Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7265–7269, September 1990
Medical Sciences

Uptake of a *nido*-carboranylporphyrin by human glioma xenografts in athymic nude mice and by syngeneic ovarian carcinomas in immunocompetent mice

(boronated porphyrin/boron neutron capture therapy/brain tumors/hepatotoxicity/thrombocytopenia)

S. B. KAHL*, D. D. JOEL†, M. M. NAWROCKY†, P. L. MICCA†, K. P. TRAN†, G. C. FINKEL†, AND D. N. SLATKIN†

*Department of Pharmaceutical Chemistry, University of California–San Francisco, San Francisco, CA 94143-0446; and †Medical Department, Brookhaven National Laboratory, Upton, NY 11973

Communicated by M. Frederick Hawthorne, May 7, 1990 (received for review January 19, 1990)

ABSTRACT **A tetraphenylporphyrin bearing four dicarbollide ($[B_9C_2H_{11}]^-$) cages linked to the *o*-phenyl ring positions by anilide bonds, known as boronated tetraphenylporphyrin (BTPP), has been synthesized in excellent yield from tetra-(*o*-aminophenyl)porphyrin and carborane carbonyl chloride followed by base-assisted cage opening and ion exchange to give the highly water-soluble potassium salt. Preliminary studies showed that BTPP accumulates in liver and in a syngeneic ovarian carcinoma, but not in normal brain parenchyma, of mice infused with BTPP subcutaneously for 6 or 7 days via surgically implanted osmotic minipumps. In this study, the uptake of boron was measured in human gliomas xenografted subcutaneously to athymic nude mice in which BTPP was infused intraperitoneally or subcutaneously or both for 3 or 7 days by using similar minipumps. Immunocompetent mice bearing a syngeneic ovarian carcinoma were similarly infused to provide comparative data. Bulk concentrations of boron up to 18 μg/g of glioma and up to 45 μg/g of carcinoma were observed when up to 102 μg/g of tissue was present in the liver after 7 days of BTPP infusion. Glioma boron concentrations were increased by ≈80% on the average (up to 33 μg/g) when correspondingly greater amounts of BTPP were infused in only 3 days. Cell counts and chemical tests on blood samples from individual mice indicate that BTPP causes moderate hepatotoxicity and thrombocytopenia. This hepatohematic toxicity syndrome should be taken into account if BTPP or a similar agent is used for boron neutron-capture therapy (BNCT) of human malignancies.**

The use of boron-containing substances in the treatment of human cancer is based on the high probability of reaction of the stable isotope boron-10 with slow neutrons, which produces an α-particle and a recoil lithium-7 particle with combined kinetic energies of ≈2.4 MeV and a 478-keV γ photon.

The photons may be quantified to measure ^{10}B nondestructively (1). Boron neutron-capture therapy (BNCT) is a form of binary therapy in which ^{10}B [currently delivered to patients with gliomas as $Na_2B_{12}H_{11}SH$ (2) or with melanomas as *p*-boronophenylalanine (3)] preferentially accumulates in a tumor before the tumor is irradiated by slow neutrons. The α and lithium-7 particles have ranges in tissue of only 9 and 5 μm, respectively. Thus, ionizing radiation is deposited preferentially in and around the tumor. A tumor ^{10}B concentration of ≈20 μg/g should be sufficient to retard tumor growth by using existing neutron beams. A tumor-to-normal-tissue ^{10}B concentration ratio exceeding 1:1 is desirable for BNCT to have advantage over other irradiation procedures.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Porphyrins and other macrocyclic nitrogen heterocycles accumulate in a variety of tumors. Although the explanation for this phenomenon remains controversial, it is the basis for photodynamic therapy (PDT), another potent binary cancer treatment (4). This communication reports *in vivo* toxic effects, tissue distribution, and tumor affinities of boronated tetraphenylporphyrin (BTPP), the synthesis of which was first reported in 1983 (5).

MATERIALS AND METHODS

Preparation of BTPP. The piperidinium salt of the non-^{10}B-enriched open-cage carboranylporphyrin was synthesized and converted to its potassium salt by a modification of the method previously described (6). The purple microcrystalline piperidinium salt was dissolved in a minimum volume of 60% aqueous acetone and passed slowly through a light-shaded column of Dowex 50W grade no. 50X2-100 ion-exchange resin in the potassium form. The eluate was lyophilized, redissolved in a minimum volume of 30% aqueous acetone, and passed again through the resin. The product was lyophilized to yield a purple, spontaneously electrostatic powder, heated *in vacuo* at 60–80°C overnight, and then stored under argon while shaded from ambient light.

Biolocalization Studies. *Experiment 1 (see Table 1).* Human malignant glioma (U-87 MG) cells maintained in culture (7) were transplanted to two subcutaneous sites on the flanks of 10 7- to 10-week-old female athymic (genotype *nu/nu*; NIH Swiss albino background) nude mice (E1–E10). When tumors reached 5–10 mm in diameter, the mice weighed 27–34 g (median, 28 g). On that day (day 0 of experimentation), one large osmotic minipump (Alza; Alzet 2001, ≈220 μl, ≈1 μl/hr) was implanted intraperitoneally (i.p.), and two smaller pumps (Alzet 1701, ≈170 μl, ≈1 μl/hr) were implanted subcutaneously (s.c.) in each of three mice (E7, E9, and E10). In three mice (E1, E3, and E4) one pump (Alzet 2001) was implanted i.p., and in two mice (E6 and E8) one pump (Alzet 1701) was implanted s.c. One large (i.p.) and two small (s.c.) mock pumps were implanted in a 27-g mouse (E2), and one no. 1701 pump containing solvent only (0.5% $NaHCO_3$ in H_2O) was implanted i.p. in a 34-g mouse (E5). One day before implantation, Alzet pumps 2001 and 1701 were loaded with solutions that contained ≈29.2 mg pf BTPP potassium salt per g of solvent and then refrigerated until use. Mice were almost continuously shaded from direct light after pump implantation during these experiments.

On day 5 of experimentation, ≈400–700 μl of blood was drawn from the scalpel-nicked ventral tail artery of mice E1–E10 (except from mouse E5, which was found dead in its

Abbreviations: BTPP, boronated tetraphenylporphyrin; BNCT, boron neutron-capture therapy.

cage) into microtubes containing EDTA for analysis in an automated blood cell counter (System 9000; Serono–Baker Instruments). On day 5 also, three untreated, aged >1 year male nude mice of the same strain (body weight, ≈30 g) were selected for experimentation, and two of these (CNTL1 and CNTL2) underwent similar blood analysis.

On day 7, 38 days after tumor transplantation, mice E1–E10 were euthanized by ether inhalation. Blood samples were taken in EDTA-treated tubes for blood cell analyses and in heparinized tubes for chemical analyses of plasma (Ektachem; Eastman Kodak). Tissues (plasma, liver, and glioma) were analyzed for ^{10}B by neutron-induced γ spectroscopy (1). The subcutaneous gliomas weighed ≈0.4–1.0 g each. Boron concentrations were calculated by multiplying ^{10}B concentrations by 5.0, reflecting the approximate 20% natural abundance of ^{10}B. Whole-body boron doses (μg per g of body weight) were determined from weights of tared and filled pumps and from boron concentrations of diluted aliquots of the solutions loaded into the pumps. These were corrected for residual boron after the infusions by soaking dismantled (flow moderator separated from pump body) osmotic pumps in 3 or 4 ml of water for several weeks and then measuring boron in 1.00 g aliquots of the soaking solutions. Air-dried, thin sections of snap-frozen (by immersion in isopentane cooled with liquid N_2) liver, glioma, and brain tissues from several mice were examined by violet/ultraviolet-induced red fluorescence (see Fig. 3) as described (6).

On day 7, some residual, refrigerated, light-protected BTPP solution (29.3 mg/g of solvent) was loaded into 10 Alzet 1003D pumps (≈88 μl, ≈1 μl/hr) for surgical implantation into two previously untreated male nude mice [CNTRL1 (three i.p. and two s.c. pumps) and CNTRL2 (five i.p. pumps). Three days later, these two male mice and a third previously untreated non-tumor-bearing male mouse [UNTR] were euthanized for similar blood and tissue analyses.

Experiment 2 (see Table 2). Fourteen 9- to 13-week-old female nude mice bearing similar s.c. xenografted human gliomas were each implanted with four (in mouse 11, five) Alzet no. 1003D pumps i.p. The pumps had been filled with a solution of ≈27 mg of K_4BTPP per ml in 0.5% $NaHCO_3$. After about 3 days of i.p. infusion, during which time the mice were shielded from direct light, the mice were euthanized under deep ether anesthesia. Tissues (liver, spleen, kidney, glioma, and whole blood) were analyzed for boron as described (see Table 1). Several nude mice were photographed under long-wave ultraviolet/violet illumination (see Fig. 2 *Top*). Major vital organs and a xenografted human glioma of mouse 6 and of an untreated nude mouse were photographed in white light (Fig. 2 *Middle*) and in long-wave ultraviolet/violet light (Fig. 2 *Bottom*) shortly after euthanasia.

Experiment 3 (see Table 3). BTPP was infused subcutaneously into 14 young C3HeB/FeJ mice bearing i.p. and s.c. transplanted syngeneic ovarian carcinomas with osmotic minipumps (6). Nineteen days before surgical implantation of the pumps (day −19), subcutaneous ovarian carcinomas were initiated in the suprascapular region by using the procedure as described (6). On day −16, ≈10^5 carcinoma cells suspended in 0.1 ml of phosphate-buffered saline were injected i.p. in mice N1–N10. By day −3, pea-sized subcutaneous tumors were seen. On day 0, Alzet 2001 pumps (≈220 μl, ≈1 μl/hr) were loaded with a solution of 28.7 mg of K_4BTPP crystals per ml of 0.5% $NaHCO_3$. The pumps were inserted beneath the dorsal thoracic skin under ether anesthesia (N1–N4, two pumps; N5–N8, one pump), positioned so that pump flow orifices were caudad to the suprascapular tumors. The subcutaneous fasciae around the tumors were not breached during pump insertion. One (N5–N8) or two (N9–N12) mock pumps were similarly inserted to provide mice N1–N12 with comparable surgical stress.

Mice were weighed during the week after pump implantation. On day +4, ≈400–700 μl of blood were drawn from the ventral tail artery for cell counts and chemical analyses. On day +7, the mice were euthanized under deep ether anesthesia, and right ventricular blood was obtained for similar analyses.

RESULTS AND DISCUSSION

The structure of the BTPP anion (Fig. 1) consists of four dicarbollide open cages ($[B_9C_2H_{11}]^-$) bound to a tetraphenylporphyrin framework by anilide bonds. Such compounds are efficient boron carriers capable of bearing up to 40 or more boron atoms per molecule. Four isomeric orientations of the carborane cages are possible, but the ^1H NMR spectrum of BTPP is consistent only with a highly symmetric species. This limits the probable orientations to α^4 (four anionic cages on the same side of the porphyrin plane) or $\alpha\beta\alpha\beta$ (alternating above and below the plane). Since coulombic repulsions resulting from four negative charges on the same face are likely to be large, the potential energy is probably minimized in the alternating $\alpha\beta\alpha\beta$ orientation, which is therefore the most probable.

The nominal weight percentage of boron in the tetrapotassium salt is 26.5%, but the material isolated after ion exchange, lyophilization, and heating *in vacuo* contains residual water of hydration and is hygroscopic. Boron concentrations of infusion solutions were determined directly to assess boron loaded in pumps. Measured boron content of K_4BTPP solutions used in these experiments gave an average boron weight percentage of about 20 ± 2% for the solid material, suggesting that the material contains about 25% water by weight.

Red fluorescence from BTPP-infused nude mice under long-wave ultraviolet illumination was remarkable (Fig. 2). Subcutaneous gliomas formed ≈5- to 10-mm diameter prescapular bulges (Fig. 2 *Top*). Cut sections of boronated gliomas were pink, which is attributed to superimposition of ultraviolet-induced red fluorescence, green autofluorescence, and violet reflection. Other organs showed intense red fluorescence: the spleen, liver, and kidney as well as the abdominal wall immediately adjacent to the infusion pump outlets. The more intense fluorescence of spleen and liver relative to tumor is expected (Table 1). Although no organ of

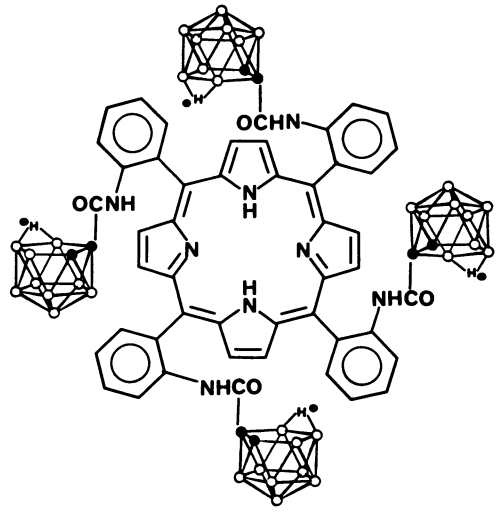

FIG. 1. Chemical structure of BTPP. In the borane cages, small open circles (○) represent BH units and small closed circles (●) represent CH or C if substituents are attached to C.

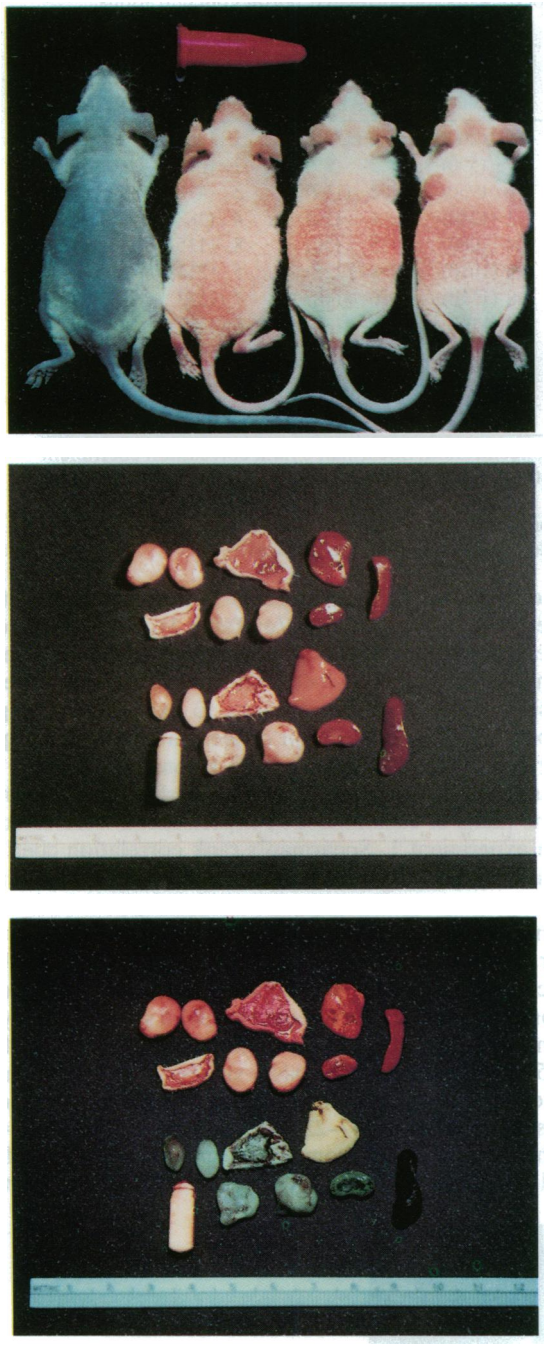

FIG. 2. (*Top*) Appearances of an untreated nude mouse (leftmost) and three BTPP-infused nude mice (experiment 2; mice from right to left: nos. 13, 12, and 6) photographed during illumination by long-wave ultraviolet/violet light. A translucent plastic microcentrifuge tube contains the red-fluorescent gel formed from interaction of mouse serum and BTPP crystals (see text). (*Middle*) White-light photograph of organs from necropsies on nude mouse 6 (experiment 2) (rows 1 and 2) and of an untreated nude mouse bearing similar xenografted human gliomas (rows 3 and 4). Numbered from left to right, objects 1 and 2 (rows 1 and 3) and objects 2 and 3 (rows 2 and 4) are cut sections of subcutaneous, xenografted human gliomas. Object 3 (rows 1 and 3) is the abdominal wall viewed from the parietal peritoneum. Object 4 is the surface of the liver in rows 1 and 3 or the cut surface of one kidney in rows 2 and 4. Object 1, row 2, is the undersurface of the skin that overlay the tumor in row 1. The right-most objects are two spleens with long axes vertical in the photograph. Object 1, row 4, is one of the four pumps removed from mouse 6 at necropsy. (*Bottom*) Same objects as in *Middle* but illuminated only by long-wave ultraviolet/violet light.

untreated mice showed more intense green autofluorescence than did the liver, the livers of BTPP-infused nude mice were invariably deep red. Necropsy demonstrated that the red fluorescence of the skin overlying the tumors in Fig. 2, seen most prominently in mouse 13, is attributable mainly to fluorescence of normal dermal tissue rather than to fluorescence of the underlying glioma.

Microscopic examination of thin (≈8 μM) cryostat sections of frozen tissues (experiment 1) revealed red fluorescence of boronated tissues (Fig. 3) and absence of red fluorescence from brain tissue as expected (6). Cytoplasmic fluorescence from the glioma of mouse E4, in which the bulk boron concentration was ≈5 μg/g, was weak. Most of this fluorescence was seen as a meshwork of thin irregular lines seemingly associated with cytoplasmic membranes (Fig. 3*d*). However, in the glioma of mouse E7, in which boron was >3-fold more concentrated, most of the fluorescence appeared to be diffusely distributed in the cells (not shown). These images suggest that the internalization of membrane-associated BTPP may proceed more efficiently at high than at low extracellular concentrations of BTPP.

The whole-body boron doses in Table 1 are upper limit estimates only, since a portion of the boron loaded into pumps may not have leached from dismantled pumps into the soaking solutions. Boron concentrations in packed erythrocytes were so low as to be attributable to adsorbed plasma. The arithmetic mean of liver/glioma boron ratios (Table 1, E1–E10), after each ratio is weighted multiplicatively by relative glioma boron concentration, is 5.0. The mean of liver/whole-body boron ratios, after each ratio is weighted by relative liver boron concentration, is 1.3. Thus, the anticipated glioma/whole-body boron dose ratio is 0.26. However, the observed mean of glioma/whole-body ratios, after each ratio is weighted by glioma boron concentration or by whole-body boron dose, was only 0.17 or 0.14, respectively. These calculations suggest that bulk concentrations of boron in xenografted human gliomas of nude mice (μg per g of tumor) are likely to be considerably less than one-quarter of whole-body boron administered (μg of boron per g of body weight) when BTPP was delivered during 7 days of continuous infusion.

Certain plasma enzyme levels indicate that BTPP is hepatotoxic (Table 1). Hematoxylin/eosin-stained sections of formalin-fixed livers showed diffuse fatty change. Focal hepatic subcapsular coagulative necrosis, present only in mouse E7, was associated with extraordinary levels of plasma enzymes and urea nitrogen. This might have been caused by mechanical pressure of the intraperitoneal pump on the liver vasculature, which incited hepatorenal toxicity.

The liver of mouse CNTL2, which showed only slight fatty change, accumulated 38% more boron in 3 days than did the liver of E7 in 7 days (data not shown). Blood platelets in CNTL2 at day 3 were normal in number, unlike those in E7, which were about 5-fold reduced at day 7. Such observations suggested to us that 3-day infusions of BTPP might allow delivery of as much boron to gliomas as 7-day infusions, perhaps with less toxicity.

Table 1 lists the ordinal rank numbers for parameters (plasma, blood, and glioma boron concentrations; blood platelet count; plasma enzyme concentrations) that yield rank correlation coefficients (r_S) with the rank of liver boron concentrations (the reference parameter) by Spearman's test. The probabilities of correlation of the rank of liver boron concentration with the ranks of each of these seven parameters are: plasma boron, >99%; blood boron, >99%; xenografted human glioma boron, 90%; blood platelets, 95%; plasma alanine aminotransferase, >99%; plasma aspartate aminotransferase, 98%; and plasma alkaline phosphatase, >99%. There was no significant rank correlation between estimated whole-body boron doses and liver boron concen-

Table 1. Ordinal rank numbers and Spearman's rank correlation coefficients for correlated boron concentrations, platelet count, and plasma enzymes (day 7 of experiment 1)

		Boron concentration				Enzyme activity†		
Mouse	Dose*	Liver	Plasma	Glioma	Platelets	ALT	AST	ALP
E7	92	1 (102)	1 (44)	1 (18)	8 (200)	1 (1596)	1 (3260)	1 (275)
E4	27	2 (67)	2 (20)	6 (5)	7 (590)	3 (89)	2 (618)	3 (95)
E1	26	3 (56)	4 (16)	4 (10)	6 (950)	2 (119)	3 (478)	4 (93)
E10	78	4 (50)	3 (20)	3 (13)	1 (1240)	4 (70)	4 (383)	2 (133)
E9	89	5 (50)	5 (8)	2 (14)	4 (1080)	5 (50)	8 (115)	6 (40)
E8	32	6 (15)	6 (7)	5 (5)	5 (1010)	8 (28)	5 (211)	5 (41)
E3	39	7 (8)	8 (0)	8 (2)	3 (1110)	6 (48)	7 (133)	7 (39)
E6	28	8 (7)	7 (4)	7 (4)	2 (1190)	7 (37)	6 (167)	8 (33)
		Correlation coefficient and probability						
r_S		+1.00	+0.95	+0.64	−0.74	+0.91	+0.83	+0.90
P [$r_S \neq 0$]			0.99	0.90	0.95	0.99	0.98	0.99

The correlation reference parameter = ordinal rank of liver boron concentration; Spearman's rank correlation coefficient = r_S. The numbers in parentheses are the measured boron concentration (μg of boron per g of tissue), platelet count (10^3/mm^3), or plasma enzyme activity (units/liter).

*Dose of boron delivered by osmotic pumps to the animal (μg of boron per g of body weight).

†ALT, alanine aminotransferase; AST, aspartate aminotransferase; and ALP, alkaline phosphatase.

trations. Thus, it was inferred that liver boron concentration is the preferred index of boron delivery by BTPP to the mouse for these experiments.

The inverse correlation of platelet counts in peripheral blood with average liver boron concentrations (Table 1) suggests that mice receiving BTPP are at risk for moderate thrombocytopenia. However, nonplatelet cell constituents of the blood were unperturbed by BTPP. Thrombocytopenia was not so severe as to be associated with petechial hemorrhages.

The addition of fresh mouse serum to BTPP crystals in the ratio of 36 mg of BTPP per ml of serum resulted in prompt jellification of the serum to form a black (when viewed in white light) red-fluorescent (Fig. 2) gel. We suggest that a more suitable vehicle for BTPP than 0.5% $NaHCO_3$, perhaps plasma albumin or Cremophor EL, might result in more uniformly delivered and possibly less toxic infusions of BTPP, since concentrated aqueous solutions of BTPP are not expected to be readily compatible with plasma or interstitial tissue fluid.

BTPP, when delivered via 3-day intraperitoneal infusions (experiment 2), yielded much higher glioma boron concentrations (mean, 21.6 μg/g; Table 2) than those obtained in experiment 1 (mean, 8.9 μg/g; Table 1). Since the livers of mice in experiments 1 and 2 underwent only moderate fatty change, we suggest that 3-day infusions of BTPP at doses that cause liver boron concentrations to exceed 185 μg/g will probably yield glioma boron concentrations >20 μg/g with tolerable toxicity. In fact, mice 1 through 5 (Table 2) demonstrate the feasibility of obtaining such concentrations.

The ranks of each mouse in experiment 2 were calculated as in experiment 1. Spearman's rank correlation coefficient for liver boron rank versus the overall boron uptake rank (8) is again highly significant [$r_S = +0.92$; P ($r_S \neq 0$) >0.998]. The glioma boron concentration rank correlated better with the liver boron concentration rank than with the overall boron concentration rank ($r_S = +0.75$ vs. $r_S = +0.56$). The correlations of overall boron uptake rank with spleen, kidney, and glioma boron concentration ranks ($r_S = +0.83$, +0.54, and +0.56, respectively) are also statistically significant but somewhat less so. Mice that acquired liver boron concentrations in the range of 147–220 μg/g lost ≈15% (range 10–19%) of their body weight from the beginning of BTPP infusion until euthanasia about 3 days later. However, similarly

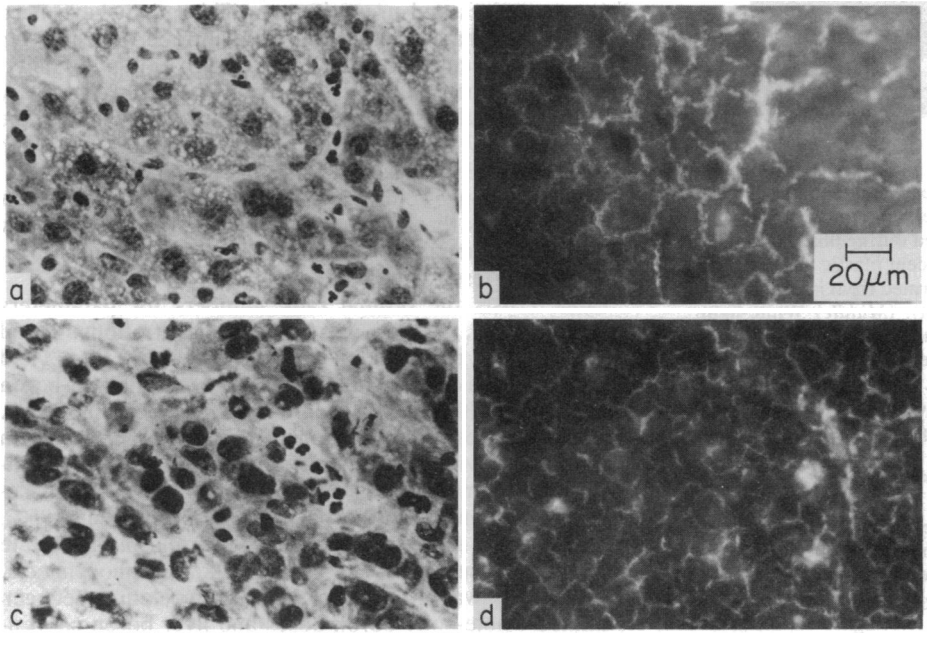

FIG. 3. Photomicrographs of ≈8-μm-thick air-dried cryomicrotome sections of tissues from nude mouse E4 (experiment 1). (*b* and *d*) Images of long-wave violet/ultraviolet-induced red fluorescence in liver and in transplanted human malignant glioma, respectively. Green autofluorescence is suppressed by barrier filters. (*a* and *c*) Representative zones of liver and glioma in contiguous, parallel ≈8-μm-thick, air-dried, hemotoxylin/eosin-stained cryomicrotome sections of liver and glioma, respectively. The 20-μm scale in *b* applies to *a*–*d*.

Table 2. Tissue boron concentrations after 3-day BTPP infusion in nude mice bearing human gliomas (experiment 2)

Mouse	Boron, μg/g of tissue				
	Liver	Glioma	Spleen	Kidney	Blood
1	220	33	189	80	38
2	213	32	221	91	11
3	192	25	179	81	—
4	186	23	219	54	—
5	186	23	108	67	33
6	185	14	172	102	—
7	174	20	163	75	—
8	174	28	204	79	46
9	168	23	148	54	31
10	154	19	148	65	—
11	147	16	125	32	—
12	144	19	69	61	—
13	135	15	87	55	—
14	129	12	84	34	35
Mean	172	22	151	66	31

light-shaded mice infused concurrently with tetraphenylporphyrin sulfonate [23.3 mg of TPPS per ml of solvent (0.5% $NaHCO_3$/Cremophore, 3:1, wt/wt)] via four i.p. Alzet 1003 D pumps lost only 6% (range 5–8%) of their body weight during the same 4-day interval. This confirms that the toxicity of BTPP is not an artifact of the osmotic pump delivery system.

To examine the uptake of BTPP in a different mouse tumor model system, we performed experiment 3 with immunocompetent mice bearing a syngeneic ovarian carcinoma, using techniques similar to those of experiment 1. The BTPP-associated thrombocytopenia and liver-associated plasma enzyme increases noted in nude mice were also observed in these immunocompetent mice (data not shown). Similarly, body weight decrements were greater in these BTPP-infused immunocompetent mice than in comparably stressed immunocompetent mice whether assessed 4 or 6 days after pump implantation (data not shown). Results of tissue boron analyses appear in Table 3. Noteworthy differences in tumor boron concentrations and in liver/tumor boron concentration ratios were observed between experiments 1 and 3. The average liver/carcinoma boron concentration ratio in experiment 3 was 1.4 (Table 3). When individual ratios were weighted multiplicatively by relative carcinoma boron concentrations, the weighted average was also 1.4. When the ratios were weighted by relative liver boron concentrations or by carcinoma tumor wet weight, the weighted average was 1.5 or 1.3, respectively. These ratios are much less than average liver/glioma ratios (≈5.0) observed in experiment 1. Moreover, with comparable concentrations of BTPP-derived boron in the liver, boron was nearly 3-fold more concentrated in ovarian carcinomas of immunocompetent mice than in human gliomas of athymic nude mice (Table 3 vs. Table 1). This disparity may be due to differences in tumor vascular permeability to BTPP (9), in local pH (10), in activity of low density lipoprotein receptors (11), or in intrinsic tumor cell affinity for BTPP.

CONCLUSION

These results demonstrate the feasibility of achieving therapeutically useful (≥20 μg/g of tissue) concentrations of boron in human gliomas and murine ovarian carcinomas by using BTPP. As gauged by rises in plasma enzyme levels, hepatotoxicities to mice from BTPP and $Na_4B_{24}H_{22}S_2$ (12) appear to be of comparable severity and to be major determinants of the limits to which malignant gliomas may be loaded with boron *in vivo* by these agents.

Table 3. Tissue boron concentrations after BTPP infusion into immunocompetent mice with syngeneic ovarian carcinomas (experiment 3)

Mouse*	Boron, μg/g of tissue		
	Liver	Carcinoma	Liver/carcinoma ratio
N3	77	45	1.7
N5	44	20	2.2
N4	36	21	1.7
N7	32	28	1.1
N8	30	22	1.4
N6	21	25	0.8
N2	18	21	0.9
N1	15	<1	—
Mean†	37	26	1.4

*Seven-day infusions except for N4 and N2, which had 4-day infusions.
†N1 was excluded from calculation.

These observations add credence to our previous identification of BTPP as a potential boron transport agent for BNCT of human malignant gliomas. We also show that concentrations of boron delivered by BTPP to xenografted human gliomas of nude mice correlate well with liver boron concentrations and that doses of BTPP expected to be useful for BNCT cause significant hepatotoxicity and thrombocytopenia. Judging from the results of experiment 2, 3-day infusions of BTPP that deliver a total dose of ≈65 mg of boron per kg of body weight are expected to yield bulk boron concentrations in human gliomas of the order of 22 μg of boron per g (range 12–33 μg of boron per g). Therefore, BTPP may be capable of contributing therapeutically useful concentrations of boron to human gliomas for BNCT without debilitating toxicity.

We thank J. C. Heinrichs, R. R. Stoutenburgh, and H. L. Ulyat for technical assistance. This research was supported in part by the U.S. Department of Energy Contract DE-AC02-76CH00016 and by the National Institutes of Health Grant CA 37961. Animals were maintained in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

1. Fairchild, R. G., Gabel, D., Laster, B. H., Greenberg, D. D., Kiszenick, W. & Micca, P. L. (1986) *Med. Phys.* **13**, 50–56.
2. Hatanaka, H. (1986) in *Boron-Neutron Capture Therapy for Tumors*, ed. Hatanaka, H. (Nishimura, Niigata, Japan), pp. 1–28.
3. Mishima, Y., Honda, C., Ichihashi, M., Obara, H., Hiratsuka, J., Fukuda, H., Karashima, H., Kobayashi, T., Kanda, K. & Yoshino, K. (1989) *Lancet* **ii**, 388–389.
4. Delaney, T. F. & Glatstein, E. (1989) *Comp. Ther.* **14**, 43–55.
5. Kahl, S. B. (1983) in *Proceedings of the First International Symposium on Neutron Capture Therapy*, eds. Fairchild, R. G. & Brownell, G. L. (Brookhaven National Laboratory, Upton, NY), Rept. BNL 51730, pp. 294–303.
6. Kahl, S. B., Joel, D. D., Finkel, G. C., Micca, P. L., Nawrocky, M. M., Coderre, J. A. & Slatkin, D. N. (1989) in *Clinical Aspects of Neutron Capture Therapy*, eds. Fairchild, R. G., Bond, V. P. & Woodhead, A. D. (Plenum, New York), Vol. 50, pp. 193–203.
7. Joel, D. D., Slatkin, D. N., Micca, P. L., Nawrocky, M. M., Dubois, T. & Velez, C. (1989) in *Clinical Aspects of Neutron Capture Therapy*, eds. Fairchild, R. G., Bond, V. P. & Woodhead, A. D. (Plenum, New York), Vol. 50, pp. 325–332.
8. Moroney, M. J. (1956) *Facts from Figures* (Penguin Books, Harmondsworth, U.K.), 3rd Ed.
9. Berenbaum, M. C., Hall, G. W. & Hoyes, A. D. (1986) *Br. J. Cancer* **53**, 81–89.
10. Brault, D., Vever-Bizet, C. & Ledoan, T. (1986) *Biochim. Biophys. Acta* **857**, 238–250.
11. Kessel, D. (1986) *Cancer Lett.* **33**, 183.
12. Marshall, P. G., Miller, M. E., Grand, S., Micca, P. L. & Slatkin, D. N. (1989) in *Clinical Aspects of Neutron Capture Therapy*, eds. Fairchild, R. G., Bond, V. P. & Woodhead, A. D. (Plenum, New York), Vol. 50, pp. 333–351.