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New carborane-based compounds for boron neutron capture therapy: binding and toxicity of ANC-1, DAC-1 and B-Et-11-OMe in cultured human glioma and mouse melanoma cells

Peter Lindström,[1] Pär Olsson,[1] Jonas Malmqvist,[2] Jean Pettersson,[3] Peter Lemmen,[4] Birgit Werner,[4] Stefan Sjöberg,[2] Åke Olin[3] and Jörgen Carlsson[1]

[1]Division of Physical Biology, Department of Radiation Sciences, Uppsala University, Box 535, S-751 21 Uppsala, Sweden. Fax: (+46) 18 18 3833 [2]Department of Organic Chemistry and [3]Department of Analytical Chemistry, Institute of Chemistry, Uppsala University, Uppsala, Sweden. [4]Institute for Organic Chemistry, The Technical University of Munich, Garching, Germany.

The toxicity and binding of the three new carborane based compounds: 2 (1,2-dicarba-*closo*-dodecaborane (12)-1(-yl-methoxy)-2-(3-amino-propyl))-1,3-propanediol, called DAC-1; 7-(3-amino-propyl)-7,8-dicarba-*nido*-undecarborate (-1) called ANC-1; and rac-1-(9-o-carboranyl)-nonyl-2-methyl-glycero-3-phosphocholine, called B-Et-11-OMe, were analyzed with cultured human glioma cells, U-343MGa, and mouse melanoma cells, B16, as biological models. The previously developed compound di-sodium undecahydro-mercapto-closo-dodecarborate (BSH), which is tested for therapy of malignant gliomas, was analyzed for comparison. In the toxicity tests the cells were exposed to the substances at cell culture medium concentrations in the range 0–50 ppm boron for 1 or 20 h and thereafter analyzed regarding growth. Growth-disturbing effects were seen for the two compounds DAC-1 and B-Et-11-OMe at the concentrations corresponding to 15 and 50 ppm boron, respectively. The compounds ANC-1 and BSH showed no growth-disturbing effects at the tested concentrations. In the binding tests, the cells were incubated for 20 h at about the highest compound concentrations that did not cause growth disturbances. The boron content in the cells was then determined by inductively coupled plasma–atomic emission spectrometry (ICP–AES) and in some cases ICP–mass spectrometry (ICP–MS). The most extensive binding was seen for DAC-1 and B-Et-11-OMe, which accumulated boron to about 100 and 60 times, respectively, compared with the concentration in the culture medium. The compound ANC-1 also accumulated boron in the cells but the boron could be easily washed out indicating no or only a weak binding. BSH did not accumulate. Further analysis should be made regarding biological properties such as intracellular compartmentalization, metabolic interference and tumor specificity of the compounds DAC-1 and B-Et-11-OMe.

Key words: Amino-carboranes, ANC-1, B-Et-11-OMe, boron neutron capture therapy, BSH, carborane, cellular binding, cellular toxicity, cultured cells, DAC-1, glioma, growth curve, ICP–AES, ICP–MS, melanoma.

Introduction

Boron neutron capture therapy (BNCT) is based on the nuclear reaction that occurs when a ^{10}B atom captures a thermal neutron and disintegrates to the highly energetic ^{7}Li and ^{4}He ion fragments with a path length of nearly one-cell diameter (6–9 μm). During their passage through the cell, the ion fragments give high linear energy transfer (high-LET) radiation.[1,2] If a high-LET ion is passing through a cell nucleus, the probability for inactivation of cell division is high.[1] The BNCT concept seems promising, due to recent developments of interesting boron compounds and due to an increasing knowledge about the basic processes involved.[3–8]

The amount of boron needed for successful treatment of tumor cells has been calculated to be about 10^9 ^{10}B atoms when the boron is in the cytoplasm or 10^8 ^{10}B atoms when located in the cell nucleus.[7,9] There are two reasons for the need for high numbers of ^{10}B atoms in the cells; first, to have a reasonable number of captures when a suitable neutron field is applied and second, to overcome the background dose delivered by captures in normally occurring elements (mainly nitrogen and hydrogen).[4,6,10] An amount of 10^8 ^{10}B atoms in the cell nucleus gives a boron-dependent dose of about

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Correspondence to P Lindström

2 Gy high-LET irradiation if a reasonable thermal neutron fluence (about 5×10^{12} n/cm^2) is applied[9] and this is most likely to give a valuable therapeutic effect.

An amount of 10^8 ^{10}B atoms per cell corresponds to an average ^{10}B concentration in the cells of about 1.5–2.0 ppm (μg boron/g tissue) if the cell diameter is about 10 μm. A practical lower limit for the requirement on the average cellular ^{10}B concentration, independent of whether the boron is in the cytoplasm or in the nucleus, can be set to 10 ppm. If the average cellular concentration is above that value, there is a good chance to inactivate cell division by boron-dependent captures.

For successful BNCT it is necessary to find compounds that can deliver the required amount of ^{10}B to the tumor cells. Low molecular weight boron compounds can be used directly if they bind specifically, or they can be conjugated to macromolecules for antigen- or receptor-mediated targeting. Compound development along these lines has been going on for at least two decades.[11,12] Examples of direct use of low molecular weight boron compounds is L-*o*-carboranylalanine, which binds in cultured malignant melanoma cells[13,14] and the two compounds BSH (sulfhydryl boron hydride) and BPA (*p*-boronophenylalanine), which are currently tried in clinical treatments of malignant gliomas[15,16] and melanomas,[17,18] respectively. Coupling of low molecular weight boron compounds to macromolecules, such as antibodies and growth factors, has been tried experimentally but not clinically.[6,7,19,20]

The aim of this study was to make some introductory investigations of the potential use of BNCT of three new boron-containing compounds. The present study involved tests of the substances' own capacity to disturb growth—which is considered to be an unwanted side effect in BNCT. The substances should only exert a toxic action upon activation with thermal neutrons. The growth disturbances were analyzed from growth curves after 1 or 20 h of exposure to the compounds. The study also involved the analysis of boron binding to the tested cells through measurements with inductively coupled plasma–atomic emission spectrometry (ICP–AES) and ICP–mass spectrometry (ICP–MS). The compounds contained natural boron (about 20% ^{10}B and 80% ^{11}B), but if the compounds are to be used for neutron capture processes, such as experimental or clinical therapy, they should be synthesized enriched in ^{10}B.

The analyzed compounds were 2(1,2-dicarba-*closo*-dodecaborane(12)-1(-yl-methoxy)-2-(3-amino-propyl))-1,3-propanediol (DAC-1); 7-(3-amino-propyl)-7,8-dicarba-*nido*-undecarborate (-1) (ANC-1) and rac-1-(9-o-carboranyl)nonyl-2-methyl-glycero-3-phosphocholine (B-Et-11-OMe). Human glioma U-343MGa cells and mouse melanoma B16 cells were applied as biological models. BSH, presently considered for clinical therapy of malignant gliomas, was analyzed for comparison.

Materials and methods

Cell lines

The two cell lines used were mouse melanoma B16[13] and human glioma U-343MGaCl2:6, called U-343MGa.[21] The cells were grown as monolayers in plastic culture flasks (Corning Glass, Corning, NY) at 37°C (humidified air containing 5% CO_2). The cell culture medium was Ham's F-10 (Kebo, Spånga, Sweden), supplemented with 10% fetal calf serum (FCS) (no. 6.9501-500; Flow, Stockholm, Sweden), L-glutamine (2 mM; no. 6.9509-100, Kebo) and PEST (penicillin 20 U/ml and streptomycin 20 μg/ml, both from Kebo), and this medium is referred to as normal culture medium. The medium was routinely changed three times a week.

Boron substances

The two compounds DAC-1 and ANC-1 were synthetized at our laboratories according to methods to be published separately. Some principles for synthesis of aminoalkyl–carboranes and carboranyl amino acids have recently been published.[22,23] The compound B-Et-11-OMe was synthetized by Lemmen and Werner.[24] The compound BSH was obtained from Callery Chemical Company (Pittsburgh, PA). Schematic structural formulas for all four substances are shown in Figure 1. All substances contained natural boron (about 20% ^{10}B and 80% ^{11}B). If applied for therapy, they should be enriched in ^{10}B. The molarities corresponding to 50 ppm natural boron were 4.6×10^{-4} for DAC-1, 5.1×10^{-4} for ANC-1, 4.6×10^{-4} for B-Et-11-OMe and 3.9×10^{-4} for BSH).

Toxicity tests

The boron substances, DAC-1, ANC-1, B-Et-11-OMe and BSH, were dissolved in warm (37°C) normal culture medium. The applied concentrations were

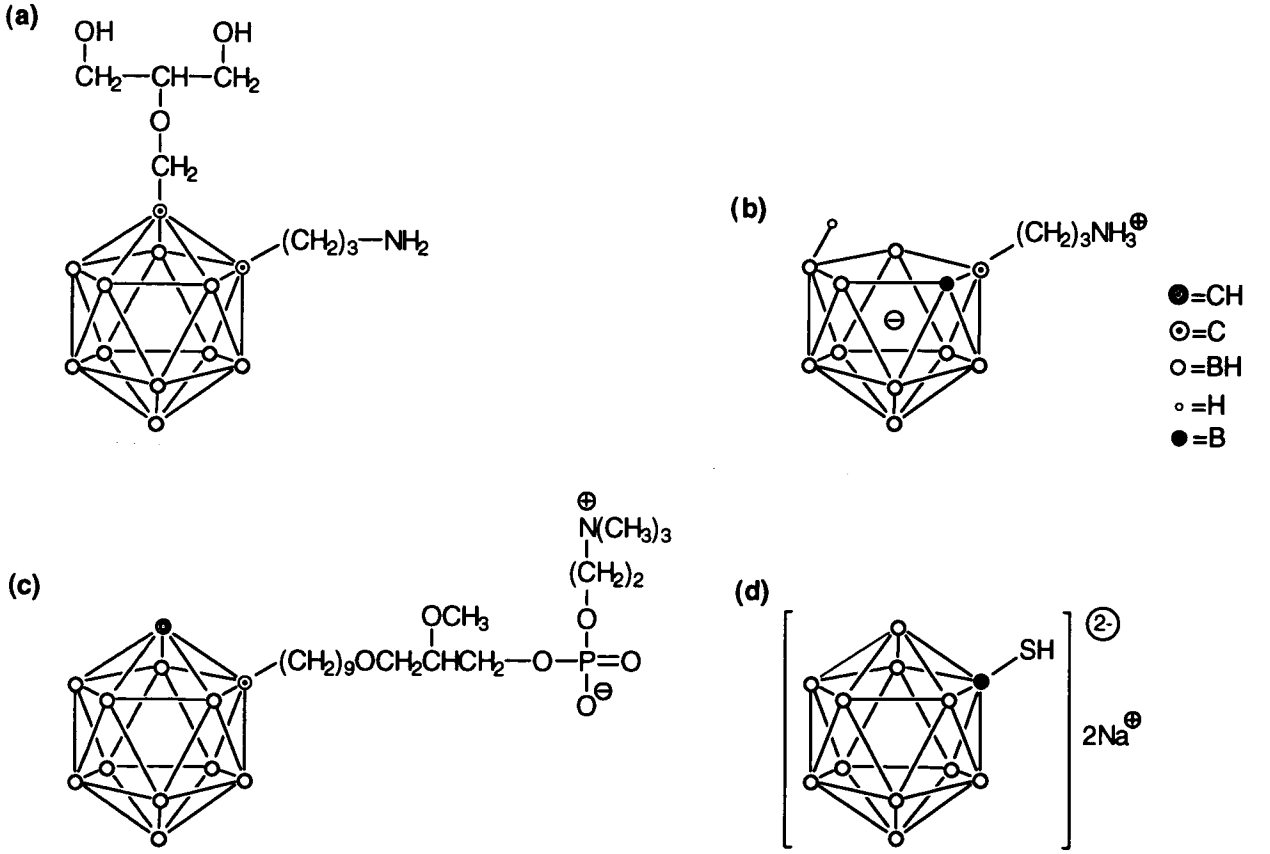

Figure 1. Schematic structural formulas of the four studied boron compounds. (a) 2(1,2-dicarba-*closo*-dodecaborane(12)-1(-yl-methoxy)-2-(3-amino-propyl))-1,3-propanediol (DAC-1); (b) 7-(3-amino-propyl)-7,8-dicarba-*nido*-undecarborate(-1) (ANC-1); (c) rac-1-(9-o-carboranyl)nonyl-2-methyl-glycero-3-phosphocholine (B-Et-11-OMe); and (d) di-sodium undecahydro-mercapto-closo-dodecarborate (BSH) or sulfhydryl boron hydride.

0.5, 1.5, 5, 15 and 50 ppm boron. The melanoma and glioma cells were plated in 35-mm culture dishes and incubated with respective compound and concentration for 1 or 20 h at 37°C. The boron-containing culture medium was then removed and the cells were washed five times with normal culture medium free of serum and then further incubated in normal culture medium. The number of cells at different periods of time during continued growth was counted in an electronic cell counter (Coulter counter model ZM; Coulter Electronics, UK) and growth curves were constructed. The growth curves were constructed as if all cells at all subcultivations should have been saved. The subcultivations were made at such intervals so that the cultures not reached confluency.

Binding of boron compounds

The boron compounds were dissolved as described above and the cells were plated in 10-cm culture dishes. The cells were incubated with the boron compounds for 20 h at concentrations not disturbing the growth. After the incubations the treated cells were divided in two groups.

The cultures in the first group were washed five times for 1 min with PBS and then harvested with a rubber policeman. The harvested cells were suspended vigourously by pipetting, to obtain single-cell suspensions. A subfraction of the cells was counted with the electronic cell counter. The remaining cells were sedimented by centrifugation at 1000 **g** for 10 min. The supernatant fluid was removed and the cell pellet was analyzed regarding boron content. These cells were termed 'washed' and the boron measured in these cells was considered to be bound to, or at least trapped in, the cells.

The cells in the second group were not washed. Instead, they were directly harvested with the rubber policeman while the boron-containing medium was still in the culture dish. The harvested cells were suspended vigourously by pipetting, to obtain single-cell suspensions. A subfraction of the cells

was counted with the electronic cell counter and the remaining cells were sedimented by centrifugation at 1000 **g** for 10 min, while they still were in the boron-containing incubation medium. The supernatant fluid was removed and the cell pellet was analyzed regarding boron content. These cells were termed 'ambient' and the boron measured in these cells was considered to be the amount of boron taken up when the boron-containing culture medium was in contact with the cells.

Corrections were made for all dilutions during preparations for boron analysis, so that the cellular concentration of boron could be given for washed and ambient cells in units of ppm (μg/g). The difference between the values for the ambient and washed cells was considered to be the boron that leaked out of the cells during the washings. Control groups of cells were, in parallel, incubated in medium without boron and then treated as described above.

Determination of boron

The reagents used were of pro analyis quality and the water was filtered through a Millipore apparatus (MQ-water). The cell pellets (about 0.1 g corresponding to 2.0–2.5 × 10^7 cells) or culture media (0.5 ml) were transferred with two 0.25 ml portions of MQ-water into a thick-walled quartz tube. The weight of the sample was determined by difference to the nearest 0.0001 g. The sample was evaporated to dryness (1 h, 125°C) and then cooled to room temperature before digestion.

All samples were digested in a steel bomb constructed by Urberg.[25] To the dried sample were added 1.5 ml of concentrated nitric acid and the bomb was sealed and placed in a heated aluminum block controlled by a temperature regulator. The samples were heated using the following temperature programme: 125°C for 30 min and 175°C for 30 min. If a larger amount of sample is to be analyzed, more reagent and a prolonged time at the higher temperature must be used. After cooling, the digest was transferred to a polypropylene tube with MQ-water to a final volume of 9.7 ml.

The boron content was measured using ICP–AES. The instrument used was a Spectroflame P from Spectro, Germany, and it was operated with a power of 1150 W. The argon gas flows were 0.7 l/min for the nebulizer, 2 l/min for the plasma and 14 l/min for cooling. The sample aspiration rate was 2 ml/min through the cross flow nebulizer. The emission of boron was measured at the 249.773 nm line and the background at 249.742 nm. Calibration was performed using standards diluted from boric acid, 1000 ppm boron (BDH, Poole, UK), in the same matrix as the sample. Blanks were run through the whole procedure and subtracted from the signal obtained from the sample.

Method control was performed by the determination of boron in bromo-methyl-*o*-carborane with a theoretical assay of 46.4% boron. For digestions in closed system the recoveries were 98–101%, but for open digestions the recoveries were often low.

The results from the ICP–AES measurements were checked by re-analyzing a set of 23 samples by ICP–MS (VG Instruments, Winsford, UK), operated at standard conditions. The samples were diluted tenfold prior to the determination, to give a matrix compatible with the instrumentation. The results from the two methods (ICP–AEC versus ICP–MS) were plotted against each other and a straight line was fitted to the data points (Figure 2). The resulting slope and intercept, with 95% confidence intervals, were 0.94 ± 0.07 and 2.9 ± 9.3, respectively, with a correlation coefficient (r) of 0.9895. That is, no evidence was found for systematic discrepancies between the two methods.

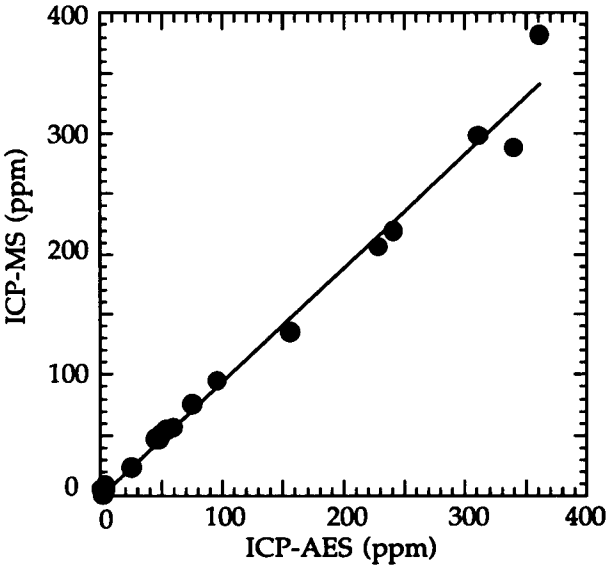

Figure 2. Boron determination in biological samples analyzed in parallel with inductively coupled plasma–atomic emission spectrometry (ICP–AES) and ICP–mass spectrometry (ICP–MS).

Results

Toxicity

DAC-1. Figure 3 shows the growth curves for the melanoma B16 and glioma 343MGa cells treated with DAC-1. The DAC-1 concentrations corresponding to boron concentrations of 5 ppm or lower did not disturb the growth in any of the studied cases. However, decreases in cell number, directly after the 1 or 20 h incubations, were seen in all cases when DAC-1 corresponding to 15 or 50 ppm boron was applied. The most severe case was 50 ppm boron on the glioma cells for 20 h, where all cells disappeared.

There were remaining cells with growth capacity in the washed dishes when DAC-1 concentrations corresponding to 15 and 50 ppm boron for glioma and 15 ppm for the melanoma cells were applied for 1 h. These cells could, after a delay of 2–3 weeks, regrow and then reach the same growth rate as the controls. Thus, fractions of cells survived these treatments.

In the case of 15 ppm boron delivered for 20 h with DAC-1 to the glioma cells and 50 ppm for 1 h or 15 and 50 ppm for 20 h to the melanoma cells, there were also remaining cells after the washes, but these cells did not recover and disappeared after some time.

The cells disappeared either because they detached from the culture dishes during the incubations or during the five washes after the incubations. This was seen as low cell counts at time zero in Figure 3 and was indicated with a vertical arrow. The cells could also disappear during the following medium changes which routinely were made three times a week during the growth analysis. This was indicated with downwards drawn lines. Detached cells were reincubated in new dishes but had no capacity to form growing cultures; these cells were therefore considered to be growth inactivated.

B-Et-11-OMe. Decreased cell numbers at the time zero were obtained in all cases (except after a 1 h exposure of the glioma cells) when B-Et-11-OMe was applied at the highest concentration corresponding to 50 ppm boron. The two types of tested cells reacted differently to such a treatment. The remaining melanoma cells recovered and grew with the same growth rate as the controls but with a delay of about 1 week. The delay was mainly due to the decreased number of cells at time zero. Examples of melanoma growth curves after 20 h of B-Et-11-OMe exposure are shown in Figure 4a. The glioma cells exposed for 20 h to 50 ppm boron through the B-Et-11-OMe administration disappeared from the cultures and these detached cells were reincubated in new dishes but had no capacity to form growing cultures and were considered growth inactivated. None of the B-Et-11-OMe concentrations corresponding to 15 ppm boron or lower induced any noticable growth disturbances.

ANC-1. There was no growth retardation after exposure to ANC-1 even after the highest concentration, corresponding to 50 ppm boron. Both the glioma and melanoma cells had, in all studied cases, the same growth rate as the control cultures. Examples of growth curves for glioma cells growing after exposure for 20 h to ANC-1 are shown in Figure 4b.

BSH. As in the case for ANC-1, there was no growth retardation after exposure to BSH. All exposed cell cultures had the same growth rate as the control cultures. Examples of growth curves for glioma cells growing after exposure for 20 h to BSH are shown in Figure 4c.

Thus, neither ANC-1 nor BSH seemed to be severely toxic, since the growth curves were, for all applied concentrations and incubation times, similar as for the control cells. In contrast, the higher concentrations of DAC-1 and B-Et-11-OMe disturbed growth. Table 1 shows a schematic summary of the results. Concentrations near the highest concentrations found not to disturb growth for of each compound and cell type were used in the binding tests below.

Binding

Table 2 shows the obtained boron values associated to the tested cells after the different compounds were applied for 20 h. The culture medium concentrations of boron were near the highest concentration found not to disturb growth for each compound and cell type (see above).

The amount of boron in control cell cultures was 0.9–4.3 ppm. Control cultures were always included in parallel to the analyzed samples exposed to the boron compounds. The culture medium itself contained about 0.1 ppm of boron.

The values for washed and ambient cells are shown in Table 2. Corrections for the boron content in non-treated cells can be made by subtracting the corresponding control culture values. The values for

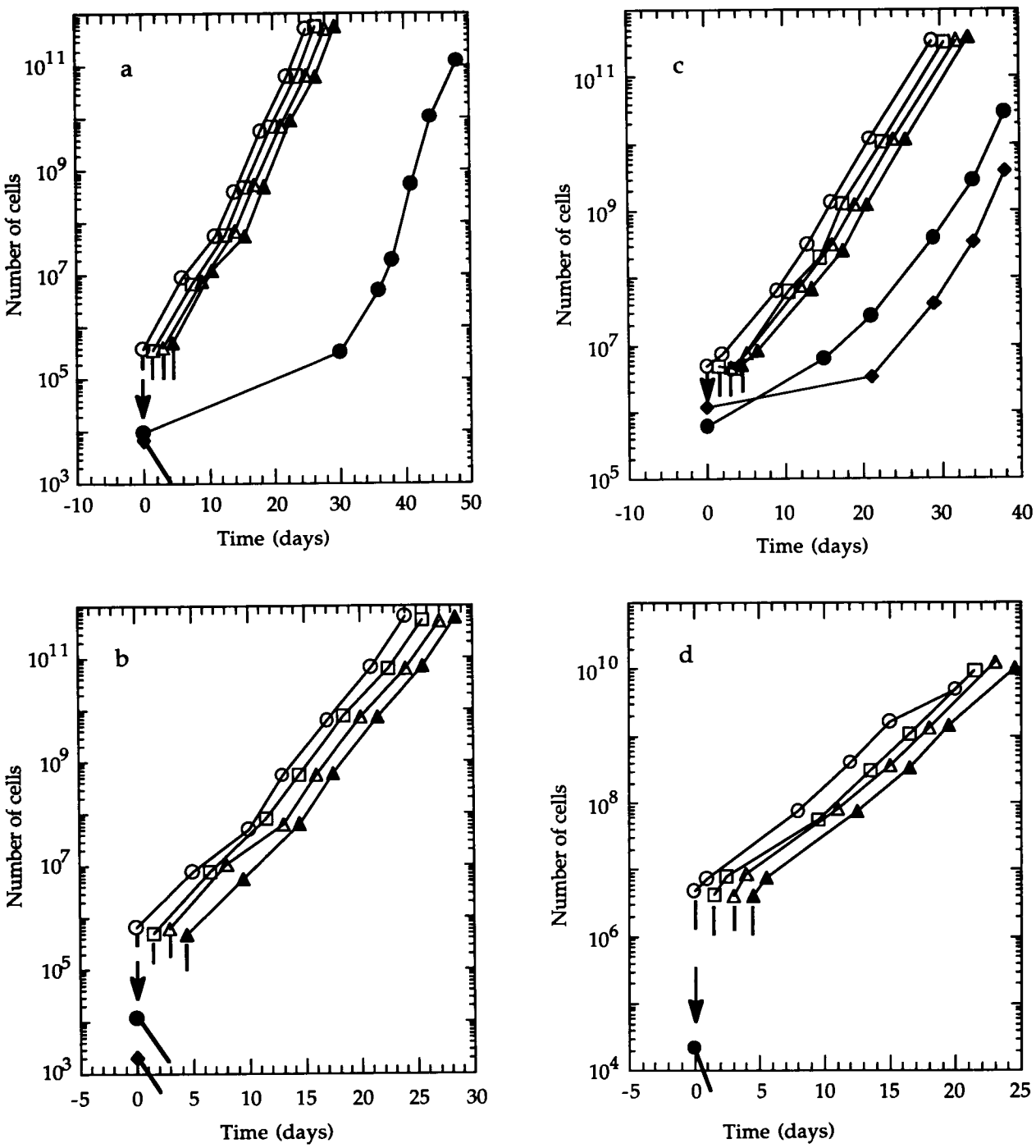

Figure 3. Growth curves for melanoma B16 (a and b) and glioma U-343MGa (c and d) cells after incubation with DAC-1 for 1 (a and c) or 20 h (b and d) at different concentrations. A decreased number of cells directly after the incubations (at time zero) is indicated with a vertical arrow. Total disappearance of cells is indicated as lines with a negative slope starting at time 0 as shown in a, b and d. The non-disturbed growth curves were displaced stepwise by 1.5 days for clarity (time zero is indicated with a short vertical bar under the first symbol in each growth curve). ○, Control cultures. The applied boron concentrations were: □, 0.5 ppm; △, 1.5 ppm; ▲, 5 ppm; ●, 15 ppm; and ◆, 50 ppm. Each point is the mean value from measurements of two to three parallel dishes. The maximal variations were in all cases less than 10%

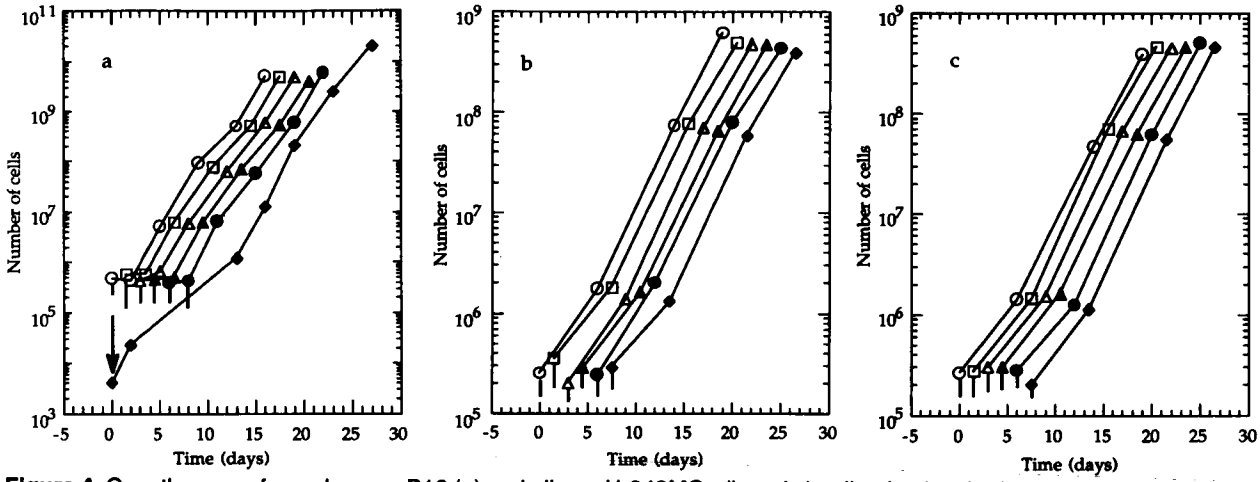

Figure 4. Growth curves for melanoma B16 (a) and glioma U-343MGa (b and c) cells after incubation with B-Et-11-OMe (a), ANC-1 (b) and BSH (c) for 20 h at different concentrations. A decreased number of cells directly after the incubations (at time zero) is indicated with a vertical arrow. The non-disturbed growth curves were displaced stepwise by 1.5 days for clarity (time zero is indicated with a short vertical bar under the first symbol in each growth curve). ○, Control cultures. The applied boron concentrations were: □, 0.5 ppm; △, 1.5 ppm; ▲, 5 ppm; ●, 15 ppm; and ◆, 50 ppm. Each point is the mean value from measurements of two to three parallel dishes. The maximal variations were in all cases less then 10%.

the washed cells showed the amount of boron that remained associated to the cells in spite of five washings and one centrifugation, and therefore could be considered as boron bound to, or trapped in, the cells. The ambient values showed the amount of boron associated to the cells when the cells were allowed to stay in contact with the boron-containing medium.

DAC-1. DAC-1 was applied to both cell types at the low concentration corresponding to 2.5 ppm boron. This was as low as the background boron concentration in the control cells. In spite of the low DAC-1 concentration, there were high boron values in the cells after the 20 h incubation. The analyzed ambient cells had boron concentrations 60–100 times higher (240–340 ppm) than the concentration in the culture medium. Large amounts of boron were retained by the cells, especially in the melanoma cells, in spite of the washings, indicating that the boron delivered by DAC-1 either bound to or was trapped in the cells.

Table 1. Schematic summary of the growth-disturbing effects exerted by the tested boron compounds

Compound (time)	343MGa, boron concentration (ppm)			B16, boron concentration (ppm)		
	≤5	15	50	≤5	15	50
DAC-1(1 h)	—	g d	g d	—	g d	c d
DAC-1(20 h)	—	c d	c d	—	c d	c d
B-Et-11-OMe(1 h)	—	—	—	—	—	g d
B-Et-11-OMe(20 h)	—	—	c d	—	—	g d
ANC-1(1 h)	—	—	—	—	—	—
ANC-1(20 h)	—	—	—	—	—	—
BSH(1 h)	—	—	—	—	—	—
BSH(20 h)	—	—	—	—	—	—

gd, growth-disturbing effect, cd, cells detached from the culture dish and died, or died without detachement; —, no measurable growth-disturbing effect.

B-Et-11-OMe. B-Et-11-OMe was applied at 8–12 ppm boron and gave a strong accumulation in the ambient cells. The concentration was 20–25 times higher in the cells than in the culture medium. However, a large fraction of the boron disappeared from washed cells and only about 20–35% of the boron remained associated to the cells after washings. Thus, the B-Et-11-OMe-delivered boron was only weakly associated to the cells. It is possible that a fraction of B-Et-11-OMe was bound or trapped, while another fraction was released. It is not possible to draw more detailed conclusions from the data obtained.

ANC-1. ANC-1 was applied at a high concentration corresponding to 50 ppm boron, which was shown not to disturb growth. This gave concentration in the ambient cells six to eight times higher than in the medium. Nearly all of this boron was released from the cells after the washings. Only 3–8% of the boron remained after the washings, and the cellular concentrations were then lower than in the originally applied incubation medium. Thus, the cells could not effectively bind or trap boron in the form of ANC-1.

Table 2. Boron content in the tested B16 and U-343MGa cells after 20 h of incubation with the different compounds

Compound/cell type	Culture medium	Control cells	Washed cells	Ambient cells
DAC-1/B16	2.5 ± 0.3	4.3 ± 0.4	229 ± 23	241 ± 24
DAC-1/U343MGa	2.5 ± 0.3	2.8 ± 0.3	156 ± 16	340 ± 34
B-Et-11-OMe/B16	12.3 ± 1.2	1.3 ± 0.1	86.0 ± 8.6	247 ± 25
B-Et-11-OMe/U343MGa	8.4 ± 0.8	0.2 ± 0.1	45.1 ± 4.5	196 ± 20
ANC-1/B16	48.4 ± 4.8	2.2 ± 0.2	9.6 ± 1.0	362 ± 36
ANC-1/U343MGa	48.4 ± 4.8	2.2 ± 0.2	26.4 ± 2.6	311 ± 31
BSH/B16	48.9 ± 4.9	0.9 ± 0.1	6.0 ± 0.6	76.2 ± 7.6
BSH/U343MGa	48.9 ± 4.9	0.2 ± 0.1	7.4 ± 0.7	87.3 ± 8.7

All values are given in ppm boron (μg boron/g tissue.)

BSH. BSH was also applied at the high concentration corresponding to 50 ppm boron. This gave a concentration only 1.5–1.8 times higher in the ambient cells than in the culture medium which was, in comparison to the other tested compounds, very low. Most of the boron was released from the cells after the washings and the remaining concentrations were down in the region below 10 ppm. Thus, the cells could not, to an appreciable extent, take up and retain boron administrated in the form of BSH.

Discussion

The amount of induced growth disturbances and the cellular uptake of boron varied for the different types of boron compounds. DAC-1 accumulated boron most efficiently and also gave the best retention. This substance was the most toxic in terms of induced growth disturbances. B-Et-11-OMe was the next best compound in terms of boron uptake and also the next most toxic compound. ANC-1 and BSH did not bind and were not growth inhibitory even at the highest concentrations applied. Thus, there seemed to be a correlation in that the most toxic substances were those which had also been taken up most efficiently by the cells. It is not known to what extent binding and toxicity are correlated for other boron compounds. However, if a substance is not taken up, or does not bind or does not in any other way interact with the cells, then that substance is probably not toxic.

The mechanism for the exerted toxicity by the highest concentrations of DAC-1 is not known. DAC-1 is an amine with one diol groups added to the carborane cage and there are many theoretical possibilities of how the toxicity could be exerted: via damage to the cell membranes (e.g. through interactions between the hydrophobic carborane part and the membranes); via cytoplasmic phenomena such as inhibition of protein synthesis; or even through direct effects in the nucleus, such as DNA damages.

The toxicity of B-Et-11-OMe, as observed in this study, is of the same order of magnitude as that of structurally-related ether lipids. Their cytotoxicity is not completely understood. The toxicity can be attributed to several biological activities, including inhibition of membrane-associated enzymes[26] and alteration of membrane fluidity.[27] Taking into consideration the knowledge about relation of chemical structure and biological action,[28] it should be possible to synthesize modified compounds of this type with minimized toxicity.

The results indicate that further analysis should be made regarding the biological properties, e.g. intracellular compartmentalization, metabolic interference and tumor specificity, of the compounds DAC-1 and B- Et-11-OMe. For example, it would be interesting to perform subcellular fractionation after administration of DAC-1 and B-Et-11-OMe, in parallel to recent investigations for BSH and some other boron compounds by Nguyen *et al.*[29] They found that BSH was mainly situated in the cytoplasm. Such an investigation could reveal the compartmentalization of DAC-1 and B-Et-11-OMe. Another possibility would be to use secondary ion mass spectrometry.[30]

It is interesting to consider the absolute values of the cellular concentrations of boron. At least 10 ppm ^{10}B have to be obtained intracellularily if a significant effect of BNCT is to be obtained. There are two reasons for the need of such a concentration in the cells; there must be a reasonable number of captures when the neutron field is applied and the background dose delivered by captures in normally occurring elements cannot be allowed to dominate. We obtained concentrations much higher than 10 ppm for ambient cells after administration of all compounds. However, in the cases with ANC-

1 and BSH, the added medium had in itself much higher concentration (about 50 ppm). Only DAC-1 and B-Et-11-OMe showed a strong accumulation in the cells and also gave really high values after the washings. We showed that both DAC-1 and B-Et-11-OMe could be administrated at really low concentrations and that they then accumulated boron strongly. Especially DAC-1 seemed to have advantageous properties in this respect, since it could be given at a low, not growth-disturbing concentration and then accumulate boron about 100 times above the level in the incubation medium.

The fact that BSH did not accumulate boron effectively in the cells was intriguing. BSH is presently tried clinically for the treatment of malignant gliomas.[16] However, it has recently been shown that BSH does not effectively deliver boron to free-prepared glioma cells. Instead, boron was found to accumulate in the central degenerative regions of cellular glioma spheroids.[31] A possible explanation to the so-far successful use of BSH for treatments of malignant gliomas might be that the substance accumulates in degenerative tumor microregions and that there might then be a slow release of the boron from these regions into surrounding viable cell layers during the neutron irradiation.[31] Further research is necessary regarding this.

It is not known whether DAC-1 and B-Et-11-OMe have any specific binding for certain types of tumor cells or whether normal cells bind these substances equally well. If all cells bind these substances, then a tumor-specific uptake can only be obtained by using other differences between tumor and normal tissues, such as differences in compound transport[32] or basic differences regarding metabolism.

Ether lipids, analogues of B-Et-11-OMe, are known to specifically accumulate in certain tumors,[33] due to their lack of a metabolizing enzyme.[34] They are considered as antineoplastic[35] or tumor-imaging agents.[36] Further experiments are needed, to see whether their reported tumor specificity holds in the case of B-Et-11-OMe.

Another possibility not evaluated is to get a specific uptake of the low molecular weight boron substances by using antibodies, antibody fragments or receptor ligands as carrier of the compounds. However, such an approach is beyond the subject of this study.

The boron concentrations given in this report were in units of ppm (μg/g). All compounds tested in this study were synthesized using natural boron containing about 20% ^{10}B and 80% ^{11}B. The boron determinations made with ICP–AES do not distinguish between the two isotopes of boron and all results given in Table 2 are for the total boron content. If the boron compounds are to be used for experiments in which captures of thermal neutrons are necessary, such as experimental or clinical therapy or distribution studies applying boron-neutron radiography, then enriched ^{10}B is preferable. The chemical synthesis is not changed by using enriched boron. The only reason for using natural boron in the present study was economical.

There were only small cell-type-dependent differences in how the boron compounds exerted their toxicity and to what extent they bound to the two types of tested cells. The glioma cells showed a tendency to withstand the 1 h treatment with 15 and 50 ppm DAC-1 better than the melanoma cells (compare the results in Figure 3a and c). This difference correlates with a lower content of boron in the glioma cells after washing (Table 2). This correlation does not have any general validity, because the number of studied cases are too few and the observed relation might be coincidental. However, it is worth noting that whereas nearly all the accumulated boron in the melanoma cells was retained after washing, this was not the case for the glioma cells. An opposite difference in sensitivity was obtained with B-Et-11-OMe. The remaining melanoma cells recovered after the exposure to 50 ppm B-Et-11-OMe, while the remaining glioma cells did not. In this case, there was no correlation with better retention of boron after washing.

More experiments are necessary to reveal the mechanisms by which DAC-1 and B-Et-11-OMe bind to cells and by which mechanism, at high concentrations, they exert their growth-inhibitory action.

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