam protons is higher in the skin than in the tumor. This implies that, for BPA, the therapeutic ratio relative to skin (tumor dose/skin dose) is lower than the therapeutic ratio relative to the normal brain endothelium.

3.3 BNCT

The factors in Table 1 allow BPA- and BSH-based BNCT irradiations of the 9L gliosarcoma to be compared based on photon equivalent (Gy-Eq) doses to the normal brain endothelium (Figure 1). The physical dose rates during BNCT were as reported[4]: 0.039 Gy/MW-min/μg ^{10}B/g for the ^{10}B(n,α)^{7}Li reaction; 0.27 Gy/MW-min for the fast neutrons; 0.093 Gy/MW-min for the ^{14}N(n,p)^{14}C reaction; and 0.19 Gy/MW-min for the total gamma dose. Endothelial doses of approximately 10 Gy-Eq and 20 Gy-Eq were targeted. Doses actually delivered with BSH were 9.5 Gy-Eq (n=6) and 17.7 Gy-Eq (n=7); and with BPA were 11.5 Gy-Eq (n=16) and 21.5 Gy-Eq (n=14). The tolerance limit of the rat brain to single-fraction photon irradiation has been reported to be approximately 17-20 Gy[6]. BPA, with a therapeutic ratio of 5:1 delivered much higher doses to the tumor than BSH with a therapeutic ratio of 1.1:1. The long-term survival data reflect these differences in tumor doses (Figure 1).

Figure 1...

Figure 1. Survival of unirradiated control rats and rats treated using BPA-based or BSH-based

BNCT. The numbers in parentheses are the doses (Gy-Eq) to the normal brain endothelium.

Figure 2 illustrates the relative dose ratios for BPA and for BSH when the brain endothelium is brought to approximately the level of tolerance. The horizontal dotted line at 20 Gy-Eq in Figure 2 represents the approximate tolerance limit of the rat brain[6]. The horizontal dotted line at 42 Gy-Eq is the approximate 50% incidence dose for moist desquamation in the rat[7].

Figure 2....

Figure 2. Doses (Gy-Eq) to tumor, brain (endothelium) and skin produced during BPA- and BSH-based BNCT at approximately the limit of brain tolerance.

4. DISCUSSION

The therapeutic ratio of over 5:1 demonstrated here for BPA-based BNCT of the rat 9L gliosarcoma is unprecedented in conventional photon radiation therapy. Extremely high doses were delivered to the tumor (> 100 Gy-Eq) while the dose to the normal brain endothelium was kept at or below the limit of tolerance. With BSH we were unable to obtain tumor/blood ratios greater than $\approx$ 0.5:1. The high levels of ^{10}B in the blood after BSH administration limit the radiation dose that can be delivered, and result in a therapeutic ratio of 1.1:1. Nevertheless, BSH-based BNCT ($\approx$ 20 Gy-Eq to the tumor) did result in $\approx$ 40% long-term survival at the brain tolerance limit. If tumor/blood ^{10}B concentration ratios $\geq$ 1:1 could be obtained with BSH, the therapeutic ratio would increase accordingly.

The CBE for BSH was based primarily on the published value for BSSB[1]. Additional experiments (Coderre, unpublished) with BSH indicated that the cell survival determined in the *in vivo/in vitro* colony forming assay was indistinguishable from that of BSSB. The values determined for both BSH and BSSB *in vitro* were also the same[1]. BSSB-based BNCT produced $\approx$ 50% long-term tumor control but only $\approx$ 3 logs of cell kill as measured in the *in vivo/in vitro* colony forming assay. It was postulated that tumor control with BSSB might be due to vascular damage rather than direct tumor cell kill[1]. If this is correct, and if this also pertains to BSH-based BNCT, the question arises as to the applicability of a CBE derived in a tumor cell kill assay if the target is the vasculature and tumor control is an indirect effect. On the other hand, the 40% survival rate for BSH-based BNCT, at a tumor dose of $\approx$ 20 Gy-Eq, does agree with the overall observed dose response of the 9L tumor to BNCT. BPA-based BNCT at a similar dose (25 Gy-Eq) produced a similar level of survival ($\approx$55%).

The 9L rat gliosarcoma is widely used and known to be refractory to conventional photon therapy. These results demonstrate the substantial therapeutic gain possible with BPA-based BNCT.

5. REFERENCES

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Table 1. Radiobiological Parameters for BNCT of the Rat 9L Gliosarcoma.

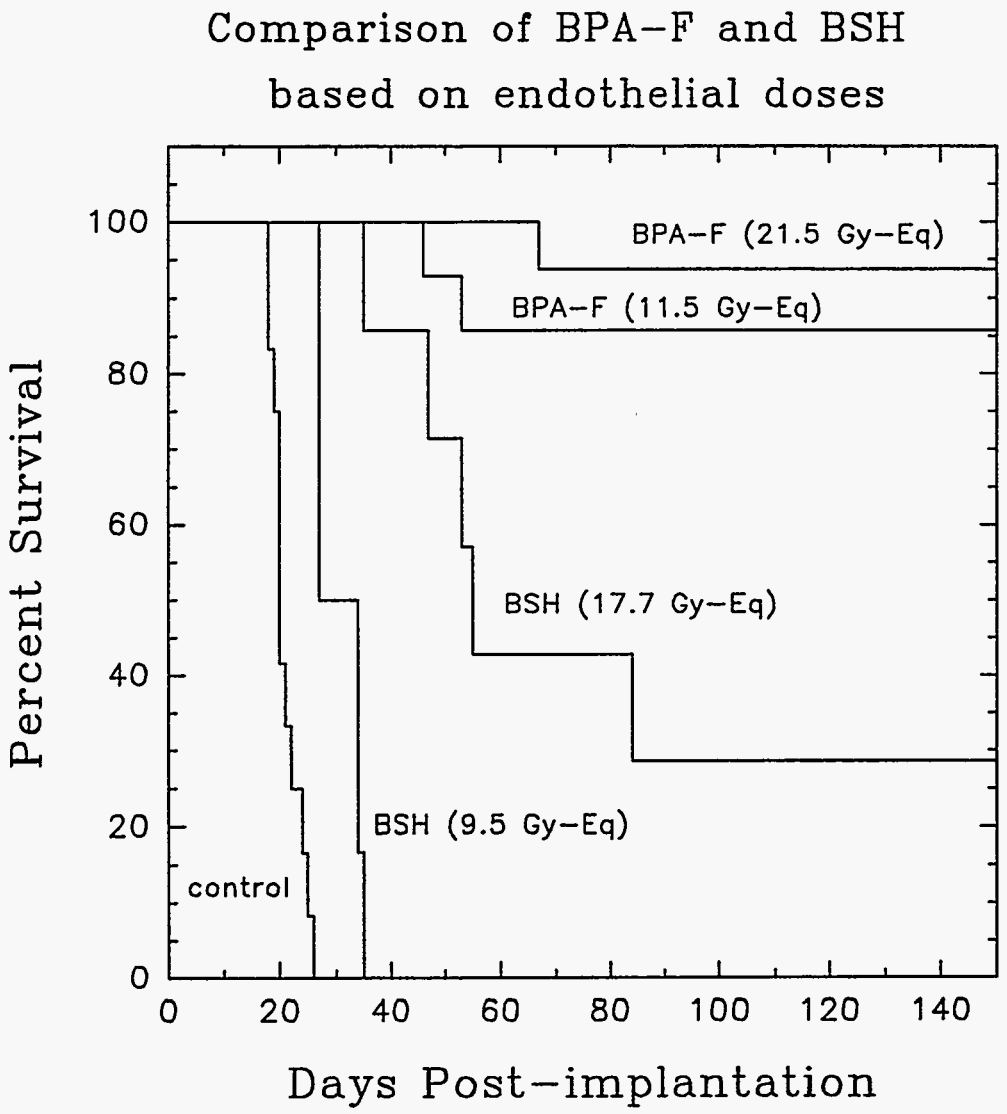
Tissue	CBE for BSH	CBE for BPA	RBE beam protons[a]
Tumor[b]	1.2	3.8	3.2
Spinal cord[c]	0.5	1.3	2.6
Skin (moist desquamation)[d]	0.6	3.7	5.1
Skin (dermal necrosis)[d]	0.9	0.7	5.1

[a]The combined proton dose from the $^{14}N(n,p)^{14}C$ reaction and the fast neutron-hydrogen recoil.

[b]Data from Reference (1).

[c]Data from Reference (2).

[d]Data from Reference (3).


Comparison of BPA-F and BSH
based on endothelial doses
Percent Survival
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Days Post-implantation
BPA-F (21.5 Gy-Eq)
BPA-F (11.5 Gy-Eq)
BSH (17.7 Gy-Eq)
BSH (9.5 Gy-Eq)
control

BNCT: Radiobiology in the rat