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Review

Targeted Drug Delivery for Boron Neutron Capture Therapy

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Purpose. Boron neutron capture therapy (BNCT) is a form of radiochemotherapy that is becoming increasingly important for the treatment of malignant gliomas, malignant melanomas and other forms of cancer. Targeted delivery of boron to tumors is a critical prerequisite for successful BNCT.
Methods. Strategies that involve synthetic chemical approaches and biochemical and biophysical approaches are employed to meet this requirement. Compounds developed for targeting to tumors include borocaptate sodium (BSH) and p-boronophenylalanine (BPA) which are currently in clinical use.
Results. Boronated porphyrins, nucleosides, nucleotides and other boronated compounds show potentials as targeting molecules. Conjugation of boron compounds to macromolecules such as monoclonal antibodies, epidermal growth factor and dextran is also employed for active or passive tumor targeting.
Conclusions. Boron delivery via microparticulate carriers such as liposomes, high density lipoproteins and microcapsules is also attractive for its potential application in BNCT.

KEY WORDS: boron neutron capture therapy; BNCT; targeted drug delivery; borocaptate sodium; BSH; boronophenylalanine; BPA.

INTRODUCTION

General Concept of BNCT

Boron neutron capture therapy (BNCT) is based on a nuclear reaction which occurs when a stable non-radioactive isotope, ^{10}B, is irradiated with low energy neutrons (Figure 1). The reaction yields intensively ionizing particles, ^{4}He (α particles) and recoiling ^{7}Li nuclei.

$$^{10}B + {}^{1}n \rightarrow \begin{cases} {}^{4}He + {}^{7}Li + 2.31\ MeV\ (94\%) \\ {}^{4}He + {}^{7}Li + 2.79\ MeV\ (6\%) \end{cases}$$

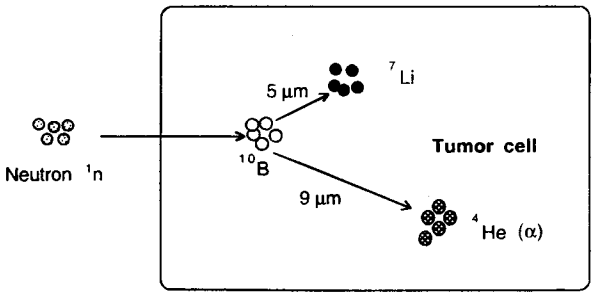

Fig. 1. Schematic representation of boron neutron capture therapy.

The nuclear fragments thus produced are highly cytotoxic and are slow moving with a pathlength of 5–9 µm (or approximately one cell diameter) in tissues. As a result, mainly the cells that have bound or taken up a ^{10}B containing agent are destroyed.

In essence, BNCT is a binary therapy with the possibility of independently manipulating each of its components (i.e. the boron compound and the neutron beam). This allows a better control in selectively destroying the tumor cells while minimizing damage to normal cells. The neutron flux (irradiation) can be started at a suitable time when the ratios of tumor/blood and tumor/surrounding tissue boron concentrations have reached an effective value. In addition, the neutron beam can be directed towards a narrow region so that other organs such as the liver and kidney, which might have accumulated significant amount of boron, are unaffected by the therapy.

^{10}B constitutes about 20% of natural boron. Since ^{10}B has a high cross-section for neutron capture, it is advantageous to employ ^{10}B—enriched compounds for BNCT. Capture reactions also occur with nitrogen and hydrogen producing protons and gamma rays, respectively. Even though the neutron capture cross-section of these elements is several times lower than ^{10}B, their high abundance in normal tissue makes these capture-reactions significant. Thus, the neutron irradiation that can be delivered depends on the tolerance of the surrounding normal tissues to the radiation produced by these capture reactions.

It is therefore necessary to deliver relatively high amount of boron to tumor cells to obtain a significant boron dependent radiation dose. The estimated boron concentration for effective therapy is in the range of 20–30 µg boron/g tissue (1). At the same time, boron concentration in the surrounding normal tissue should be kept low to minimize damage to the normal tissue.

Thus, selective delivery of relatively high amounts of boron compounds to tumor is one of the key requirements for

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the success of BNCT. This review will focus on the various approaches employed for targeting boron to tumors. Various boron compounds developed for tumor targeting will be discussed. Targeting strategies employing macromolecules such as monoclonal antibodies and epidermal growth factor will be reviewed. Microparticulates such as liposomes, high density lipoproteins and microcapsules will be discussed as potential carriers for selective delivery of boron. Detailed information on other aspects of BNCT such as physics and engineering of neutron beams, radiobiological considerations, analytical methods for boron measurements and biological and clinical studies can be found in literature (1–6).

History and Present Status

The potential of neutron capture therapy was first recognized in the 1930s. BNCT was tested in clinical trials between 1951 and 1961 at Brookhaven National Laboratory and Massachusetts General Hospital. Poor results were obtained during these trials and the trials were discontinued in 1961. Two main reasons were identified for the failure: lack of tumor selectivity of the boron compound and poor physical characteristics of the neutron beam employed (7). Subsequent to the initial trials in the U.S., Hatanaka, a neurosurgeon, began clinical tests with BNCT in Japan. He employed a tumor-selective boron compound, borocaptate sodium or BSH (to be discussed later in this review), for the treatment of brain glioma patients. The median life expectancy of the patients having this type of tumors under the conventional treatments is less than one year. More than 100 patients with grade III–IV gliomas were treated with BNCT and the mean survival time was significantly improved (8). There were several long term survivors (8). These encouraging results have revived the world's interest in BNCT. Currently, several research laboratories in the U.S., Europe, Japan and Australia are intensively working on BNCT. Since September 1994, several patients with glioblastoma multiforme have been treated with BNCT in the U.S. (9).

STRATEGIES FOR TARGETED DELIVERY OF BORON TO TUMORS

Synthesis of Tumor Localizing Boron Compounds

An antineoplastic drug should satisfy two major criteria: it should be cytotoxic and should have an affinity to the neoplastic cells. On the other hand, an ideal boron compound for BNCT should not have any cytotoxicity or therapeutic activity. The only important requirement for such a compound is to selectively target cancer cells to deliver a sufficient number of boron atoms to the cells. A number of boron compounds have been specifically synthesized for the targeting purpose.

Sulfur Containing Boron Compounds

Shortly after the initial clinical trials of BNCT, Soloway *et al.* reported mercaptoundecahydrododecaborate $(B_{12}H_{11}SH)^{2-}$ as having favorable tumor/blood ratio (10). Since then, the sodium salt of this compound, also known as Borocaptate Sodium or BSH (Figure 2a), has been widely used in BNCT. Significant accumulation of BSH in malignant tumors and the tumor/blood concentration ratio above unity have been shown

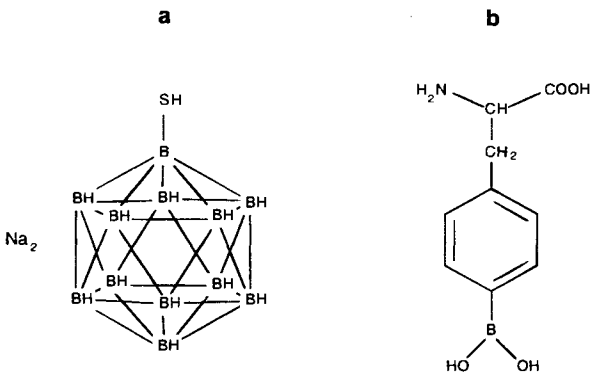

Fig. 2. Compounds in clinical use for BNCT: (a) borocaptate sodium (BSH) and (b) p-boronophenylalanine (BPA).

in experimental animals (11) and in samples from surgical patients (5, 12). BSH appears to be excluded from normal brain tissue (13, 14). The treatment employed in Japan was relatively straightforward. Approximately 4 weeks after surgical resection of tumors, patients received BSH at doses ranging from 30 to 80 mg/kg by intracarotid infusion, followed by neutron irradiation 12–16 h later. The mean tumor/blood concentration ratio obtained was 1.69 (5, 12). In more than 100 patients, no BSH related toxicity was observed at these doses (5, 13). Because of its low systemic toxicity and selective localization in tumors, BSH has been one of the most widely used compounds for BNCT. Its biodistribution and pharmacokinetics in animal models have been reported (14–17).

The mechanism for selective uptake of BSH in the tumor when compared to the blood compartment or normal brain is not clear. BSH has a potential to interact with plasma proteins via its sulfhydryl group. Selective uptake of such boronated proteins in cancer cells has been reported (6). Therefore, it has been speculated that interaction with plasma proteins is important for selective localization of BSH. However, it is not certain whether the selective uptake of BSH could be attributed to its incorporation into tumor cell proteins (5).

The dimer form of BSH, $(B_{12}H_{11}S\text{-}SB_{12}H_{11})^{4-}$, also abbreviated as BSSB, has been studied for its potential application in BNCT (15). It was reported to yield higher ratios of tumor/blood and tumor/normal brain boron concentrations than those obtained with BSH. BSSB accumulated significantly in liver and kidney, and caused marked elevation in the levels of hepatic enzymes in animals. Its hepatotoxicity appeared to be an important factor limiting its usefulness. However, the hepatotoxicity was reported to be reversible and could be minimized by a slow infusion of BSSB over an extended time period (18). The mechanism for selective uptake of BSSB is not known. However, its interaction with plasma proteins is believed to play an important role (19).

An iodinated analog of the dimer was recently synthesized for serving dual roles as a ^{10}B carrier and a radiographic contrast agent (20). Such a compound would allow for non-invasive quantification of boron in tumor by conventional computed tomography (CT). In the preliminary study, the compound appeared to be hepatotoxic. However, detailed biological evaluation of this compound is awaited.

Boronated Amino Acid Analogues

In the last ten years, there has been an increasing interest in developing boron containing amino acids. This is mainly due to some success in clinical trials for the treatment of malignant melanoma using a ^{10}B containing derivative of phenylalanine, p-boronophenylalanine (BPA) (Figure 2b). Mishima and his colleagues did pioneering work in the development of this compound. Since the initiation of clinical trials in 1987, they have treated more than twelve patients with malignant melanoma and have reported encouraging results (21). In some patients complete cure of melanoma lesions with no sign of recurrence was reported. Favorable tumor/blood and tumor/normal tissue boron ratios and tumor boron concentrations up to 30 μg/g were reported in experimental animals (22, 23). No acute toxicity was observed after a single dose of 3 g/kg over one hour infusion. However, a 4 g/kg infusion over 3 hours resulted in death in experimental animals (24). Originally, BPA was developed with a reasoning that since the biosynthesis of melanin requires phenylalanine as precursor, the boronated form of this amino acid may be selectively taken up by the melanoma cells. It has been proven that BPA does accumulate selectively in melanoma cells (22). However, it is also known that BPA is not incorporated into melanin and, hence, the accumulation in melanoma cells is only temporary (25). The current evidence suggests that BPA is taken up by melanoma cells via an amino acid transport system where it forms a complex with some melanin related compound, presumably L-DOPA (26). This complex is responsible for transient accumulation of BPA in melanoma cells. The concentration of BPA in the cell declines gradually as the complex dissociates.

In contrast to the earlier belief that BPA is selective only for melanoma, its preferential uptake in glioma was recently reported (23). Gliomas exhibit elevated levels of tyrosine hydroxylase. It was suggested that since BPA is a tyrosine analogue, it is selectively taken up by glioma cells. However, in another study on the pharmacokinetics of BPA in human patients, the tumor/blood boron ratio obtained with BPA for high grade glioma was not considered satisfactory (27).

One pharmaceutical limitation of BPA is its poor water solubility. Even though an i.v. infusion would be a simple way of administrating BPA, its use may be limited because of the low water solubility. The hydrochloride salt of PBA has good solubility but the solution has a pH of 1.5 which causes severe pain and irritation upon injection. Hence, attempts have been made to improve the aqueous solubility of BPA by complex formation with fructose (27) and cyclodextrin (25, 28). Both complexes resulted in improved bioavailability in experimental animals. BPA-fructose complex has been used clinically (27). The formulation has also been employed in recent clinical BNCT trials in the U.S. which utilized BPA as the boron delivery agent.

Stimulated by the results obtained with an amino acid containing a single boron atom (such as BPA), attempts have been made to increase the boron load by synthesizing amino acids attached to a boron cage. A boron cage consists of ten or twelve boron atoms covalently linked in a polyhedral fashion (see Figure 2a as an example). The basic idea was to develop a compound with the same affinity for tumors but containing a higher boron content. The first such compound, carboranylalanine, carried a cage containing ten boron atoms (carborane cage) attached to the amino acid alanine. Recently, improved synthesis of this compound and several new carborane containing amino acids has been reported (29). However, there is very little information available at present time on their tumor locating properties.

Boronated Porphyrins

Porphyrins are metal chelating agents which exhibit selective affinity for malignant tumors. A highly water soluble boronated porphyrin termed BOPP (Figure 3a) was reported to selectively localize in tumors at a ratio as high as 400:1 relative to normal brain tissue. The uptake and retention of BOPP was found to be approximately 20 times that of BSH (30). BOPP also accumulates significantly in liver. However, this may not pose any problem for its use in the treatment of cerebral gliomas owing to the localized neutron irradiation. More recently the synthesis of a manganese chelate of BOPP (Mn-BOPP) was reported. It has a potential of serving as a tumor selective agent for BNCT and as a proton MRI contrast enhancement agent for glioma. Mn-BOP was selectively localized in rat 9L gliosarcoma and preferentially enhanced the tumor/normal brain contrast (31). It was also taken up by melanoma cells more effectively than BPA. However, studies to evaluate the mechanism of selective uptake and toxicity are needed.

Boronated Nucleosides and Nucleotides

Since the pathlength of the high energy particles produced during the capture reaction is short, it would be advantageous if the reaction occurs inside the nucleus. Calculations have shown that the radiobiological effectiveness (RBE) of a capture reaction occurring in nucleus would be 2.5 times higher than that in cytoplasm (2). Hence, boronated nucleic acid precursors may be suitable agents for BNCT. Since the mitotic index of malignant cells is several times higher than that of surrounding normal cells, larger amounts of these agents could selectively accumulate in the rapidly multiplying tumor cells. Upon entry into the cells, they may get incorporated into nuclear DNA, or at the very least may get converted into the corresponding nucleotides and become trapped intracellularly. The first boron containing nucleoside, 5-dihydroboryl-2′-deoxyuridine (DBDu) (Figure 3b), synthesized by Schinazi and Prusoff, was shown to destroy hamster v-79 cells *in vitro* upon neutron irradiation (32). In an attempt to increase the boron load, carboranyl nucleosides have been synthesized (33). These compounds exhibited high *in vitro* and *in vivo* uptake. However, the mechanism for their cellular uptake and retention is not fully confirmed. A recent study reported that boronated nucleoside triphosphate can serve as substrates for DNA polymers and thus can potentially be incorporated in DNA (34).

It was suggested that boron containing nucleotides may prove to be better substrates for incorporation into nucleic acid (1). Recently, the synthesis of a carborane-containing nucleotide was reported (2). Even though nucleotides are not expected to cross cell membranes because of their negative charge, it has been suggested that incorporation of a lipophilic moiety, such as a carbonyl cage, may compensate for the ionic character of the molecule and facilitate membrane penetration. Tumor selectivity of such a nucleotide remains to be evaluated.

Boronated antisense oligonucleotides represent yet another class of compounds for BNCT. Antisense oligonucleotide

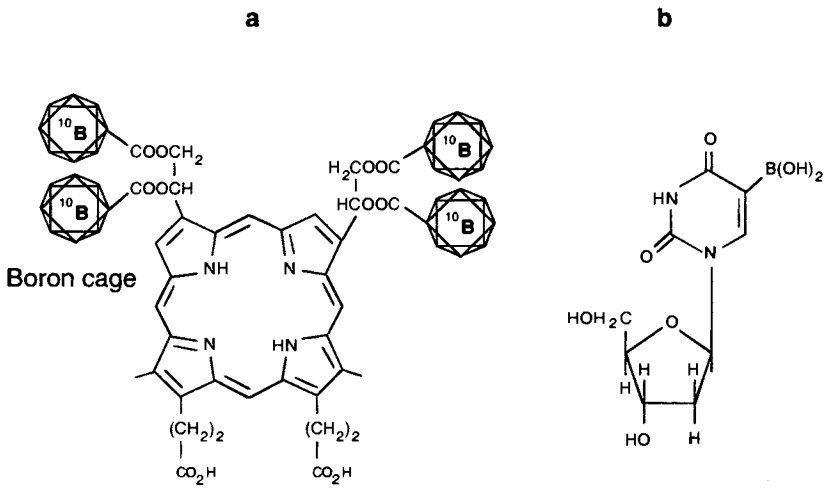

Fig. 3. Chemical structures of (a) a boron-containing porphyrin (BOPP) and (b) 5-dihydroboryl-2′-deoxyuridine (DBDu).

research is at an early stage and problems such as poor permeability through cell membranes and obtaining tumor selectivity through specific homology with nucleotide sequences remain to be solved. However, considering the fast developments in the field of antisense oligonucleotide research, it may be possible to apply this concept to BNCT in the near future. As an initial step, synthesis of a boronated oligonucleotide with a carboranyl phosphoramidate backbone was recently reported (34).

Miscellaneous Boronated Compounds

Boronated Ether Lipids. It has been demonstrated that natural ether lipids and their artificial analogues accumulate selectively in various tumors. This is based partly on the fact that the catabolic enzyme required for their cleavage, o-alkyl glycerol mono oxidase, is absent in a great number of tumors. It is, therefore, proposed that boronated analogues of ether lipids could serve as potential boron carriers for BNCT. The synthesis of a carborane containing ether lipid has been reported and its biological evaluation remains to be performed (35).

Carboranylaziridine. A carborane compound containing an aziridine group has been synthesized. This compound has the potential to alkylate DNA via the aziridine group thereby leading to the breakdown of DNA strands and placing boron in proximity to the nucleic acids (36). It has been found selective in its uptake and cytotoxicity towards cancer cells in *in vitro* studies.

Boronated Nitroimidazoles. Boronated derivatives of 2-nitroimidazole have been synthesized based on their potentials for targeting the hypoxic cell fraction in tumors. Such cell fractions, existing at low oxygen tension, may contain radiation resistant cells capable of repopulating the tumor mass after the therapy. An ortho carborane derivative of 2-nitroimidazole exhibited hypoxia selective toxicity *in vitro* in a rodent tumor model (37).

Boronated Bibenzimidazoles. Bibenzimidazoles bind to the minor groove of DNA and are widely used as fluorescent DNA stains. ^{10}B containing bibenzimidazoles have been synthesized as potential agents for carrying boron to DNA. Such compounds significantly enhanced the cell kill in *in vitro* experiments. Biodistribution studies are underway (38).

Conjugation of Boron Compounds to Macromolecules

The second general category for targeting is based on linking a compound containing a large number of boron atoms to a macromolecule carrier. Macromolecules that have an affinity for tumor cells as well as those that are passively retained in the tumor bed have been utilized.

Targeting Using Monoclonal Antibodies

Several attempts were made in the last decade to utilize monoclonal antibodies (MAbs) as boron delivery agents (1). A major requirement for the success of this approach was identified as loading the MAbs with large amount of boron without compromising their immunoreactivity. Calculations have shown that approximately 1000 ^{10}B atoms must be carried by each antibody molecule in order to achieve effective tumor concentration. Modifying a large number of sites on the antibody to achieve this resulted in loss of immunoreactivity (5). Consequently, a boronated polylysine (BPL) containing more than 1000 ^{10}B atoms per unit, was synthesized and conjugated to MAb (Figure 4a). While such an immunoconjugate retained its immunoreactivity *in vitro,* it exhibited poor tumor selectivity *in vivo* and accumulated mainly in the liver. In an attempt to improve this approach, BPL was linked to specific sites on the antibody that were distant from the antigen binding site (39). About 10^4 boron atoms were linked per antibody molecule. The conjugate showed no loss in immunoreactivity *in vitro. In vivo* evaluation of this immunoconjugate is yet to be performed. In another approach, boronated starburst dendrimers (BSD) were linked to MAbs (Figure 4b). The conjugate retained its immunoreactivity *in vitro* but was found unsuitable in *in vivo* studies, requiring further modifications of the conjugate (40). Synthesis of an extremely lipophilic oligophosphate based boron rich trailer molecule for labelling MAbs was also reported (1). One of the new approaches suggested for BNCT is the use

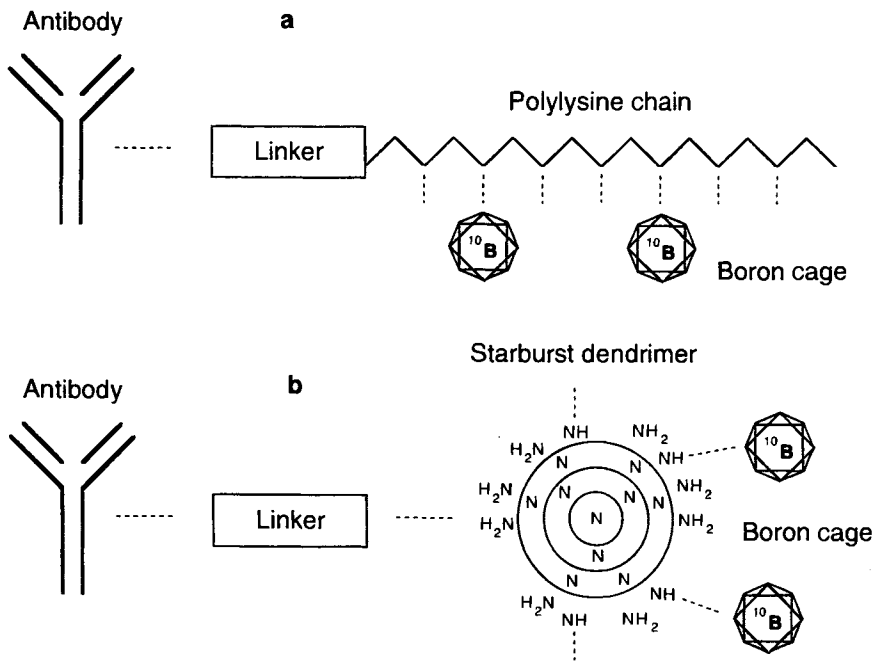

Fig. 4. Schematic representation of antibody conjugated to (a) boronated polylysine (BPL) or (b) boronated starburst dendrimer (BSD).

of a bispecific antibody which has specific affinity for tumor associated antigen as well as a boronated macromolecule. Studies evaluating the feasibility of such an approach are underway (40).

Growth Factors as Boron Targeting Agents

Growth factors and peptides may serve as potential boron delivery agents if their receptors are overexpressed in tumor cells. Epidermal growth factor (EGF) receptors are overexpressed in 25–30% of highly malignant gliomas. Boronated EGF could be used for targeting in such cases. Boronated EGF could be advantageous over boronated MAb because of the relatively smaller molecular weight of the EGF conjugate, which would allow easier penetration into tumor cells. Binding to the receptors is very specific and the conjugate would then be rapidly internalized. Synthesis and *in vitro* evaluation of a boronated starburst dendrimer linked to EGF was recently reported (41). More than 1000 boron atoms were linked to each EGF molecule. However, this resulted in a 10-fold decrease in the affinity for the EGF receptor. Questions such as *in vivo* performance of these conjugates, total boron load that can be effectively delivered to tumor cells and intracellular persistence of boron upon internalization of the complex remain to be answered. Studies are planned to address these issues (41).

Boronated Dextran Conjugates

In contrast to the use of a tumor-specific macromolecule such as MAb, the use of a boronated dextran conjugate for 'passive' tumor targeting was recently suggested (42). The idea is based on the fact that tumor vasculature has increased permeability for macromolecules and poor lymphatic drainage. This results in prolonged retention of macromolecules in tumors; also known as the enhanced permeability and retention (EPR) effect. Water soluble BSH-dextran conjugates with more than 1000 boron atoms per conjugate were synthesized (42). The biological effectiveness of such conjugates, however, remain to be evaluated.

Boron Delivery via Microparticles

The third category of boron delivery utilizes microparticulate carriers, such as liposomes and lipoproteins. Such systems can carry large amounts of boron compounds and offer some degree of tumor localizing properties.

Liposomes for Boron Delivery

The use of liposomes as a boron delivery system could be a promising approach for BNCT as they can carry relatively large quantities of boron compounds and show some degree of selective localization in tumors. It has been demonstrated in several studies that the uptake of intravenously administered drugs encapsulated in small liposomes was higher in tumors when compared to surrounding normal tissues (43). Although the exact mechanism of the selective tumor uptake is unclear, one likely mechanism is due to the increased extravasation of small liposomes in areas of leaky vasculature in a rapidly growing tumor mass. Once at the tumor site, liposomes are internalized by various pathways and thus deliver their contents intracellularly (43).

Liposomes containing a variety of boron compounds have been evaluated in experimental animals and the results have demonstrated the ability of liposomes to selectively deliver significant quantities of boron to murine tumors (44,45). Tumor boron concentrations of 30–40 μg B/g and tumor/blood boron ratios about 5 were reported. It was suggested that boron compounds that can react with intracellular proteins or be metabolized to such reactive entities would be retained for longer time

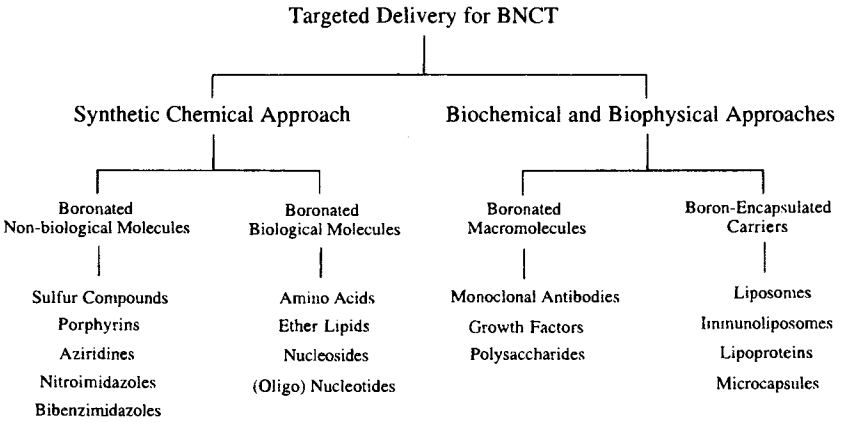

Fig. 5. An overview of the current approaches for targeted boron delivery for BNCT. The synthetic chemical approach involves the compounds which can be completely synthesized.

by the tumor cells. Steric stabilization of liposome surface by certain amphipathic lipids, such as polyethylene glycol (PEG) derivatized phospholipids, resulted in marked reduction in liposome uptake by the mononuclear phagocyte system and thus prolonged the circulation time of the liposomes containing boron compound. This presents an increased opportunity for the uptake of liposomes by tumors. Sterically stabilized liposomes containing boron compounds have been evaluated (45,46). Prolongation of circulation time was demonstrated thus allowing continued accumulation of boron agent in the tumor.

Targeting Using Immunoliposomes

As an extension to the concept of using liposomes for boron delivery, the use of immunoliposomes possessing a tumor specific antibody on the surface was proposed as a boron delivery system. The advantage is similar to conjugation of boronated polymers to MAb. Liposomes can carry a relatively large number of boron atoms, and the presence of a tumor specific antibody on the surface could improve tumor selectivity. ^{10}B containing liposomes conjugated with monoclonal antibodies specific for carcinoembryonic antigen (CEA) have been shown to bind selectively to cells bearing CEA on the surface (47). Thermal neutron irradiation of the cell culture resulted in suppressed cell growth. However, the *in vivo* utilization of immunoliposomes faces the problems of loss of immunospecificity and extensive uptake by the liver and spleen. The *in vivo* performance of this system remains to be evaluated.

Boronated Low Density Lipoproteins

Low density lipoproteins (LDL) have been suggested as tumor specific boron carrier. The density of LDL receptors is much higher in tumor cells as compared to normal cells. LDL's have a high capacity of carrying lipophilic boron compounds. Their relatively small sizes facilitate the diffusion from vascular to extracellular sites. Binding with cell surface receptors leads to internalization of LDL and results in intracellular delivery of its contents. Carborane carboxylic acid esters of fatty alcohols have been shown to effectively replace the cholesterol ester core in human LDL (48). The *in vitro* cell culture uptake of these boronated LDL was consistent with a receptor mediated binding mechanism.

Boron Delivery via Microcapsules

Gadolinium (Gd) neutron capture therapy is similar to BNCT in principle where ^{157}Gd, because of its large cross-section, is used as a neutron capture agent. One of the approaches suggested for selective delivery of high amount of Gd is to deliver Gd in microcapsules to tumors via a feeding artery (49). This may result in temporary intratumoral embolization of the microcapsules leading to Gd retention in tumor. Intraperitoneal injection of Gd-microcapsules followed by neutron irradiation resulted in significant increase in survival time, when compared to placebo microcapsules in experimental animals inoculated intraperitoneally with tumor cells (49). Such an approach could be applied to BNCT. However, factors such as the size of the microcapsules, release of boron agent from the microcapsules, and its uptake and retention in tumor cells will have to be considered.

SUMMARY AND FUTURE PROSPECTS

The targeted delivery of boron to the tumor sites involves a number of approaches. These approaches can be grouped into two main categories: the synthetic chemical approaches and the biochemical and biophysical approaches. The approaches which have been studied to date are summarized in Figure 5 for a clear overview.

The synthetic chemistry for BNCT is unique when compared to that for other therapeutic agents. In general medicinal chemistry, compounds which have defined therapeutic effects are synthesized. The compounds may also be required to have certain degree of targeting or absorption capacities. A compound for BNCT is, however, solely developed for targeted boron delivery with no consideration of therapeutic effects. The general requirement is to achieve a suitable tumor/blood or tumor/normal tissue ratio of boron after administration. As described above, many boron-containing compounds have been synthesized and tested by attaching boron to various chemical compounds with defined targeting capacities. These compounds involve a spectrum of molecules spanning non-biological identities (e.g. the sulfur compounds) and biological molecules (e.g. amino acid analogues). The compounds are grouped under the synthetic chemical approach because they can be completely synthesized. A boron-rich structure, such as the boron cage, is often used to increase boron delivery efficiency.

The biochemical and biophysical approaches for BNCT are to utilize suitable carriers to enhance the delivery of boron compounds to tumor sites. The methods are generally similar to those which have been used and tested for other therapeutic agents (e.g. the methods using monoclonal antibodies, liposomes and microcapsules for targeted delivery). The delivery of boron to tumors requires an acute accumulation of boron before applying neutron irradiation to destroy tumor cells. A chronicle or long-term administration of boron compounds is generally not necessary. Therefore, the intravenous administration is sufficient and other routes of administration are not advantageous. However, localized administration, such as intracerebral administration, for enhancing the targeted boron delivery has been suggested (50).

BNCT holds a promising future as a radiochemotherapeutic treatment for various tumors. One of the key requirements for the success of BNCT is to selectively deliver sufficient quantities of boron to tumors. As described in this review, significant research is being carried out to meet this requirement. While BSH continues to show promising clinical results in the treatment of glioblastoma multiforme, more studies are needed to establish the molecular basis for its selective uptake. The other clinically used compound BPA, which was conventionally employed for the treatment of malignant melanoma, is also showing promise in the treatment of other types of cancer. Research continues to establish the biochemical basis of its uptake and to enhance the boron delivery by increasing its boron load. A number of new boron compounds have been synthesized and a variety of new approaches have been suggested for their selective delivery. Most of the researches are in the developmental stages although *in vitro* and limited *in vivo* studies have generated a number of promising leads. Detailed animal studies will be needed to fully explore the potential of each of these compounds or the delivery approaches. It is very likely that these extensive research efforts may generate a suitable strategy for selective boron delivery to tumors and thereby benefit the development of BNCT.

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