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IN VIVO STUDIES IN NCT WITH A BORONATED PORPHYRIN AND TUMOR GROWTH DELAY AS AN END POINT

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INTRODUCTION

The robust carrying capacity of the porphyrin molecule and its propensity for localizing in tumor justified the pursuit of synthesizing a porphyrin labeled with boron for use in BNCT. However, problems associated with poor solubility impeded the utility of the molecule. Until BOPP was synthesized (1), porphyrins were promising, but impractical.

After in vitro experiments had demonstrated the biological efficacy of BOPP and had confirmed its intracellular localizing ability (2), in vivo studies were carried out using BALB/c mice. The KHJJ murine mammary carcinoma was selected because, like glioblastomas, it is radioresistant (TCD_{50} of ~54 Gy)[1]. Irradiation of tumors to the TCD_{50} in a single fraction was precluded because the accompanying whole body dose is lethal. This problem was overcome by the use of fractionated doses of radiation. BOPP was administered either as 3 0.5 ml injections per day over 2 days, or by continuous i.v. infusion, 2 ml per day over three days (3) for a total dose of ~42 μg ^{10}B/gbw. Boron-10 distribution in the tumor at the time of irradiation was ~20 μg/g. Fig. 1 is a neutron capture radiogram showing boron distribution with BOPP. Note that normal brain is free of boron, indicating that BOPP respects the blood-brain barrier.

Irradiations were carried out at the thermal beam port at the Brookhaven Medical Research Reactor (BMRR) at a reactor power of 1 MW. Mice were anesthetized with ketamine and rompun, then taped to a LiF-epoxy collimator with a centrally-truncated hole (1.5 cm aperture) to accommodate the tumor which was carried on the thigh of the mouse. 6.4 MW min was used for the 3 fraction experiment; 10.3 MW min for the 5 fractions.

Because a tumor control dose could not be delivered, the end point selected for these experiments was tumor growth delay. This necessitated knowledge of the growth characteristics

[1]The tumor was kindly provided by Dr. Sara Rockwell.

Figure 1

of the tumor. Tumor was implanted on the thigh of thirty mice and volume was determined by daily measurements of length (l), width (w), and thickness (h), corrections for the hemi-ellipsoidal shape of the tumor were made using the formula V=0.524 lwh, as recommended in (3). The tumor volume ,V, increased exponentially with time, t (days), according to the equation:

$$V = V_0 e^{(0.35-0.50V_0)\,t} \qquad (1)$$

This relationship pertained during the course of a ten-fold increase from the initial volume, V_0 where 0.02 $cm^3 < V_0 < 0.28\ cm^3$. The analysis of tumor growth delay was based on the time span between the first irradiation and a ten-fold increase in tumor volume. The growth delay ratio (GDR) is the ratio of time to reach $10 \cdot V_0$ for the treated animal relative to the unirradiated control, normalized to the same initial V_0 Multiple linear regression analysis was applied for the data and the GDR was determined from the equation:

$$GDR = 0.996\, e^{(0.127t+0.012\,[^{10}B])} \qquad (2)$$

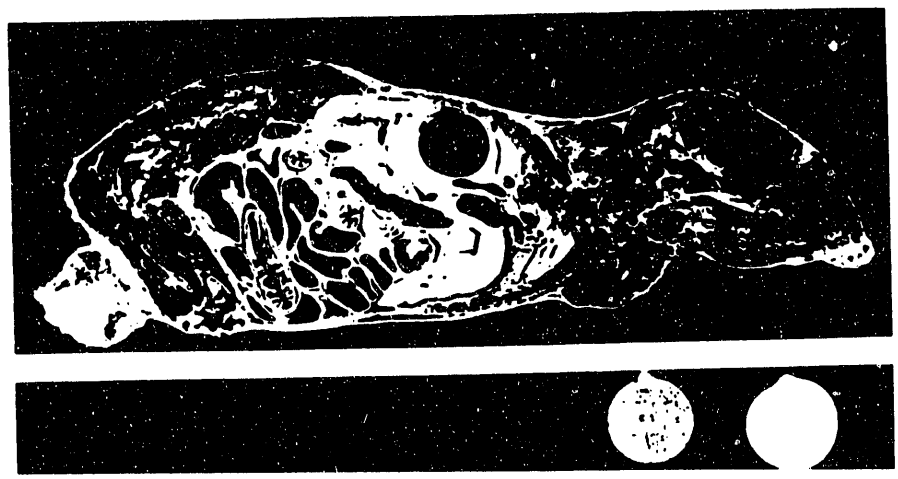
The resulting correlation coefficient was 0.982.

Physical dose rates and effective doses for the various mixed-field radiations are shown in Table I, along with the total effective dose per MW min for each component radiation. The effective dose was determined using Dose ·RBE/MW min. as described in (4). Dosimetric techniques are detailed in (5).

Comparisons are made between a three and a five fraction experiment. In the former, BOPP increased the effectiveness of the neutron irradiations by ~60% at 6.4 MW minutes of irradiation, and in the latter, ~95% at 10.3 MW minutes. Relative to the untreated control mice, tumor growth was retarded by factors of 4 and 6, respectively. Results are shown in Table II.

Although the biological efficacy of BOPP has thus been demonstrated in an <u>in vivo</u> system, it is uncertain that the conditions for exhibiting the advantage of the compound to its fullest extent were met. BOPP appears to remain systemic for long periods of time. The adrenal glands, spleen or liver might be the storage organs for BOPP, since high levels of the drug are present in these organs and are observed in tumor after a considerable period of time (unpublished data). It is likely that the drug recirculates systemically. In these experiments, BOPP was delivered prior to the first fraction. If BOPP has similar propensity for acting at the site of the endothelium as does the hematoporphyrin derivative HPD, vascular damage from the first fraction could have prevented the recirculation of the compound (6). Therefore, the advantage of boron retention in tumor would have been lost. To overcome this obstacle, we will have to consider alternative modes of neutron delivery, e.g., hypo-fractionation. At any rate, the boronated porphyrin, BOPP, is a clear candidate for clinical application in NCT, and further studies are warranted.

The above assessment of the efficacy of a boron-containing compound was accomplished using only megawatt minutes as a measure of the amount of exposure, i.e., it was not necessary resort to absorbed dose, RBE or the products of the two. In a companion paper,



DISCLAIMER

Table I. Dose Rate at 2 mm Depth in a Murine Tumor Irradiated at the BMRR* Power Level = 1 MW

Component	Gy (per min)	Effective dose rate RBE x Gy/min**	Percent of total effective dose rate
Fast Neutrons from the reactor	.22	.44	16.5
Extrinsic photons	.085	.085	3.2
$^{14}N(n,p)^{14}C$ reaction products	.11	.22	8.2
2.2 MeV photons from $^{1}H(n\gamma)^{2}H$	.03	.03	1.1
^{7}Li and α from $^{10}B(n,\alpha)^{7}Li$	1.01	1.89	71.0
TOTAL		2.7	100.0

*A uniform and identical extracellular and intracellular mass concentration of $2.4 \cdot 10^{-2}$ ^{14}N, $1.03 \cdot 10^{-1}$ ^{1}H and $2 \cdot 10^{-5}$ ^{10}B was assumed (fraction by weight). Electron equilibrium was assumed to exist at this depth.
**An RBE value of 2 was assumed for the fast neutrons and the $^{14}N(n,p)^{14}C$ reaction. A value of 2.5 was used for the $^{10}B(n,\alpha)^{7}Li$ reaction.

Table II

Irradiation Time (MW min) (n = fractions)	$\mu g^{10}B$/gbw	Initial tumor volume (cm^3)	Growth to $10 \cdot V_0$ (days)	Growth Delay Ratio	Dose (Gy·RBE)
	----	0.074±.013	7.4	----	0
0					
6.4[a] (n=3)	----	0.074±.013	17.6±8	2.4±.12	4.6
0	----	0.104±.024	7.8	----	0
6.4[b] (n=3)	49	0.104±.024	28.2±2.6	3.82±.40	16.7
0	----	0.050±.005	7.1	----	0
10.3[c] (n=5)	----	0.050±.005	22.5±1.5	3.27±.21	7.4
0	----	0.043±.004	7.0	----	0
10.3[d] (n=5)	37	0.043±.004	44.5±1.5	6.33±.22	26.8

Letters refer to the number of animals in the experiment.
a=21 b=10 c=18 d=17

Table III

Initial Tumor Volume (cm_3)	Growth to $10 \cdot V_0$ (days)	Growth Delay Ratio	Dose (Gy)
0.090±.036	8.9±2.9	-----	0
0.129±.065	23.4±8.2	2.63	27.2

in which an in vitro system was used, we demonstrated the substantial difficulty of using the Dose • RBE approach for this purpose. We now show that the same difficulties exist when an in vivo system such as that employed here is used in an attempt to use the Dose • RBE approach.

In preliminary experiments, we first compared the tumor growth delay obtained in an x-ray study using the murine mammary tumor model. Two groups (six mice each), one x-irradiated and one control, were used. Tumors were irradiated to a total dose of 27.2 Gy using a 100 kVp x-ray source filtered with 1.5 mm Al. Data are shown in Table III. The GDR in this experiment was 2.63 days, yielding a photon equivalent dose (PED) of 27.2 Gy. With reactor irradiations, a total dose of 4.6 Gy yielded a GDR of 2.4 days (Table II). Based upon the RBE assumption listed in Table I, the Dose •RBE was given as 4.6. The discrepancy between the PED of 27.2 Gy and the effective dose (D_{EF}) of 4.6 Gy, is a factor of 6. Clearly, the Dose •RBE method in these preliminary experiments and using RBE values that have frequently been used, did not predict correctly the severity of biological effect. We intend to seek an explanation for the discrepancies in additional experiments. Fortunately, the effectiveness of boron compounds in vivo can be established by using MW •min as a measure of exposure, without need to resort to the Dose •RBE method.

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