CONF-9609193--10
BNL-64237

CARBORANE-CONTAINING METALLOPORPHYRINS FOR BNCT

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INTRODUCTION

For BNCT of malignant brain tumors, it is crucial that there be relatively high boron concentrations in tumor compared with normal tissues within the neutron-irradiated treatment volume. Fairchild and Bond [1] estimated that major advances in BNCT should be possible if ratios of ^{10}B concentrations in tumor to those in normal tissue (e.g. brain and blood) were at least 5:1. Given that the only current boron carrier being tested clinically in the U.S., *p*-boronophenylalanine [BPA] [2], yields tumor:blood and tumor:brain ratios of about 3:1, the criteria for new boronated compounds should be to at least match these ratios and maintain tumor boron concentrations greater than 30 μg B/g.

Although previously tested boronated porphyrins have not only matched but surpassed these ratios, it was at a cost of greater toxicity [3]. Chemical and hematological assays of blood analytes showed marked thrombocytopenia, a decrease to about one-tenth the normal concentration of platelets circulating in the blood, in addition to abnormalities in concentrations of circulating enzymes, that indicated liver toxicity [3,4]. The physical appearance and behavior of the affected mice were different from those of mice injected with solvent only. Although thrombocytopenia and other toxic effects had disappeared after a few days, previously tested porphyrins would not be safe to infuse into patients for BNCT of potentially hemorrhagic malignant tumors in the brain such as glioblastoma multiforme and metastatic melanoma.

We synthesized a different boronated porphyrin, tetracarboranylphenylporphyrin, [TCP] and inserted nickel, copper, or manganese into its coordination center (Figure 1). Biological studies of NiTCP in mice [5] and of CuTCP in rats show that these compounds elicit little or no toxicity when given at potentially therapeutic doses.

MATERIALS AND METHODS

NiTCP was synthesized from 3,4-diacetic acid pyrrole dimethylester [6] and 3-*o*-carboranylmethoxybenzaldehyde [7] as described [5]. CuTCP was synthesized in an analogous manner except that copper acetate was used instead of nickel acetate. CuTCP was purified by flash chromatography using silica and 1% acetone in dichloromethane as eluent. The yield of CuTCP is typically 40% similar to NiTCP starting with the pyrrole and benzaldehyde.

Solutions containing 1.67 and 2.27 mg NiTCP/ml were prepared for murine studies by dissolving NiTCP in Cremophor EL [CRM] and propylene glycol [PrG]. Saline (0.9% NaCl) was added dropwise to the solution to give final concentrations of 3-9% CRM and 6-18% PrG v/v [8]. For rat studies the solutions were filtered through an 8 μm filter and the concentration ranged between 2.4 and 4.0 mg CuTCP/ml. A solution containing 100 mg BPA/ml was prepared as a fructose complex [9].

Female BALB/c mice (Taconic Farms, Germantown, NY) weighing 20-24 g were implanted with KHJJ mammary carcinomas. A 1-3 mm^3 tumor fragment was implanted s.c. in the dorsal thorax of each mouse with an 18-gauge trocar. Serial i.p. injections of NiTCP were initiated when the tumors weighed between 50 and 100 mg (ten days after tumor implantation) using a NiTCP solution of 0.02 ml/gbw per injection. Mice were kept in darkness at night and in subdued light during the day with access to food and water *ad libitum*.

Under deep Halothane anesthesia leading to euthanasia, right ventricular blood was collected in Microtainer (Becton-Dickinson, Rutherford, NJ) tubes containing EDTA for hematological analyses and boron concentration measurements of whole blood and in Microtainer™ tubes containing lithium heparin for chemical analyses and enzyme assays of blood plasma. Tumor, brain, and liver were sampled at necropsy for boron analyses.

Male Fisher 344 rats were implanted with multiple subcutaneous 9L gliosarcomas (2×10^6 cells in 100 μl of growth medium). The animals were prepared for i.v. infusion using small-animal swivels with spring tethers, silicone tubing, and syringe pumps [10]. When tumors were approximately 100 g (11 days after tumor implantation) CuTCP was infused over a 48-hr period. Tumors were surgically removed and retroorbital venous blood was sampled at 0, 1, 2, 4, and 6 days after the end of infusion. Tumor and blood were assayed for boron and blood was analyzed for toxicity.

Direct current plasma-atomic emission spectroscopy (ARL/Fisons Model SS-7) was used (detection limit: 0.1 μg/ml). Samples (50-130 mg) were digested at 60°C with sulfuric acid:nitric acid (1:1). Triton X-100 and water were added to give final concentrations of ≈50 mg tissue/ml, 15% total acid v/v and 5% Triton X-100 v/v.

RESULTS AND DISCUSSION

NiTCP was given to tumor-bearing BALB/c mice at 2 total doses; 160, and 244 μg NiTCP/g body weight [gbw]. Mice given the lower dose of 160 μg NiTCP/gbw were compared to mice given 900 μg BPA/gbw complexed with fructose. The resulting boron concentrations in tumor, blood and brain are shown in Table 1. At 6-hr post-injection, there was a median of 27 μg B/g in tumors of mice given NiTCP and only about half this amount in mice given BPA. Blood boron was about double that in tumor at this time point, but at 78 hr, the tumor-to-blood ratio was 85:1 and the tumor-to-brain ratio was 10:1. However, the tumor boron concentration was only 17 μg B/g.

Following injections of 244 μg NiTCP/gbw, the boron concentrations in tumor, brain, and blood at 3 time points are also shown in Table 1. At 41 hr after the last injection, a median of 69 μg B/g was found in tumor tissue with a tumor:blood ratio of 3.6:1. At 90 hr, a median of 43 μg B/g was found in tumor tissue with a tumor:blood ratio of >200:1. The tumor:brain ratios are ≈10:1 at both time points.

The physical appearance and behavior of mice given NiTCP were no different from those given only solvent or saline. At necropsy, tissues and organs appeared normal in mice given NiTCP and few differences in chemical, enzymatic, or hematological values from those obtained from mice given solvent only. Of particular importance is that thrombocytopenia was never observed. A 3-month study using BALB/c mice with no tumors given 270 μg NiTCP/gbw showed no differences from control mice given only solvent [5].

For studies of radiotherapeutic dosimetry in neutron-capture therapy, rats bearing intracerebral 9L gliosarcomas will be used. Drug administration methods will be optimized for tumor boron concentration, tumor:blood boron ratios and tumor:brain boron ratios using rats bearing subcutaneous tumors, which permits serial sampling. Since i.v. infusion is used clinically and is feasible in rats but not convenient in mice, this was used to administer CuTCP.

Rats were infused with a starting dose of 110 mg CuTCP/kg over a 48-hr period using a total infusion volume of 9.6 mL. Blood and tumor were sampled 0, 1, 2, 4, and 6 days after the end of infusion and animals were killed on day 6. The boron concentrations are shown in Table 2. As with the i.p. injections in mice, the boron levels in tumor decreased with time, although, at a much slower rate than the boron in blood. The optimal time for neutron irradiation appears to occur 2 days after the end of infusion, when the average tumor boron concentration is ≈23 μg B/g and the tumor:blood ratio is ≈39:1.

In order to increase the tumor boron concentration, the total dose was increased to ≈200 mg CuTCP/kg with a total infusion volume of 19.2 ml. Tumor and blood were sampled at 0, 1, and 2 days after the end of infusion. Animals were killed at 2 days to determine brain boron. The results

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in Table 3 show that a median of 38 μg B/g was found in tumor on day 2, which is sufficient for neutron-capture therapy. However, the tumor:blood ratio was only 1.5:1. The tumor and blood boron values were extrapolated beyond 48 hr on the assumption that they decayed exponentially. Tumor and blood boron values of 32 and 2 μg B/g, respectively, were estimated using this method, which indicate that therapy would be possible at 96 hr. The brain boron values were ≈2 in both cerebellum and cerebrum at the 2-day time point giving a tumor:brain boron ratio of 20:1.

Similar to mice given NiTCP, rats given CuTCP showed no abnormal behavior patterns nor did their physical appearance differ from animals given solvent only. Results of chemical, hematological, and enzymatic blood tests of rats showed no differences from rats given only solvent.

In conclusion, data from mice and rats have shown not only that both NiTCP and CuTCP can deliver large quantities of boron to tumor with high tumor:blood and tumor:brain boron ratios, but can accomplish this with little or no toxicity. The much slower clearance of boron in tumor than from blood might allow fractionation of neutron irradiation without reinfusion of drug.

ACKNOWLEDGEMENTS

This research was supported by United States Department of Energy contract DE-AC02-76CH00016.

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Table 1. Boron concentrations in various tissues from BALB/c mice bearing KHJJ tumors given either BPA (single i.p. injection) or NiTCP (6 i.p. injections over 32-hr period) at 2 different doses at different time points. Tabulated values are median followed by the range.

Drug	**BPA**	**NiTCP**	**NiTCP**	**NiTCP**	**NiTCP**	**NiTCP**
Period after injections	**6 hr**	**6 hr**	**78 hr**	**17 hr**	**41 hr**	**90 hr**
Dose (μg/gbw)	900	160	160	244	244	244
Dose (μg B/gbw)	43	36	36	54	54	54
n	7	8	8	6	6	6
Tumor (μg B/g)	14 (11-21)	27 (20-43)	17 (9-29)	52 (37-83)	69 (40-92)	43 (28-47)
Blood (μg B/g)	5 (1-9)	56 (33-74)	0.2 (0.1-0.2)	92 (60-123)	19 (16-42)	0.2 (0.1-0.4)
Brain (μg B/g)	7 (3-11)	7 (3-11)	2 (0.4-6)	2 (1-2)	6 (2-9)	5 (3-12)

Table 2. Boron concentrations in tumor and blood from Fisher rats bearing multiple subcutaneous 9L gliosarcomas given CuTCP at 2 different doses by 48-hr i.v. infusion. Tabulated values are median followed by the range. Number of animals was 3 in each group.

Period after end of infusion	**48 hr**	**0 hr**	**24 hr**	**48 hr**	**96 hr***
Dose (mg/kgbw)	110	200	200	200	200
Tumor (μg B/g)	23 (17-33)	52 (35-58)	44 (30-52)	38 (36-46)	33
Blood (μg B/g)	0.6 (0.5-0.6)	385 (341-428)	144 (142-151)	26 (25-35)	2
Brain (μg B/g)				2 (1-2)	

*extrapolated assuming exponential decay

Legend to Figure 1.

Chemical structures of NiTCP, CuTCP, and MnTCP. Open circles represent C or CH and shaded circles represent BH.