New Boron Delivery Agents

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ABSTRACT:

This review compiles current research on the development of boron delivery drugs for boron neutron capture therapy (BNCT) that was presented and discussed at the National Cancer Institute (NCI) Workshop on Neutron Capture Therapy that took place on April 20-22, 2022. The most used boron sources are icosahedral boron clusters attached to peptides, proteins (such as albumin), porphyrin derivatives, dendrimers, polymers, and nanoparticles, or encapsulated into liposomes. These boron clusters and/or carriers can be labelled with contrast agents allowing for the used of imaging techniques, such as PET, SPECT and fluorescence, that enable quantification of tumor-localized boron and their use as theranostic agents.

1. Introduction

BNCT is a binary therapy treatment form of radiotherapy based on the ability of ^{10}B nuclei for capture low energy neutrons and subsequent fission of the resulting excited nuclei to produce high-linear energy transfer α-particles and recoiling lithium-7 nuclei as shown in equation (1):

$$^{10}B + {}^{1}n \rightarrow {}^{7}Li^{3+} + {}^{4}He^{2+} + \gamma + 2.4\ MeV \qquad (1)$$

The biologically abundant nuclei ^{12}C (0.0034 barn), ^{1}H (0.33 barn), and ^{14}N (1.8 barn) show negligible interference with the ^{10}B (n,α)^{7}Li neutron capture reaction due to their much smaller nuclear cross sections in comparison with ^{10}B (3,838 barns). Since the high-linear energy transfer particles have < 10 μm path length in tissue, the BNCT effect is localized to the ^{10}B-containing cells. Two boron delivery agents have been used in BNCT clinical trials for malignant brain tumors, melanomas, and squamous cell carcinomas: sodium mercaptoundecahydro-*closo*-dodecaborate ($Na_2{}^{10}B_{12}H_{11}SH$), designated BSH, and (*L*)-4-dihydroxy-borylphenylalanine, known as L-BPA, often delivered as a water-soluble fructose or sorbitol complex. Since May 2020, the company Stella Pharma is allowed to market Steboronine® (generic name: Borofalan), which is ^{10}B-enriched (99%) L-BPA as its D-sorbitol complex. Also recently, the technology for generation of neutrons employing accelerators has been developed in various countries, making the development of novel, selective boron carriers an important and much needed task. Over the last decades, new boron delivery agents emerged for application in BNCT of various cancers. These agents possess

several advantages over BSH and L-BPA, including the delivery of higher amounts of boron selectively to tumor cells.

1.1 Requirements of a Boron Agent for BNCT

The success of BNCT is highly depending on the selective accumulation ability of ^{10}B in the tumor cells and its intracellular biodistribution. Ideally, the boron agents used in BNCT should be able to maintain the ^{10}B concentration in tumor to a level of approximately 30 µg ^{10}B/g tumor where an antitumor effect can be expected during neutron irradiation, and be safe with low systemic toxicity. In addition, the tumor tissue concentration/normal tissue concentration (T/N) and the tumor tissue concentration/blood concentration (T/B) ratios must be high (> 5:1), while at the same time being rapidly expelled from normal tissue and blood after neutron irradiation. Furthermore, boron agents must comply with the International Council for Harmonization of Pharmaceutical Regulations (ICH) guidelines for "neoplastic agents".

In March 2020, an accelerator-based BNCT for head and neck cancers using L-BPA was approved by the Pharmaceuticals and Medical Devices Agency in Japan, making BNCT a much more accessible treatment. Although L-BPA is known to actively accumulate into cancer cells via the L-type amino acid transporter 1 (LAT-1), which is overexpressed in many cancer cells, there are still many patients to whom L-BPA is not applicable, which makes the need for new boron agents even more urgent. In addition, because of the rapid clearance of L-BPA, a high-dose infusion (500 mg/kg body weight) is often performed to maintain ^{10}B concentration in blood.

The development of new boron carriers for BNCT has focused on small molecules of high boron content and boron compounds conjugated with biomolecules. Unlike boron-based pharmaceuticals, boron carriers for BNCT should be highly tumor selective and essentially nontoxic. Thus, compounds with a very large boron content[1,2,3,4,5] and tumor selective have been developed, employing specific shuttle systems that accumulate the BNCT agent within tumor cells by internalization processes.[1,2,6,7,8] These include boron compounds conjugated with biomolecules, such as peptides, growth factors, antibodies (mAbs), carrier proteins, and/or to porphyrin derivatives, which enable the use of photodynamic therapy (PDT) as adjuvant to BNCT, and tumor detection via optical microscopy.

2. Boron clusters as scaffolds for BNCT

Aromatic compounds that play important roles in biochemistry find numerous applications ranging from drug delivery to nanotechnology or biological markers. The group of C. Viñas reached an important achievement in demonstrating experimentally and theoretically that neutral and anionic carboranes, as well as anionic metallabis(dicarbollides), display 3D global aromaticity.[9] Based on the relationship between stability-aromaticity, they opened new applications of boron clusters as key components in the field of new materials for healthcare.[10] One of the most promising boron delivery systems are icosahedral boron clusters, such as carborane, metallacarborane and their derivatives, due to their high boron content and low toxicity in biological systems.

The group of C. Viñas has developed several strategies to prepare high boron containing nanomaterials for multimodal therapies, including in BNCT, by using icosahedral boron clusters that consist of their attachment onto nanocarriers, such as dendrimers,[11] polymers,[12] nanoparticles (gold,[13] magnetic[14] or quantum dots[15]), leading to payloads with a high boron density. Parallel to their use as BNCT plus chemotherapy and/or phototherapy and/or hiperthermaltherapy agents simultaneously, boron clusters are excellent scaffolds for diagnostic and therapeutic labelling, opening the door to a wide range of biomedical applications.

Regarding carboranes in biomedicine, research has been focused on the development of new multifunctional hybrid (carboranyl + anilinoquinazolines)[16] and nanoparticles as nanocarriers and/or, as anticancer drugs that, exhibiting desirable *in vitro* antitumor activities against F98, HT29, A172 and hCMEC/D3 cancer cells lines, offer the possibility of dual-action (chemotherapy+BNCT[16] and thermotherapy+BNCT[14b]), may result in significant clinical benefits for cancer treatment, particularly for glioblastomas.

About the icosahedral metallacarboranes, the anionic metallabis(dicarbollides), [3,3-M(1,2-$C_2B_9H_{11}$)$_2$]$^-$, (M= Co, Fe) are the most studied.[17] The 3D aromatic Na[3,3-Co(1,2-$C_2B_9H_{11}$)$_2$] forms hydrogen and dihydrogen bonds that participate in its self-assembling, water solubility and aggregates' formation.[18] Metallabis(dicarbollides) have attracted much attention in biology because are inert to biochemical reactions. The Na[3,3-Co(1,2-$C_2B_9H_{11}$)$_2$] possesses the ability to

readily cross cellular membranes[19] not being cytotoxic, but cytostatic, and cells recover following its removal.[19c] Having performed experiments in a round-bottom flask on a chemical scale, the C. Viñas group showed that [3,3-Co(1,2-$C_2B_9H_{11}$)$_2$]$^-$ and some of its halogenated derivatives interact with biomolecules (amino acids,[20] proteins,[21] ds-DNA[22] and glucose[23]). They observed these interactions *in vitro* experiments by changing the round-bottom flask to a cell and the solutions to the cell physiological components. The chemical scale studies were done individually, whereas the cell study incorporates the effect of all the interacting biomolecules. The group of C. Viñas went a step ahead by using SR-FTIRM to understand and detect that this anion modifies biomolecules (proteins, DNA and lipids) and concentrates in the cells' nuclei after their cellular uptake.[24] Recently, Na[3,3-^{57}Fe(1,2-$C_2B_9H_{11}$)$_2$] demonstrated multitherapies' activity.[25]

Furthermore, carboranes and Na[3,3-Co(1,2-$C_2B_9H_{11}$)$_2$], which can be labelled with contrast agents such as ^{124}I and ^{125}I for *in vivo* markers by PET and SPECT nuclear imaging techniques make these clusters to be very good scaffolds as theranostic agents,[26] accumulating selectively in the tumor tissue for diagnosis and multimodal therapies, speeding up action and diminishing secondary effects.

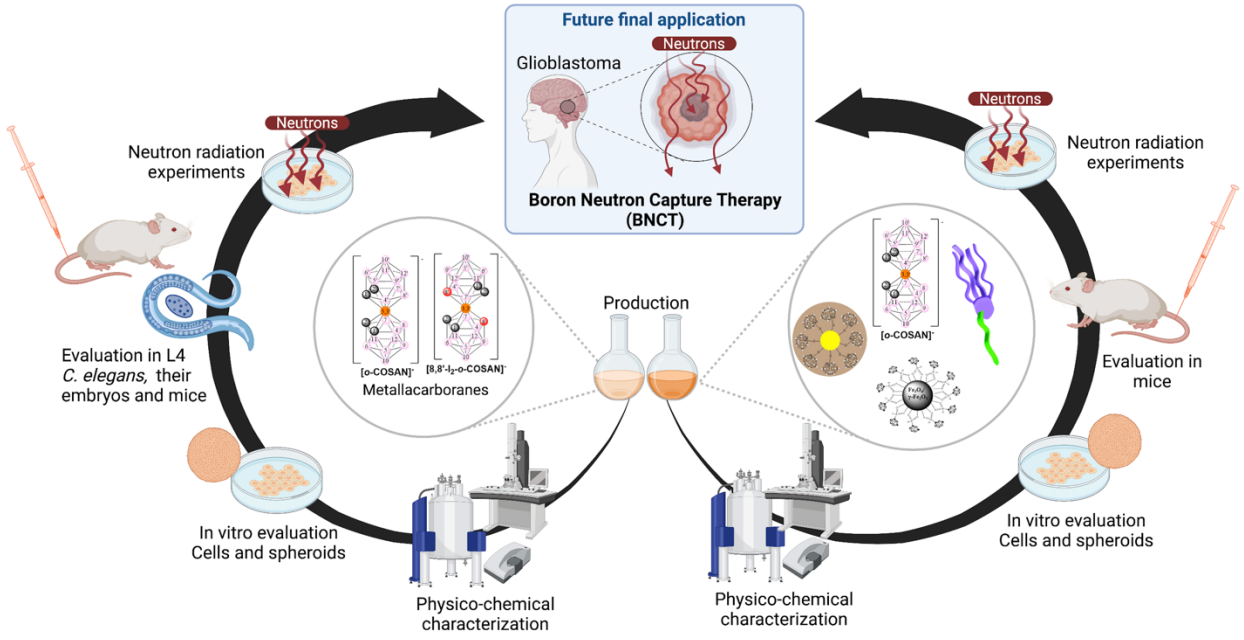


Figure 1. Carboranes and metallabis(dicarbollides) on the road to anticancer therapies: From synthesis, characterization, cellular uptake, and in vitro / in vivo biological evaluations to neutron irradiation to defeat cancer.

3. Carborane-Peptide Conjugates as Selective Agents for BNCT

Recently, peptides have captured much attention as therapeutic compounds.[27,28] Cell-penetrating peptides play an important role in BNCT research for shuttling high amounts of boron into cancer cells. Michiue et al. showed high uptake of a compound bearing three arginine (R, Arg) moieties and BSH (BSH-3R) in the tumor, investigated with DOTA-^{64}Cu fused to BSH-3R and studied by PET which is promising for clinical use.[29] This approach was enhanced to eight BSH molecules and 11 arginine molecules in a dendritic lysine structure.[3] Peptide derivatives with boron moieties such as boronated starbust dendrimers or BSH, applicable for BNCT, have been described.[30,31,32] Furthermore, carboranes have been integrated into the peptide sequences of well-known therapeutic peptides for the selective delivery of a large amount of boron to tumor cells. Roesch et al. focused on agonists for the somatostatin receptor, which is overexpressed in many neuroendocrine tumors. Their developed carborane-conjugated Tyr^3-octreotate derivatives showed high internalization rates binding affinities to the somatostatin receptor subtype 2 in the nM range depending on the spacer length between the carborane and the cyclic peptide.[33] Another attractive target is integrin $\alpha_v\beta_3$, which is overexpressed in various proliferating endothelial and tumor cells. *In vitro* cell adhesion assays of cyclic Arg-Gly-Asp (RGD) peptides conjugated with BSH or *ortho*-carborane demonstrated the high binding affinity of the conjugates to integrin $\alpha_v\beta_3$.[34] Furthermore, biodistribution studies showed a comparable tumor uptake but a significantly longer retention in tumors compared with BSH.[34,35] These few selected examples already showcase the suitability of carborane–peptide conjugates as potential boron carriers for BNCT. Other peptides, which were suggested as shuttle systems for the targeted delivery of pharmaceuticals into the tumor cells, are somatostatin (SST), epidermal growth factor (EGF), neurotensin, substance P, gastrin-releasing peptide (GRP), insulin-like growth factor (IGF), alpha-melanocyte stimulating hormone (α-MSH), cholecystokinin (CCK), vascoactive intestinal peptide (VIP), bombesin (BN), and neuropeptide Y (NPY).[28,36]

The Hey-Hawkins and Beck-Sickinger's groups focus on combining tumor-selective small peptides as highly selective G protein-coupled receptor agonists with *meta*-carborane derivatives for targeted delivery (Figure 2).[1,2,37,38,39] They have devised efficient syntheses for novel boron compounds, which provide a combined tumour-targeting system: carborane-containing amino

acids and carboxylic acids for incorporation in suitable peptides.[38,39,40,41,42] The first tumor-selective peptide-carborane conjugates prepared comprised *closo*-carborane-modified neuropeptide Y analogs[43,44] or metallacarborane derivatives;[45] another approach was the incorporation of *meta*-carboranes in novel ghrelin receptor agonists.[37] However, a very high carborane loading (more than two carborane clusters attached to a peptide including 36 amino acids) resulted in loss of solubility or aggregation in aqueous media, resulting in decreased potency and higher EC_{50} values.[1,2] Therefore, carborane derivatives bearing water-soluble groups, namely carbohydrate moieties, specifically galactosyl groups, were employed to compensate the hydrophobic character of the carborane clusters (up to eight modified carboranes attached to the same peptide comprising 36 amino acids).[1] Furthermore, the change from D-galactosyl to L-galactosyl groups increased the selectivity of these derivatives due to a lower unspecific uptake of bioconjugates into liver tissue.[2]

In summary, the combination of tumor-targeting peptides and carboranes covalently linked to the peptide represents a very efficient shuttle system to transport large amounts of boron into respective target cells and can be considered as a promising approach in tumor-selective boron shuttle system for BNCT.[46]

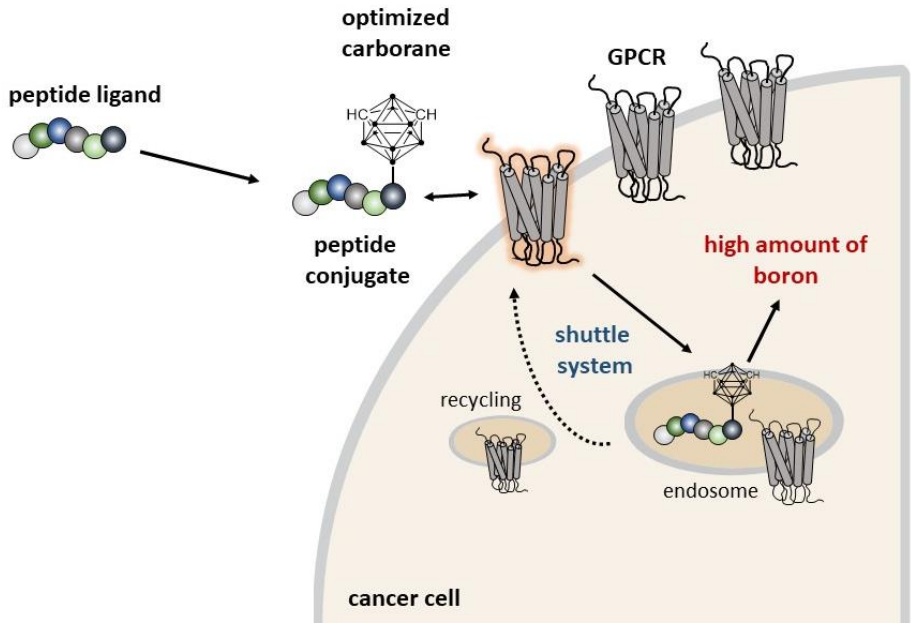


Figure 2. Tumor-selective peptides as highly selective G protein-coupled receptor (GPCR) agonists with *meta*-carborane derivatives facilitate targeted delivery of high amounts of boron to specific tumor cells.

4. Boron-Albumin Conjugates for BNCT

To improve the efficacy of boron transport, Nakamura's group focused on serum albumin as a boron biocarrier. Serum albumin is the major component of plasma proteins and accounts for approximately 55% of the human plasma proteins. Albumin is known to accumulate in malignant tumor and inflamed tissues due to its enhanced permeability and retention (EPR) effect. The Nakamura's group chose *closo*-dodecorate ($B_{12}H_{12}$) as a boron source due to its exceptional stability and high boron content. They designed and synthesized a maleimide-functionalized *closo*-dodecaborate (MID; Fig. 3A) suitable for conjugation with serum albumin at Cys34, which has the only free SH group among 35 Cys residues.[47] Interestingly, MID was found to bind not only to Cys34 but also to lysine residues in bovine serum albumin (BSA) under physiological conditions.[48] MID-BSA conjugates accumulated in colon 26 cells in a concentration-dependent manner. MID-BSA conjugates efficiently accumulated in mouse tumors, in contrast to boronated liposomes that were highly distributed in other organs, such as liver, kidney and spleen, 12 h after administration.[49,50] Administration of 7.5 mg[^{10}B]/kg of MID-BSA conjugates showed significant tumor growth inhibition in tumor-bearing mice irradiated with thermal neutrons.

Further, the Nakamura's group focused on the cyclic RGD (cRGD) peptide, which is known to bind to the integrins that overexpress in many cancer cells and designed cRGD-MID-BSA.[51] The bioorthogonal modification method of stepwise binding of c[RGDfK(Mal)] peptide and MID to BSA allowed the formation of cRGD-MID-BSA conjugates (Fig. 3A). Selective accumulation of cRGD-MID-BSA was observed against U87MG cells overexpressing integrin αvβ3. In vivo fluorescence live imaging of near infrared dye (Cy5)-conjugated cRGD-MID-BSA and MID-BSA revealed that cRGD-MID-BSA accumulates more selectively than MID-BSA (Fig. 1B). In vivo boron neutron capture therapy (BNCT) studies revealed that the cRGD peptide ligand combination promoted the accumulation of MID-BSA into tumor cells in U87MG human glioblastoma xenograft models. After neutron irradiation, significant tumor growth suppression was observed at a cRGD-MID-BSA dose of 7.5 mg [^{10}B]/kg. In summary, albumin was found to act as a carrier for boron to tumor, and cRGD conjugation of boronated albumin was effective in accumulating in U87MG human glioblastoma cells in vivo.

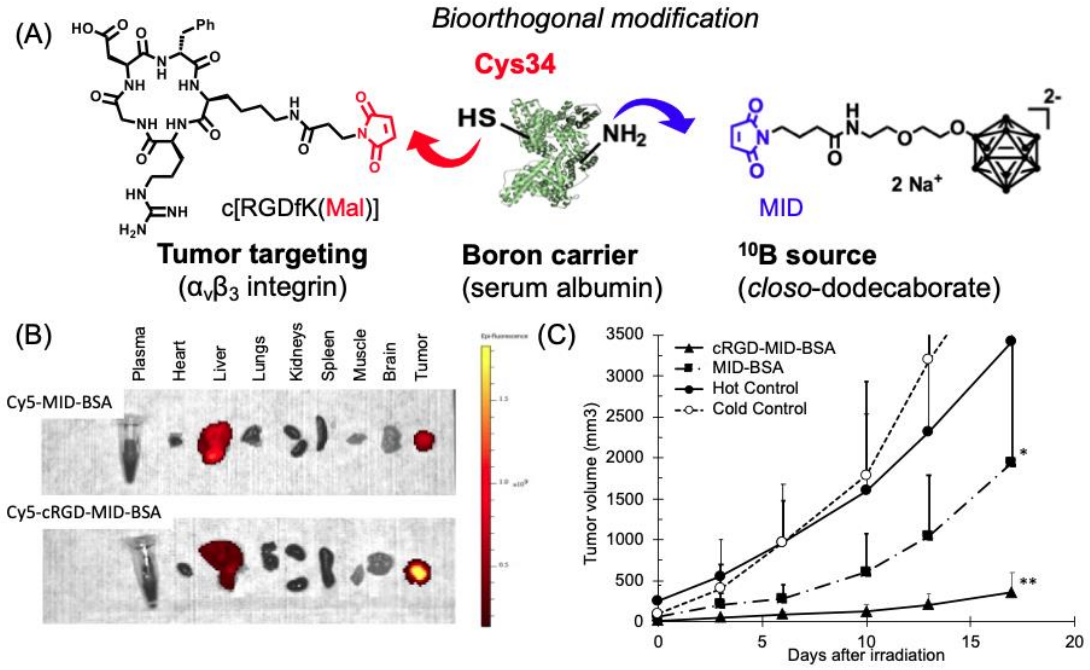


Figure 3. Design of cRGD-MID-albumin conjugates (A), their ex vivo imaging (B), and BNCT effect using U87MGxenograft model.

5. Boronated Porphyrin Derivatives for BNCT

The preferential accumulation of porphyrin-based compounds within certain tumors vs. nearby normal tissues, and their current use in PDT,[52,53,54] led to their investigation as boron delivery vehicles for BNCT. These properties along with their low dark toxicity, high chemical stability, and fluorescence properties led to their development as BNCT agents, as well as BNCT/PDT dual sensitizers. Several boronated porphyrin derivatives and their metal complexes were reported in the 1990s, including BOPP and VCDP.[55,56,57] These porphyrin derivatives were evaluated in multiple *in vitro* and in *vivo* studies that revealed high tumor uptake, favorable localizing properties and retention ability in tumor bearing mice, with high T/B and T/N boron concentration ratios (up to 20:1). It was also reported that changing the delivery of boronated porphyrins from i.v. injection to convection enhanced delivery (CED)[58,59,60] dramatically increased the boron concentration in tumor as well as the T/B and T/N boron ratios, and that the combined administration of BOPP and L-BPA increased the tumor uptake compared with BOPP or L-BPA alone.[61]

More recently, porphyrin conjugated to boron clusters, including carboranes, nido-carboranes and cobaltabis(dicarbollides), via hydrolytically-stable carbon-carbon links were reported by the Vicente group.[62,63] Up to 12 boron clusters were introduced onto a porphyrin macrocycle, resulting in compounds of 35-45% boron by weight, with the potential to deliver a high amount of boron to tumor cells.[64,65] Despite the bulkiness of the boron clusters at the periphery of porphyrin macrocycles, some of these compounds were shown to interact with DNA and thereby produce *in vitro* DNA damage following light activation, making them highly promising as BNCT/PDT dual sensitizers.[66,67]

With the goal to further increase the tumor uptake of boronated porphyrins, the Vicente group investigated the conjugation of a boronated porphyrin to the cell-penetrating peptide from the human immunodeficiency virus I transcriptional activator HIV-1 Tat (48-60) with the sequence GRKKRRQRRRPPQ. This sequence was found to significantly enhance the uptake of the boronated porphyrin into T98G tumor cells.[68] Similar observations were found upon the conjugation of polyamines, due to up-regulation of the polyamine transport system in tumor cells.[69] However, all boronated porphyrin investigated were found to have low BBB permeability hCMEC/D3 brain endothelial cells, in part due to their high molecular weights and high degree of hydrophobicity, which could jeopardize their use for glioblastoma treatment unless delivered via CED. On the other hand, boron dipyrromethenes, known as BODIPYs, have lower molecular weight, higher solubility, and lower toxicity compared with porphyrin derivatives, while displaying high fluorescence quantum yields (ϕ_f ~ 0.50). The Vicente group recently reported a series of BODIPYs bearing one or two boron clusters and investigated their tumor cell uptake, toxicity and BBB permeability.[70,71] These studies showed that boronated BODIPYs exhibited low toxicity, high cellular uptake and moderate BBB permeability, in part due to their low molecular weight (< 400 Da) and favorable hydrophobic properties (log *P* ~ 1.50).

In summary, porphyrin derivatives are an important class of pharmacological agents for application in a variety of cancer treatments. The ability of boronated porphyrin derivatives for generation of singlet oxygen upon light activation, makes them suitable for tumor treatment by PDT as adjuvant therapy to BNCT. In addition, their fluorescence properties and unique ability for metal complexation and functionalization allows the detection of tumor-localized boron prior to

irradiation, via optical imaging, SPECT, and/or PET. On the other hand, boronated BODIPYs are emerging as very promising BNCT agents due to their low molecular weights, low toxicity, high tumor cellular uptake, high fluorescence, and moderate BBB permeability.

6. Boron-Rich Liposomes as Nanoscale Delivery Agents for BNCT

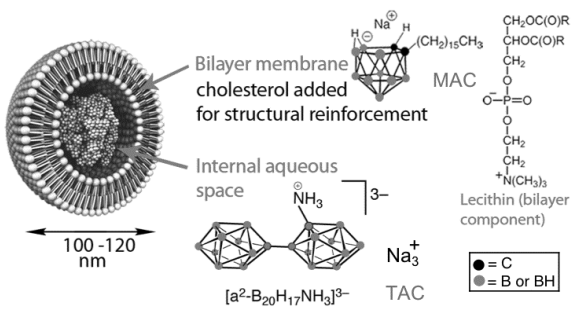
Another approach to boron agent development leverages nanotechnology, as explored by Jalisatgi's group. Through this protocol, nanoparticles are targeted to cancerous tissue, with each particle carrying many boron atoms. Nanoparticles of optimal composition are widely observed to accumulate selectively in tumor tissue due to the Enhanced Permeability and Retention (EPR) effect. Furthermore, the surface of each nanoparticle may be modified to enhance stability, plasma circulation time, and tissue specificity. As demonstrated in Jalisatgi's laboratories, liposomal nanoparticles of varying compositions are promising boron agents for BNCT.[72,73,74] These agents selectively deliver large quantities of boron to tumor tissues.

Liposomes are spherical nanoparticles comprised of a lipid bilayer shell encapsulating an aqueous core. The liposome formulations investigated in our laboratories are small unilamellar vesicles, or SUVs and range in size between 100 to 130 nanometers in diameter. As liposomes possess both hydrophobic and hydrophilic environments (lipid bilayer and aqueous core), two modes of boron incorporation are available. Hydrophilic, water-soluble species may be encapsulated in the aqueous vesicle interior, and hydrophobic (lipophilic or amphiphilic) species may be embedded within the lipid bilayer.

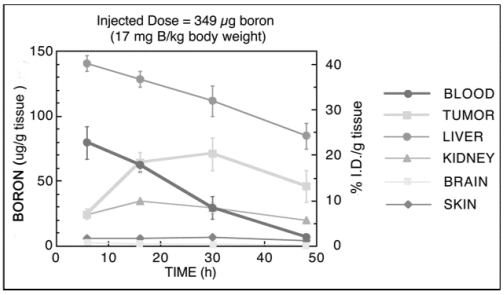
The liposome project was initiated at UCLA by Professor M. Frederick Hawthorne in the 1990s and continued at the University of Missouri International Institute of Nano and Molecular Medicine (MU-I^2NM^2). This work has shown that small unilamellar liposomes (MAC-TAC liposomes) are very promising boron delivery agents since they are targeted to the cancer cell interior and or endothelial cells in the tumor vascular supply. This results in high selectivity for tumor as opposed to blood and normal tissue. This mechanism provides a long therapeutic time window. These MAC-TAC liposomes selectively accumulate in tumor tissue over a period of 30 to 48 hours giving sufficient time for neutron irradiation procedures.[52,53] These exquisite tumor-

targeting materials are non-toxic to mice, hamsters, dogs and cats. Hopefully this will also be true in the case of humans.

Nearly all therapeutic applications of liposomes rely on the stability of the liposome bilayer. This bilayer protects the encapsulated compounds from physiological degradation, thereby providing boron agents with a prolonged circulation lifetime. Liposomes constructed from pure, saturated phospholipids are particularly notable for their high stability and long survival half-lives in human plasma. Because of the similarity of liposomes to biological membranes and their construction from natural body constituents they exhibit extremely low toxicity and may be safely administered without serious side effects.



Typical Liposome Constituents and Structure.



Typical Biodistribution of Liposomes Containing $Na_3[a^2\text{-}B_{20}H_{17}NH_3]$ and $Na[C_2B_9H_{11}(CH_2)_{15}CH_3]$ in **BALB/c Mice Bearing EMT6 Tumors**.

Figure 4. Murine biodistribution of boron in BALB/c mice bearing subcutaneous EMT6 tumors, incorporating $Na_3[a2\text{-}B_{20}H_{17}NH_3]$ and $Na[C_2B_9H_{11}(CH_2)_{15}CH_3]$ anion species in liposomes, injected dose 349 μg B (17 mg B/kg body weight).

Extensive research was conducted optimizing liposome formulation in conjunction with animal biodistribution studies. This work made use of liposomes which contain a water-soluble $[B_{20}H_{17}NH_2R]^{-2}$ polyhedral borane anion derivative and/or an amphiphilic nido-carborane anion species, $[nido\text{-}7\text{-}CH_3(CH_2)_{15}\text{-}7,8\text{-}C_2B_9H_{11}]^-$, embedded in the bilayer. These liposomes contain 3-5 weight percent boron and are able to deliver therapeutic doses of 30 ppm, or greater, selectively to tumor tissues. Figure 4 is a graph representing a typical biodistribution experiment in BALB/c mice bearing subcutaneous EMT6 mammary adenocarcinoma.

Liposomes are prepared by the probe ultrasonication of a dried lipid film composed of equimolar quantities of distearoylphosphatidylcholine (DSPC) and cholesterol (CH) in the presence of a hydrating solution (buffer or aqueous borane salt solution) incorporating $K[nido\text{-}7\text{-}CH_3(CH_2)_{15}\text{-}7,8\text{-}C_2B_9H_{11}]$ in the lipid bilayer, and encapsulating $Na_3[1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8]$. The

liposome suspension is purified by gel filtration on Sephadex and sterilized by microfiltration through a 0.2 μm membrane. The boron content of the liposome suspension is determined pre-injection by ICP-AES, and the liposomal size distribution determined by dynamic light scattering.

Microwave assisted digestion followed by inductively coupled plasma-optical emission spectroscopy was utilized to determine the biodistribution of boron in various tissues. Single and double-injection protocols were explored to optimize boron content in the tumor 48 to 72 h subsequent to the initial injection. Significant tumor response for a single BNCT treatment was demonstrated by growth curves versus a control group. Vastly diminished tumor growth was witnessed at 14 days in mice.[52]

Toxicity issues were not apparent in these experiments and therapeutic boron concentrations were retained in tumor for many hours. In these experiments tumor boron concentration surpasses that of blood between 10-20 h post-injection, with the maximum measured tumor boron concentration after 30 h. This produces a significant concentration of boron in the tumor (up to 60 ppm at 48 h) with very favorable T/B and T/N ratios (> 5.5). Results similar to these were obtained using Fisher rats with subcutaneous RG2 tumors. Hamsters bearing chemically induced tumors were treated in Argentina in a collaboration with DOE/ Argentina Atomic Energy Commission using MAC-TAC liposomes and the nuclear facilities available there. As in the case of mice, hamsters tolerated the therapy very well with no loss due to toxicity or radiation effects in both the mouse and hamster cases. Many remissions were observed. All in all, approximately one hundred hamsters were treated in this manner.[75] Furthermore, in order to establish the viability of liposomes as a drug delivery system in a large animal, a preparation of blank (boron-free) liposomes was administered to a canine subject at a dose comparable to those proposed. During this experiment, the dog appeared normal and healthy. Likewise, the blood chemistry analyses (16 factors measured) indicated no significant impact.

The University of Missouri Vet School initiated a series of distribution studies employing dogs with head-and-neck carcinoma treated with MAC-TAC liposomes. A small number of cases involving tumor bearing dogs and MAC-TAC liposomes exhibited clinical symptoms resembling CARPA syndrome (complement activation-related pseudoallergy). These dogs developed a

marked fever four hours after liposome infusion followed by profound neutropenia. After twelve hours the temperature of these animals had normalized and blood work demonstrated a rebound leukocytosis.

In vitro biodistribution studies of these liposomes in the EMT6 murine mammary carcinoma cell line show the localization of TAC in cytoplasm specifically in lysosomes and localization of MAC in the cell membrane. These biodistribution studies were done by incubating MAC/TAC liposomes with EMT6 cells for 24h. After 24 h, the cells were washed to remove any excess of liposomes present in the culture media. The cells were then lysed and cell component separated by filtration. Cell membrane components and the cytoplasm components were separately analyzed for boron by ICP-OES and mass spectrometry methods. Mass spectrometry analysis showed that TAC remains in the cytoplasm and MAC in cell membrane.

7. Conclusions and Outlook

From the works collected in the previous pages, it can be appreciated that chemists undertook the challenge of discovering new and more effective boron delivery agents for BNCT, by conjugating boron clusters to various carriers, including peptides, proteins (such as albumin), porphyrin derivatives, and liposomes. In some cases, the boron agents have the ability to bind to DNA and localize in close proximity to cell nucleus, which can enhance their biological efficacy. Pre-clinical studies suggest that alternative administration methods of boron agents, such as CED, could be used for the delivery of large amounts of boron to tumors, and that the combination of different boron carriers can lead to higher BNCT efficacy, compared with the use of a single boron agent. Furthermore, to facilitate treatment planning and maximize the tumor killing effect with minimal damage to normal tissues, the neutron irradiation treatment should be applied at the highest T/N and T/B boron concentration ratios. Therefore, promising BNCT agents containing easily detectable moieties, such as a fluorescence label, or a PET, SPECT or MRI agent, which play a prominent role in tracking and quantifying tissue localized boron, treatment planning and outcome.

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