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RADIOBIOLOGY OF BORON NEUTRON CAPTURE THERAPY: PROBLEMS WITH THE CONCEPT OF RELATIVE BIOLOGICAL EFFECTIVENESS

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The radiation dose delivered to cells *in vitro* or *in vivo* during boron neutron capture therapy (BNCT) is a mixture of photons, fast neutrons and heavy charged particles from the interaction of neutrons with nitrogen and boron. The concept of relative biological effectiveness (RBE) has been developed to allow comparison of the effects of these radiations with the effects of standard photon treatments such as 250 kVp x-rays or ^{60}Co gamma rays. The RBE value for all of these high linear energy transfer radiations can vary considerably depending upon the experimental conditions and endpoint utilized (c.f. Fukuda, 1989). The short range of the particles from the $^{10}B(n,\alpha)^{7}Li$ reaction make the precise subcellular location of the ^{10}B atom of critical importance. The microscopic distribution of the ^{10}B has a decided effect on the dosimetry. Monte Carlo simulations have shown that, at the cellular level, there is a profound difference in the probability of cell kill depending on the location of the ^{10}B relative to the nucleus (Gabel, 1987). Convenient analytical techniques for the detection of boron at the cellular and subcellular level remain to be developed. Different boron-delivery agents will almost certainly have different distribution patterns at the subcellular level. For equivalent ^{10}B concentrations at the macroscopic level (e.g. μg ^{10}B/gram wet tissue), different boron-delivery agents may have vastly different cytotoxic effects. The application of a single RBE value for the $^{10}B(n,\alpha)^{7}Li$ reaction to different boron-delivery agents without some experimentally determined compensatory factor for subcellular localization could lead to gross under- (or over-) estimates of the actual absorbed dose.

The effect of BNCT with the amino acid *p*-boronophenylalanine (BPA) was compared with the effect of 250 kVp x-rays on a pigmented B16 melanoma subclone, both *in vitro* and *in vivo*. Generally accepted RBE values were applied to the relevant components of the Brookhaven Medical Research Reactor (BMRR) thermal neutron beam, however, there were still discrepancies when the resulting dose response curves were compared with the response to 250 kVp x-rays.

METHODS

B16 melanoma (subclone G3.12; Stackpole, 1985) cells were maintained in DMEM to which 5% (v/v) fetal bovine serum, 1% (v/v) antibiotics (penicillin, 100 IU/ml, amphotericin-B, 0.25 μg/ml and streptomycin 100 μg/ml) and 1% (v/v) L-glutamine (200 mM) were added. Solid tumors were initiated in C57B1/6 mice by s.c. injection of 2 x 10^5 cells in 0.1 ml of growth medium. Tumors were utilized after $\approx$ 12-14 days of growth when they weighed $\approx$ 50-100 mg. Irradiations at the BMRR have been previously described for *in vitro* (Gabel, 1984) and *in vivo* (Coderre, 1988) conditions. Clonogenic survival was assayed in tumors removed <5 min post-treatment, which were then minced, trypsinized, cell-counted in suspension, and plated for colony-forming assay. BPA (35 μg ^{10}B/ml) was added to the cell growth medium 24 hours prior to irradiation and was present in the medium during reactor irradiation. Mice were dosed with intragastric (ig) slurries of 15 mg of the L-enantiomer of BPA in 0.5 ml water. Two ig doses given 5 hours apart resulted in $\approx$ 40 μg ^{10}B/g tumor at the time of irradiation (3 hours after the second dose).

RESULTS AND DISCUSSION

Dosimetry. The dose from BNCT irradiations is expressed as gray-equivalent (Gy-Eq), calculated by summation of physical doses (Gy) of the component radiations multiplied by appropriate relative biological effectiveness (RBE) factors. RBE values of 2.3 and 2.0 have been assumed for the ionizing particles from the $^{10}B(n,\alpha)^{7}Li$ and $^{14}N(n,p)^{14}C$ reactions, respectively, and 2.0 has been assumed for fast neutrons. BNCT *in vivo* resulted in the following dose rates to the tumor (at 1 MW reactor power) for the various beam components: fast neutrons, 0.23 Gy/min; gamma, 0.12 Gy/min; $^{14}N(n,p)^{14}C$ reaction, 0.13 Gy/min; and $^{10}B(n,\alpha)^{7}Li$ with 40 μg ^{10}B/g tumor, 2.13 Gy/min. BNCT *in vitro* produced the following dose rates: fast neutrons, 0.15 Gy/min; gamma, 0.07 Gy/min; $^{14}N(n,p)^{14}C$ reaction, 0.05 Gy/min; and $^{10}B(n,\alpha)^{7}Li$ with 35 μg ^{10}B/ml (ambient), 1.0 Gy/min.

In vitro irradiations. Figure 1 shows cell survival *versus* dose (Gy-Eq) following irradiation *in vitro* with either x-rays (D_0 = 1.8 Gy), BNCT (D_0 = 1.1 Gy-Eq) or with the BMRR thermal neutron beam in the absence of BPA (D_0 = 1.5 Gy-Eq). In theory, all three survival curves should superimpose if all of the RBE assumptions are accurate and if RBE correction alone is sufficient. It is possible that cells exposed to BPA in culture accumulated ^{10}B to levels higher than in the surrounding medium, resulting in an underestimate of the dose.

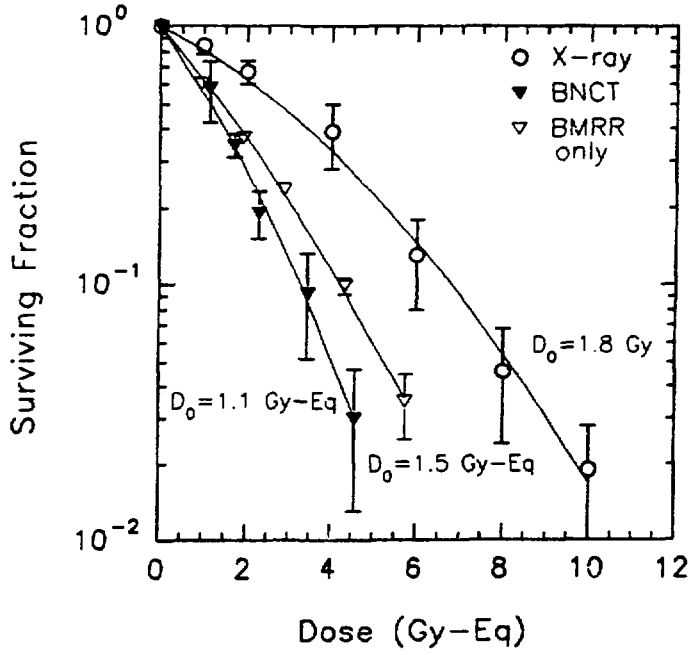

Figure 1. Survival of B16 melanoma cells exposed *in vitro* to x rays, BNCT or reactor irradiation only (no BPA). Each point is the mean of 3-5 experiments, 3 dilutions per point with 5 replicate plates per dilution.

In vitro irradiations. Figure 2 shows clonogenic cell survival in tumors irradiated *in vivo* with either x-rays (D_0 = 4.3 Gy) or BNCT (D_0 = 2.0 Gy-Eq). The presence of hypoxic cells could account for the lower cell kill by x-rays *in vivo*. Interestingly, the cell kill produced by BNCT *in vivo* (D_0 = 2.0 Gy-Eq) is close to that produced by x-rays *in vitro* (D_0 = 1.8 Gy; Fig. 1).

DISCLAIMER

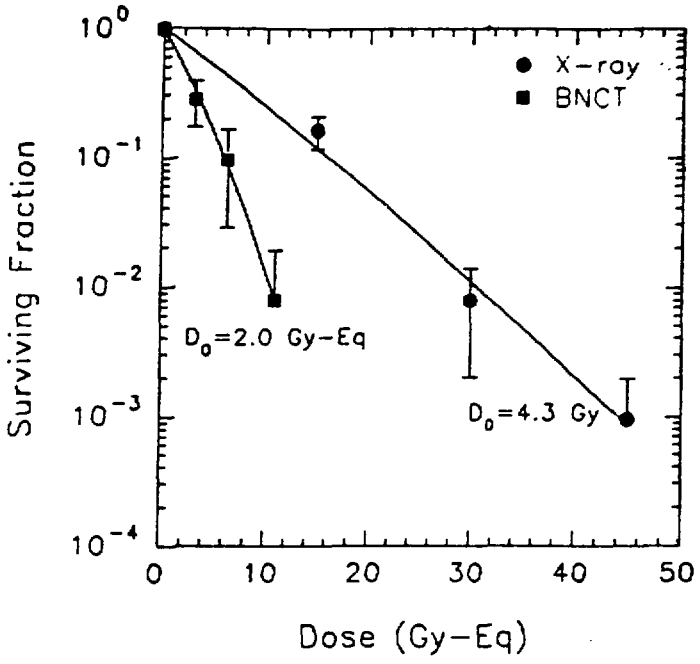

Figure 2. Clonogenic survival in solid tumors determined by colony forming assays <5 min after either BNCT or x-ray irradiation. Each point is the mean (± SD) of 4-6 tumors; 3 different dilutions were plated per tumor with 5 replicate plates per dilution.

The different response of the B16 melanoma cells to irradiation with 250 kVp x-rays *in vitro* (D_0 = 1.8 Gy; Fig. 1) or *in vivo* (D_0 = 4.3 Gy; Fig. 2) is most likely due to the resistance of hypoxic cells *in vivo* to photon irradiation; the magnitude of the oxygen enhancement ratio for cells in culture versus air-breathing mice was 2.4 (ratio of D_0 values). The different response of the B16 melanoma cells to BNCT *in vitro* (D_0 = 1.1 Gy-Eq; Fig. 1) or *in vivo* (D_0 = 2.0 Gy-Eq; Fig. 2) is unlikely to be due to an oxygen effect; the gamma component of the total tumor dose was only ≈ 5%. It is possible that non-uniform distribution of ^{10}B within the tumor resulted in a fraction of the cells receiving a lower dose.

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