Anti-Cancer Drugs 1995, **6**, pp. 744–748

Uptake and distribution of the boron-containing ether lipid B-Et-11-OMe in tumor-bearing mice

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Ether lipids in general are accumulated in tumor tissue with a favorable tumor/healthy tissue ratio. The uptake of the boron-containing analog *rac*-1-(9-*o*-carboranyl)nonyl-2-methyl-glycero-3-phosphocholine (B-Et-11-OMe) was studied in C3H mice bearing the murine mammary carcinoma AT17 and in BALB/c mice bearing an osteosarcoma. Boron concentrations of tumor, blood, liver and kidney were followed up to 48 h by inductively coupled plasma emission spectrometry and inductively coupled plasma mass spectrometry. Boron concentration in AT17 mamma carcinoma rose up to 2 mg/kg and the tumor/blood ratio rose to 0.5. The bulk was taken up by the liver. Osteosarcoma did not take up B-Et-11-OMe. This result constitutes a significant contrast to the behavior of published (non-boron-containing) analogs. It is interpreted in terms of critical micellar concentration (CMC). Whereas earlier work with ether lipids was done well below CMC, this study was undertaken above. Further studies will concentrate on syntheses of high CMC analogs.

Key words: AT17, B-Et-11-OMe, BNCT, boron neutron capture therapy, C3H mice, carborane, ICP-AES, ICP-MS, mammary carcinoma, osteosarcoma.

Introduction

Boron neutron capture therapy (BNCT) is a fascinating concept of binary cancer treatment.[1] It depends on the products of the $^{11}B(n_{th},\alpha)^{7}Li$ reaction and their high linear energy transfer (LET). The energy produced, about 2.4 MeV, is deposited within the order of the diameter of one cell. This results in a high probability of double-strand breakage and other fatal damage. One single incident can, in principle, destroy a cancer cell.

The successful implementation of this promising modality strongly depends on the availability of appropriate boron-containing substances. These must accumulate preferentially within tumor cells and should be non-toxic. Different classes of boron compounds are at present being inspected as promising candidates of such BNCT drugs.[2] These include, among others, boron-containing porphyrines,[3] antibodies,[3,4] amino acids,[5] saccharides,[6–8] and targeting systems for unspecific compounds like the use of LDL[9] and liposomes.[10,11]

We have proposed boron-containing ether lipids for this purpose[12] because these are expected to accumulate selectively in tumors. This expectation is based on known facts. The concentration of natural ether lipids in a variety of natural and artificial tumors is enhanced with respect to the corresponding healthy tissue.[13] The artificial ether lipid Et-18-OMe (**2**) shown in Figure 1 is accumulated with a relatively high tumor/healthy tissue ratio.[14] Its concentration in cell culture was found to be up to 18% of the total phospholipid content of plasma membranes.[15] Encouraged by these results we have synthesized the carborane substituted derivative **1**[12] and analogs.

The concept is further confirmed by observations on the ^{125}I-containing analog **3**. This was accumulated with time and reached a tumor/blood ratio of 30:1 in a human colon carcinoma and 8:1 in a human melanoma xenograft in mice.[16] This compound proved to be useful for γ-scintigraphic tumor visualization.

A preceding study in cell culture showed the concentration of **1** associated with both mouse melanoma (B16) and human glioma (U-343MGa) cells was 20–25 times higher than in culture medium. However, this ratio was diminished to 5–7 after a thorough washing.[17]

The following studies were undertaken to determine whether this result also holds *in vivo*.

Dedicated to Professor Dr Ivar Ugi on the occasion of his 65th birthday.

Financial support was given by the Fonds der chemischen Industrie (PL) and by Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen (TA). The work was also sponsored by the European Collaboration for BNCT.

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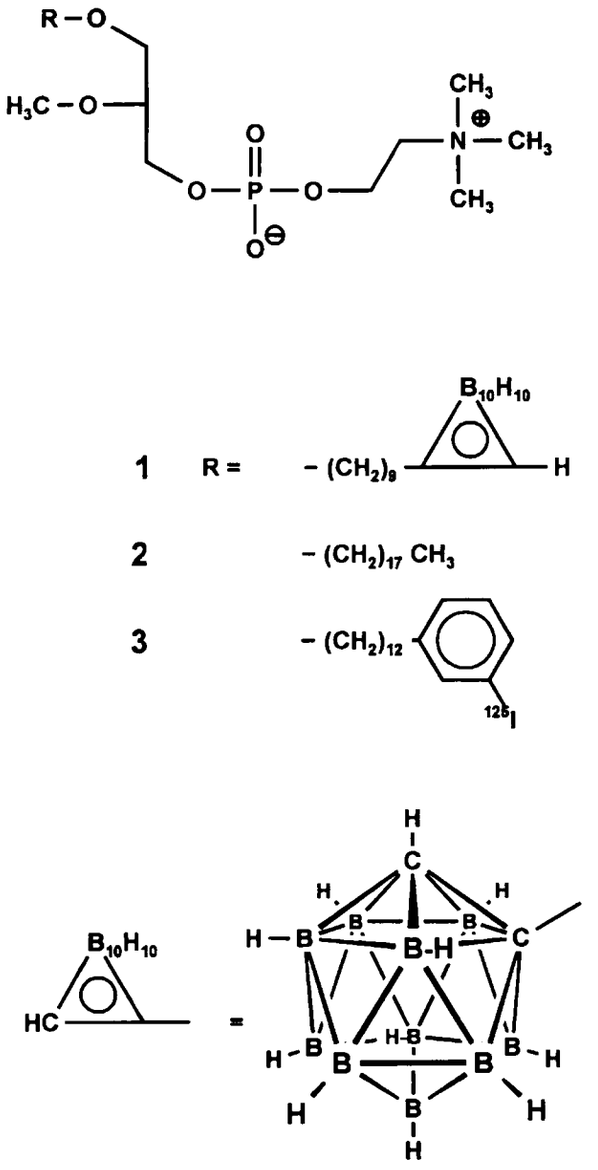

Figure 1. Structural formulas of the studied boron compound *rac*-1-(9-*o*-carboranyl)nonyl-2-methyl-glycero-3-phosphocholine (B-Et-11-OMe) (**1**) and its analogs *rac*-1-octadecyl-2-methyl-glycero-3-phosphocholine (Et-18-OMe) (**2**) and *rac*-1-(ω-3-iodophenyl)-dodecyl-2-methyl-glycero-3-phosphocholine (**3**).

Materials and methods

Tumor model

AT17 mammary carcinoma was isotransplanted into the right abdominal wall of female C3H mice. AT17 is known as a slow growing adenocarcinoma.[18,19] It rarely develops necroses, even up to large tumor volumes. Like human epithelial tumors, it has the tendency to keratinize. AT17 in general is not well vascularized, it is hypoxic. Originally this tumor has been induced by irradiation.

The second tumor model used was an osteosarcoma transplanted into the right hind leg of female BALB/c mice. The animals were sacrificed at different times and the boron contents of tumor, blood, liver and kidney were measured by means of inductively coupled plasma emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS).

The animals were supplied by Charles River (Sulzfeld/Main, Germany) Permission for this experiment was given by Regierung von Oberbayern, no. 221-2531-57/92.

The compound *rac*-1-(9-*o*-carboranyl)nonyl-2-methyl-glycero-3-phosphocholine (B-Et-11-OMe) (**1**) was synthesized as described earlier[12] and dissolved in physiological saline (3 mg/ml). In general, 0.5 ml of this solution was administered i.p. to mice. This resulted in an initial averaged dose of about 50 mg/kg body weight of compound **1** (about 10 mg/kg B).

Boron analysis

A microwave digestion procedure, followed by ICP-AES and by ICP-MS was applied for the determinations of boron in samples of mammary carcinoma, osteosarcoma, blood, liver and kidney.[20]

Microwave digestion decomposes the biological matrix as well as the injected compound **1**. The samples (60–2500 mg) were weighed into teflon vessels of 150 ml volume. After the addition of 25 ml 65% v/v ultrapure nitric acid the samples were digested in a MLS-1200 Mega apparatus for 27 min. The obtained homogenous colorless solutions were diluted with 2% v/v nitric acid to a total volume of 10–25 ml. Triton X-100 (100 μl) was added as a detergent. The samples were analyzed by two independent analytical techniques, ICP-AES and ICP-MS. The boron concentrations were determined by external calibration using two independent lines in both spectroscopic methods. The correlation of the two techniques was checked in the concentration ranges 1–250 ng/ml and 10–400 μg/ml.[17]

A sequentially operating ICP-AES plasma emission spectrometer Plasma 40/400 of Perkin Elmer was used for the determination of boron concentrations at 249.773 and 249.678 nm wavelength. The sample aerosols are produced using a cross-flow nebulizer.

The quadrupole ICP-MS ELAN 5000 of Perkin El-

mer & SCIEX equipped with an autosampler AS-90, a platinum cone, a nickel skimmer cone, a quartz injector tube in the Fassel torch, a Meinhard nebulizer of type C and a Scott-type glass chamber was used for the presented experiments. Boron was determined on the lines of 10 and 11 a.m.u. Rhodium in a concentration of 10 ng/ml was used as an internal standard for the ICP-MS measurements.

Results are given as the mean value of five animals (controls: three) ± SEM.

Toxicological observations

Side effects were observed about 1 min after the end of injection: the mice started running fast and jumping, and emitted short screams. Then they hid in the bedding and fell into a somnolence-like state with heavy breathing, but remained extremely sensitive to noise. Between 4 and 5 min after injection the animals awoke and returned to inconspicuous behavior. For the remaining time, up to 48 h, the animals appeared completely normal. The symptoms were concentration dependent: 1.5 mg B-Et-11-OMe dissolved in 0.3 ml physiological saline induced the same symptoms, but more intense. Dilution to 0.6 or 0.9 ml reduced intensity somewhat. Rapidity of injection had no influence. In the control group, which received the same volume of physiological saline, this symptom was not seen.

Results

The concentration of boron in some types of tissue at various times is summarized in Table 1 for AT17-bearing mice.

Even at the earliest time examined, the concentration of boron in the liver is high. It passes a broad peak at about 1.5 h and decreases; after 12 h the boron is virtually cleared from this organ. The concentration in the kidney remains essentially constant in the beginning; also this organ was cleared of boron after 12 h. Boron concentration in blood was about 10 mg/kg after 0.5 and 1 h. It decreased rapidly during the first hours; later the decrease slowed down. The concentration of boron in the tumor was about 2 mg/kg after 0.5 h. Its concentration in the tumor decreased more slowly compared with blood within the first 12 h. Thus, the tumor/blood ratio rose up to 0.79. This ratio reached a plateau of about 0.5 (area 2, Fig. 2).

The osteosarcoma did not accumulate significant amounts of boron.

Table 1. Time-dependent boron concentration (mg/kg) in tissues of C3H mice bearing a AT17 carcinoma

Time (h)	Liver[a]	Kidney[b]	Blood[c]	Tumor[d]
0.5	41 ± 2	4.6 ± 0.3	9.8 ± 0.7	1.9 ± 0.2
1	53 ± 5	4.8 ± 0.3	9.3 ± 2.2	1.5 ± 0.5
1.5	68 ± 9	4.8 ± 0.9	7.5 ± 1.4	1.0 ± 0.2
2	53 ± 3	4.8 ± 0.3	4.4 ± 0.8	2.1 ± 0.3
4	48 ± 6	6.1 ± 0.5	3.1 ± 0.5	1.3 ± 0.1
12	3.4 ± 1.6	1.5 ± 0.4	1.7 ± 0.6	1.4 ± 0.4
24	0.8 ± 0.4	0.6 ± 0.3	0.5 ± 0.1	0.24 ± 0.08
48	0.6 ± 0.1	0.28 ± 0.05	0.4 ± 0.2	0.18 ± 0.05

Controls: [a] 0.3 ± 0.01, [b] 0.67 ± 0.09, [c] 0.10 ± 0.06, [d] 0.09 ± 0.04 mg/kg.

Discussion

These results differ significantly from those expected in analogy to the literature.[14,16] The main difference appears to be: whereas compounds **2** and **3** were retained, redistributed and taken up by the tumor for several days, in the present experiment compound **1** is cleared rapidly from the organism, obviously via uptake into the liver. The disappearance of compound **1** from tumors is significantly more slowly than from blood as long as the concentration in blood is higher. However, when the concentrations become comparable, the clearance rates become comparable too. Thus, the tumor/blood ratio did not rise. The gross retention of the compound does not seem to be governed by different metabolic rates, as anticipated, but by cellular uptake. This uptake by tumor cells appears to be insufficient.

We interpret this finding as follows. The experiments with compounds **2** and **3** have been performed with trace amounts (3×10^{-8} mol/l in the case of compound **2**[14] and 3×10^{-5} mol/l in the case of compound **3**,[16] calculated from specific activities given). We have used a more concentrated solution, 5.5×10^{-3} mol/l compound **1**, which is in the order of magnitude needed for BNCT. This concentration was estimated to be the maximal tolerable dose of compound **2** in man.[21]

The low concentrations used for compounds **2** and **3** are most probably below the critical micellar concentration (CMC). In both cases, to our best knowledge, the CMC of compounds **2** and **3** has not been reported. It is expected to be in the order of magnitude of 10^{-3} to 10^{-4} mol/l. In contrast, 5.5×10^{-3} mol/l compound **1** appears to be above the CMC. (This assumption is backed by ^{1}H- and

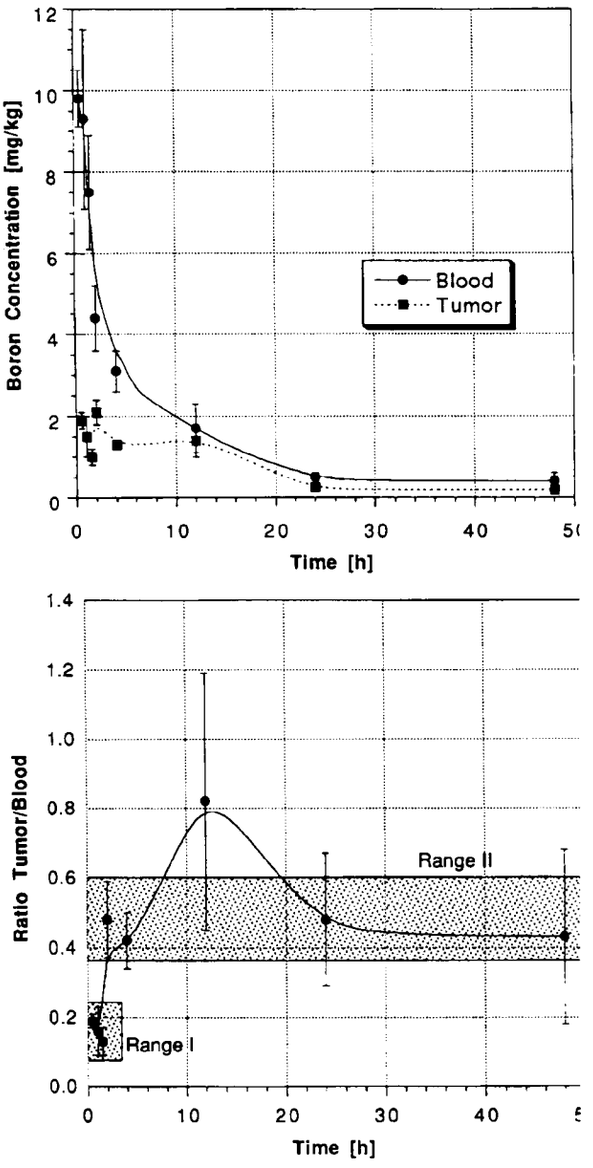

Figure 2. (a) Variation of boron content in blood and tumor of C3H mice bearing a AT17 mamma carcinoma after i.p. administration of 50 mg/kg B-Et-11-OMe. (b) Ratio of these concentrations.

^{31}P-NMR spectroscopy: lineshapes in the spectra of compound **1** strongly suggest the formation of aggregates.) Preliminary attempts to determine CMC by NMR shows it to be below 1.8×10^{-4} mol/l. NMR signals do not change down to this low concentration. Examination by light scattering shows three different aggregated species: a major component of particles measuring about 100 nm (probably vesicles), a second of about 1–10 nm (probably micelles) and an intermediate one of about 40–50 nm (measurement by Professor G Cevc).

Based on this assumption, fundamentally different pathways of cellular internalization must be taken into account. At low concentration (sub CMC), molecular diffusion constitutes an effective major pathway. However, at higher concentrations (above CMC), substances have to be taken up by pinocytosis of micelles. This pathway of uptake is much less effective. This difference appears not to have been taken into account in the case of clinical experiments with compound **2**.

Experiments in cell culture[17] also suggest that at least a major proportion of compound **1** is not internalized but bound extracellularly.

To conclude, a boron-containing ether lipid, structurally modified to exhibit a significantly elevated CMC and administered below this concentration, appears to be more promising under this rationale in BNCT. Further work on this is in progress.

Acknowledgments

We are grateful to Professor G Cevc for the light scattering measurement, Professor F Baumgärtner for the possibility of IPC-AES and ICP-MS analyses, and M Bannasch for expert technical assistance.

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(Received 17 July 1995; received in revised form 28 July 1995; accepted 31 July 1995)