Pharm Res (2015) 32:2824–2836
DOI 10.1007/s11095-015-1718-y

EXPERT REVIEW

Advancements in Tumor Targeting Strategies for Boron Neutron Capture Therapy

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Received: 12 March 2015 / Accepted: 18 May 2015 / Published online: 2 June 2015

ABSTRACT Boron neutron capture therapy (BNCT) is a promising cancer therapy modality that utilizes the nuclear capture reaction of epithermal neutrons by boron-10 resulting in a localized nuclear fission reaction and subsequent cell death. Since cellular destruction is limited to approximately the diameter of a single cell, primarily only cells in the neutron field with significant boron accumulation will be damaged. However, the emergence of BNCT as a prominent therapy has in large part been hindered by a paucity of tumor selective boron containing agents. While L-boronophenylalanine and sodium borocaptate are the most commonly investigated clinical agents, new agents are desperately needed due to their suboptimal tumor selectivity. This review will highlight the various strategies to improve tumor boron delivery including: nucleoside and carbohydrate analogs, unnatural amino acids, porphyrins, antibody-dendrimer conjugates, cationic polymers, cell-membrane penetrating peptides, liposomes and nanoparticles.

KEY WORDS Boron neutron capture therapy · BNCT · Cancer · Neutron radiation · Targeted delivery

ABBREVIATIONS

^{10}B	Boron-10
^{18}F-BPA	4-borono-2-^{18}F-fluoro-phenylalanine
BBB	Blood–brain barrier
BNCT	Boron neutron capture therapy
CPP	Cell-membrane penetrating peptide
DNA	Deoxyribonucleic Acid
EGFR	Epidermal growth factor receptor
EPR	Enhanced Permeability and Retention effect
^{157}Gd	Gadolinium-157
GdNCT	Gadolinium neutron capture therapy
GBM	Glioblastoma multiforme
ABCPC	1-amino-3-boronocyclopentanecarboxylic acid
BPA	L-boronophenylalanine
BSH	Sodium borocaptate
H_2PzCOB	1-methyl-*o*-closocarboranyl-2-hexylthioporphyrazine
i.v.	Intravenous
L-DOPA	L-3,4-dihydroxyphenylalanine
LCOB	*o*-closocarboranyl β-lactoside
mAbs	Monoclonal antibodies
NPs	Nanoparticles
PET	Positron Emission Tomography
RES	Reticuloendothelial system (RES)
H_2TCP	Tetra-(4-nido-carboranylphenyl) porphyrin
T/B	Tumor/blood
T/N	Tumor/normal tissue

INTRODUCTION

Boron neutron capture therapy (BNCT) is an emerging cancer treatment modality that utilizes the neutron capture reaction of boron-10 (^{10}B) and subsequent nuclear fission reaction to produce cellular death [1–4]. BNCT has the capability to provide the regional selectivity of radiation therapy with significantly less destruction to surrounding healthy tissue. This principle is obtainable because BNCT utilizes a lower energy neutron beam compared to the traditional higher energy x-ray or gamma particles used in ionizing-radiation therapy [4]. Since the neutron beam is non-ionizing in nature, primarily only tissues that contain neutron absorbing isotopes (such as ^{10}B) will undergo nuclear fission and subsequent tissue

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destruction. Boron has a neutron-capture cross section that is three orders of magnitude greater than other common nuclei in our body [2]. Additionally, because the neutron beam alone does not cause significant cellular death, the neutron beam field can be extended to irradiate the tissue surrounding the tumor to help eradicate micro residual disease and subsequent tumor recurrence or metastasis [5]. Therefore, assuming adequate neutron beam penetration, the efficacy of BNCT is ultimately limited by the selective tumor accumulation of boron containing agents.

Traditionally BNCT utilized a lower energy thermal neutron beam ($E_n < 0.5$ eV) which facilitated the neutron capture and fission reaction of ^{10}B [6]. However, in order to increase neutron beam penetration depth, clinical practice has adapted using an epithermal neutron beam ($0.5\ eV < E_n < 10\ keV$) [4]. The higher energy neutron beam is clinically significant because it increases neutron beam penetration through the skull and thick tissues. Upon ^{10}B neutron capture, the resulting unstable ^{11}B isotope undergoes a nuclear fission reaction ($^{10}B(n,\alpha,\gamma)^7Li$) to release an alpha particle (^4He), lithium-7 (^7Li) ion and gamma radiation corresponding to 2.31 MeV (94% of time) or 2.79 MeV (6% of time) (Fig. 1) [6]. The breadth of cell destruction is limited by the path lengths of the aforementioned linear energy transfer particles, typically 5–9 microns [6]. It is important to note that BNCT does result in a background dose of radiation administered to non-boron containing tissues. This is a direct result of low linear energy transfer gamma rays (a direct result of neutron capture by tissue hydrogen atoms) and high linear energy transfer protons (resulting from either the scattering of fast neutrons or from neutron capture by nitrogen atoms) [6].

Gadolinium neutron capture therapy (GdNCT) is an alternative neutron capture therapy modality which uses gadolinium-157 (^{157}Gd) [2]. For the ^{157}Gd neutron capture reaction, the majority of the energy is released as long range gamma radiation, while 0.63% of the time this emission occurs as Auger and conversion electrons [7]. If gadolinium is incorporated or in close proximity to deoxyribonucleic acid (DNA), the generated Auger electrons can enhance the cytotoxic effect through double strand breaks. The efficacy of GdNCT and BNCT has been compared in the treatment of several canine cancer models [8]. For canine oral melanoma, BNCT achieved full tumor regression in 78% of dogs ($N = 14$), compared to only 44% in the GdNCT treatment arm ($N = 14$). In the setting of osteosarcoma, both BNCT ($N = 1$) and GdNCT ($N = 8$) treatment arms illustrated full tumor regression. While further studies with GdNCT agents are clearly warranted, this review will focus on tumor targeting strategies for BNCT agents.

In order for BNCT to become a viable therapeutic option, the radiation dose delivered to the tumor must exceed the background radiation healthy tissue receives from non-specific neutron absorption. The efficacy of BNCT primarily depends on the selective accumulation of boron in the tumor tissue compared to the surrounding healthy tissue and blood. Generally the following requirements must be satisfied for a successful BNCT agent [1–4, 6, 9–11]: (1) A tumor ^{10}B concentration of approximately 20–35 μg/g of tumor (ppm range); (2) Selective tumor/normal tissue (T/N) and tumor/blood (T/B) concentration ratios above unity and preferably 3:1 or higher; and (3) Minimal systemic cytotoxicity and rapid clearance from blood and normal tissue.

Despite the potential of BNCT becoming an alternative (or adjunct) treatment modality for glioblastoma multiforme (GBM) [12–23], melanoma brain metastases [12, 24], adenocarcinoma liver metastases [25–30], hepatocellular carcinoma [31] and recurrent head and neck cancer patients [32–36], only two drugs have been investigated in these BNCT clinical trials: L-boronophenylalanine (BPA) and sodium borocaptate (BSH) [10]. The vast majority of patients diagnosed with the aforementioned malignancies undergo palliative care options. To put the severity of these diagnoses into perspective, consider the 2- and 5- year survival rates of newly diagnosed GBM are 10 and 1%, respectively [37]. High grade glioma patients enrolled in BNCT clinical trials have affirmed that BNCT is tolerated well, has comparable (or fewer) side effects than conventional radiation therapy, typically requires only 1–2 treatment sessions, and the median survival times are comparable to standard of care (radiation and temozolomide treatment) [15, 38]. Clinical findings such as this encourage further investigation of

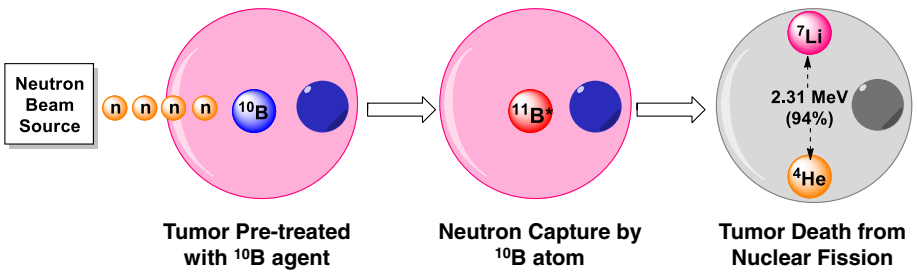

Fig. 1 Schematic representation of tumor destruction by BNCT.

BNCT as a therapeutic option. However, the suboptimal T/B and T/N ratios commonly achieved with BPA and BSH treatments require more selective agents to be developed [2]. This review aims to highlight the recent advances in boron delivery methods and reemphasizes the dire need for more selective boron delivery agents.

CURRENT AGENTS FOR BNCT CLINICAL TRIALS

BPA and BSH are the most extensively investigated agents in BNCT clinical trials, traditionally utilized in the treatment of melanoma and glioblastoma multiforme, respectively (Fig. 2) [2, 4, 39]. Since the ^{10}B isotope only has a natural abundance of 19.9%, BNCT agents must be enriched with ^{10}B during synthetic preparations to be maximally effective. The following sections highlight the preclinical and clinical progress made with BPA and BSH.

Boronophenylalanine

The L-BPA enantiomer structurally resembles the amino acid L-phenylalanine, and is used clinically due to its higher accumulation in cancer cells compared to the D-enantiomer or racemic mixture (Fig. 2) [40]. Due to the poor solubility of

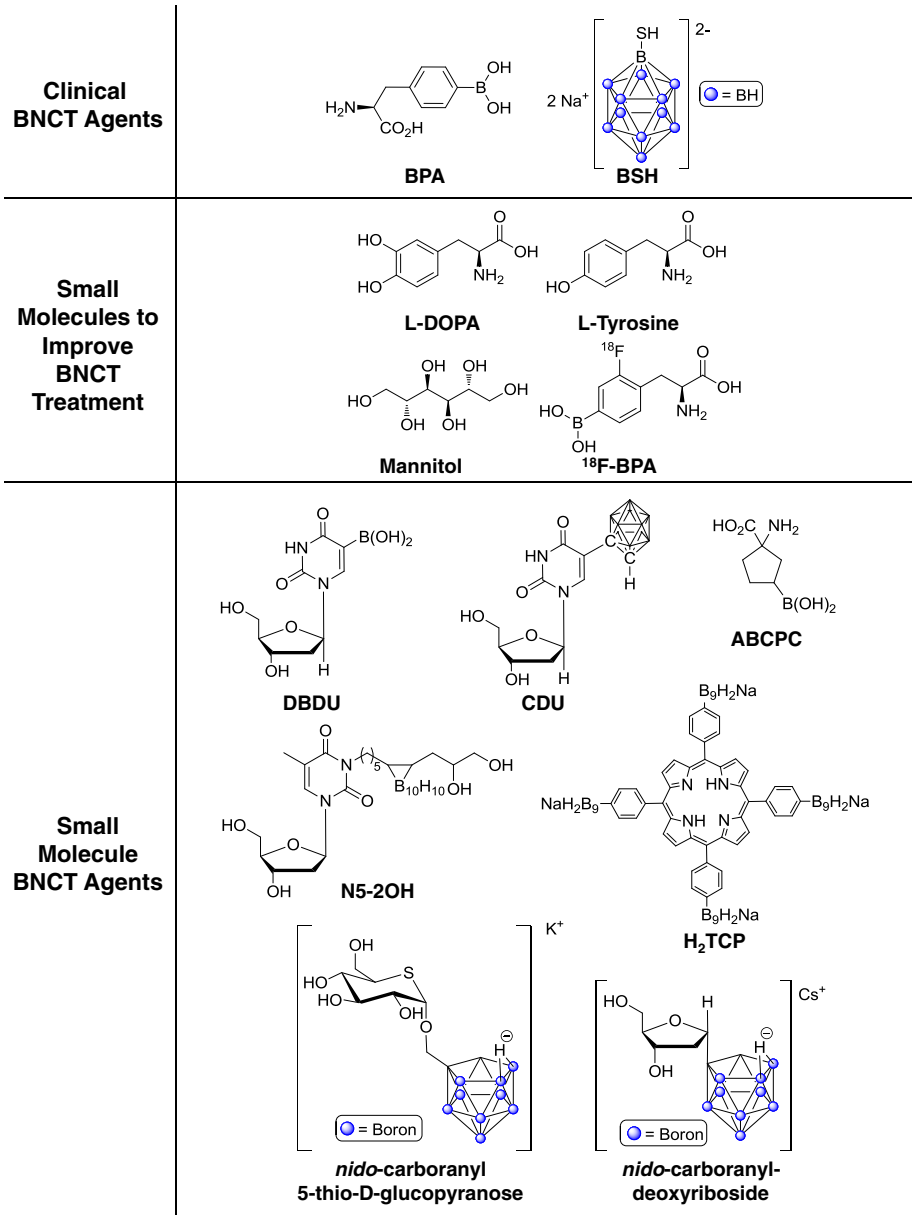

Fig. 2 Chemical structures of some small molecule agents used in BNCT. L-boronophenylalanine (BPA) and sodium borocaptate (BSH) have been the most extensively studied agents clinically. L-3,4-dihydroxyphenylalanine (L-DOPA), L-tyrosine and mannitol have all been utilized to improve BPA and BSH tumor uptake. 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-BPA) has been studied as a dual modality PET/BNCT agent. The following small molecules have all been proposed as novel BNCT agents: 5-dihydroboryl-2′-deoxyuridine (DBDU), 5-(1-*o*-carboranyl)-2′-deoxyuridine (CDU), 1-amino-3-boronocyclopentanecarboxylic acid (ABCPC), 3-carboranyl thymidine derivative (N5-2OH), tetra-(4-nido-carboranylphenyl) porphyrin (H_2TCP), *nido*-carboranyl 5-thio-D-glucopyranose and *nido*-carboranyl deoxyriboside.

BPA in water, BPA is often administered as a BPA-fructose adduct [41]. However, the short shelf life of BPA-fructose after preparation makes clinical administration challenging [42]. There is a well-established tradeoff between hydrophilicity of BPA agents and their cytotoxicity (with increasing water solubility there is a marked decrease in BPA derivative cytotoxicity in melanoma cells) [41]. Although hydrophilic BPA will increase solubility and enable intravenous (*i.v.*) administration, this will decrease its blood–brain barrier (BBB) penetration [6].

The accumulation of BPA in cancer cells relies on the higher metabolic rate in these cells. BPA is also structurally analogous to tyrosine, which is a precursor for melanin synthesis [43]; therefore, use of BPA in melanoma treatment relies on the principle that melanoma cells will have higher melanin synthesis [6]. An additional mechanism proposes that BPA is taken up through the L-type amino acid transporter [43, 44]. This system can transport neutral amino acid analogs containing aromatic side chains [45]. Since human L-type amino acid transport 1 expression is upregulated in a wide range of cancers (including brain tumors), this allows agents like BPA to preferentially accumulate in cancerous tissues compared to surrounding normal tissues [37, 46]. Additionally, BPA may preferentially accumulate in brain tumors compared to normal parenchyma due to a compromised BBB integrity within the tumor vasculature [13]. Since BPA uptake is largely an active process, subpopulations of quiescent cancer cells may have lower BPA uptake, thereby decreasing BNCT efficacy [23].

The biodistribution of BPA in GBM, melanoma, and head and neck cancer patients achieved T/B ratios ranging from 1.1 to 3.6 [12–15], T/N ratios of 1.1–2.9 [12, 15, 36], and tumor boron levels in the range of 1.8–34.8 ppm [12, 13, 15, 17, 36]. This data illustrates that more selective and targeted agents are urgently needed.

Sodium Borocaptate

BSH is an anionic carborane derivative and is administered as a sodium salt (Fig. 2). Due to its anionic nature, BSH is thought to preferentially accumulate in brain tumors compared to normal parenchyma because of BBB disruption unique to the tumor [28, 47, 48], and in contrast to BPA, BSH accumulates passively and not by active transport [23]. Early studies of BSH have noted that boron levels in normal brain are sometimes not even detectable [49]. An additional notable difference is BSH contains 12 boron atoms per molecule while BPA contains only a single boron atom. Therefore, given an equal molar accumulation of BSH and BPA within a tumor, BSH will have delivered a 12-fold higher concentration of boron atoms compared to BPA, thereby facilitating the 20–35 ppm ^{10}B levels required for effective BNCT. However, their administration is limited because of poor water solubility and cytotoxicity [50].

The biodistribution of BSH achieved T/B ratios ranging from 0.9 ± 0.4 to 1.2 ± 0.4, T/N ratios in the range of 0.7 ± 0.1 to 3.6 ± 0.6, and boron levels in the tumor ranged from 0.7 to 84.2 ppm [20, 28, 32]. Again, this data illustrates that more selective agents are urgently needed.

Techniques to Improve BPA and BSH Uptake in Cancer Cells

Since there is a precedent of using BPA and BSH in clinical trials, techniques that increase their delivery to a tumor are a desirable concept. One common strategy is the pretreatment of cells with an amino acid analog such as L-3,4-dihydroxyphenylalanine (L-DOPA) (Fig. 2). L-DOPA is structurally similar to BPA, and both of these molecules may enter the cell through the L-type amino acid transport system [44]. It is presumed that this transport system utilizes a substrate coupled antiport (exchange) mechanism [44]. Therefore, pretreatment with a specific amino acid such as L-DOPA can improve the subsequent accumulation of BPA via this antiport mechanism (L-DOPA is exchanged for intracellular uptake of BPA). This mechanism has been validated with *in vitro* and *in vivo* studies using C6 glioma cells. Pretreatment of C6 glioma bearing rats with L-DOPA increased the BPA uptake in the tumor 2.7-fold higher than the BPA-only treated group, while accumulation in normal brain tissue did not vary significantly between the two groups [44].

In addition to L-DOPA, pretreatment of rat 9 L gliosarcoma cells with L-tyrosine (Fig. 2) resulted in a near 2-fold increase in BPA tumor uptake [43]. While pretreatment with L-tyrosine increased uptake, simultaneous administration of BPA and L-tyrosine actually resulted in decreased BPA uptake. These results further support that an antiport mechanism can be utilized to improve BPA tumor accumulation.

Another technique used to improve boron accumulation in tumors is BBB disruption by a hyperosmotic agent such as mannitol (Fig. 2). Intracarotid administration of BSH or BPA to F98 glioma-bearing rats resulted in T/N ratios of 8.2 ± 1.3 and 5.9 ± 2.0, respectively; but when combined with BBB disruption by mannitol, the BSH and BPA T/N ratios increased to 12.3 ± 4.7 and 7.5 ± 4.3, respectively [51]. Subsequent studies illustrated that co-administration of BPA or BSH with mannitol increased mean survival time of F98 glioma rats [52, 53]. However, disruption of the BBB by mannitol has nonspecific effects and may be limited since this technique can also promote boron uptake in healthy brain tissue [54]. Focused ultrasound techniques may be an alternative strategy to improve BPA uptake compared to traditional BBB disruption strategies [54].

In addition to improving tumor uptake of boronated compounds, an optimal response to BNCT depends critically on

performing the neutron irradiation at the time of maximal boron accumulation (or highest T/N ratio). One of the biggest limitations to BNCT is the ability to reliably determine when maximal boron accumulation has occurred in a patient (especially considering the variability that exists between patients). This challenge can potentially be overcome by positron emission tomography (PET) guided BNCT using a dual modality agent. The main advantage of a dual modality agent for BNCT is the ability to monitor the real-time boron accumulation within the patient's tumor. One example of a dual modality BNCT agent is 4-borono-2-^{18}F-fluoro-phenylalanine (^{18}F-BPA), a radiolabeled derivative of BPA (Fig. 2). In head and neck cancers, ^{18}F-BPA uptake significantly correlated with the uptake of ^{18}F-fluorodeoxyglucose [55]. Tumor/normal tissue ratios ranging from 1.5 to 7.8 have been reported with ^{18}F-BPA administration for numerous tumor types (malignant gliomas, malignant melanomas and various head and neck cancers) [56]. Furthermore, ^{18}F-BPA has been shown to be preferentially taken up by L-type amino acid transporter 1 in human glioblastoma cells [45]. Inhibition experiments demonstrated that BPA administration significantly decreased ^{18}F-BPA uptake, indicating ^{18}F-BPA may be a suitable imaging agent to estimate BPA uptake in glioma patients [45]. A myriad of strategies for the radio halogenation of boron clusters has been reported previously [3]. PET-guided BNCT has the potential to determine whether a patient will even benefit from BNCT, and this ultimately redefines the selection criterion of candidates for clinical trials [56].

NOVEL BORON DELIVERY AGENTS

With the modest T/N ratios achieved with BPA and BSH administration, the necessity for new boron delivery methods is obvious. The following sections aim to overview novel boron delivery systems and their relative advantages and limitations (summarized in Table I).

Nucleoside and Carbohydrate Analogs

Boronated deoxyribose derivatives have been investigated as a novel approach to improve boron uptake in tumor cells due to their higher metabolic activity [57]. These agents are a primary substrate for human thymidine kinase-1 and achieve their tumor selectivity through subsequent phosphorylations which entraps them intracellularly [58]. The first designed agents consisted of a boronic acid moiety or a carborane cage structure attached to the C-5 position of 2′-deoxyuridine (Fig. 2) [58]. A more recent generation of nucleoside derivatives are 3-carboranyl thymidine analogs, in which the carborane group is attached with a linker to the N-3 position of thymidine [58]. Barth *et al.* have demonstrated superior drug uptake of the 3-carboranyl thymidine derivative N5-2OH compared to BPA in glioma *in vivo* biodistribution studies (Fig. 2) [59]. With convection-enhanced delivery (a catheter is used to deliver drug into the tumor) BPA achieved boron tumor levels of 68.3±17.9 ppm, while N5-2OH achieved levels of 40.7±11.3 ppm. However, N5-2OH accumulated more selectively (T/N=8.5) compared to BPA (T/N=3.6).

In addition to thymidine analogs, Hosmane *et al.* have synthesized and evaluated a series of carborane-appended 5-thio-D-glucopyranose [60] and deoxyribose derivatives [57] as promising BNCT agents (Fig. 2). Previous generations of carbohydrate boron carriers commonly link the carborane moiety to the carbohydrate core using a glycosidic linkage; however, under physiologic conditions this linkage is susceptible to hydrolysis. To circumvent this stability concern, carborane-appended derivatives of 5-thio-D glucopyranose (a non-metabolized carbohydrate) and deoxyribose (containing a carbon-carbon linkage between the carborane and carbohydrate) have been evaluated [61]. While both *nido*-carborane and *closo*-carborane carbohydrate derivatives were prepared with each scaffold, it was determined that *nido*-carborane derivatives were significantly less cytotoxic compared to their *closo*-carborane counterpart. Further studies with the *nido*-carborane derivatives of 5-thio-D-glucopyranose and deoxyribose illustrated preferential accumulation of these agent in hepatocarcinoma (SK-Hep1), prostate cancer (DU-145) and bladder carcinoma (T-24) models compared to BPA, BSH or BPA/BSH treatments [61]. Additionally, treatment of a murine squamous cell carcinoma cell line (SCC-VII) with *nido*-compound illustrated a lower survival fraction compared to BPA after neutron irradiation. Further studies with carborane-appended carbohydrates may warrant clinical trials with these agents.

Carboranes linked to a DNA binding unit have also been explored as a novel boron delivery vehicle [50]. DNA targeting is achieved by the interaction of a 5,6,7-trimethoxyindole moiety with DNA, analogous to its function in the anticancer agent duocarmycin A [50]. Several hydroxymethylcarborane compounds were synthesized, and the two most promising derivatives had cytotoxicity values (ED_{50}) of 32 and 42.5 μM in human bronchial carcinoma cells (A549) and 7.5 and 10 μM in B-16 human melanoma cells. Treatment of B-16 cells with 10 μM of either hydroxymethylcarborane compound resulted in maximal intracellular boron levels of 2.3 and 3.7 ppm per 10^7 cells after just 3 hours. In contrast, a 1000 μM BPA solution required a 24 h incubation to achieve comparable levels (3.1 ppm per 10^7 cells) [50]. Numerous other classes of boronated DNA-binding molecules have been explored [3]."

Overall, 3-carboranyl thymidine analogs and carborane-appended carbohydrate derivatives have the potential advantage of intra-nuclear accumulation (DNA incorporation) that

Table I Summary of novel boron delivery systems for BNCT

Boron Delivery Vehicle	Proposed Mechanism of Accumulation	Advantages	Disadvantages
BPA	Cell membrane diffusion or uptake by L-type amino acid transporter [43].	Minimal cytotoxicity, clinical trial experience, membrane permeable.	Low percent boron composition, T/N ratio usually <4, short shelf life of fructose analog [42].
BSH	Compromised BBB due to rapid angiogenesis in brain tumor [48].	High percent boron composition, clinical trial experience, low uptake in normal brain tissue.	Net charge hinders cell membrane diffusion, cytotoxic, T/N ratio usually <4.
Nucleoside and Carbohydrate Analogs	Tumor accumulation via kinase mediated trapping [58].	Intra-nuclear accumulation (DNA incorporation) may lower dosing requirements for effective BNCT.	Human thymidine kinase-1 is cell cycle dependent; therefore treatment response may be cell cycle dependent.
Unnatural Amino Acids	L-type amino acid transporter upregulation in tumor increases uptake; accumulation occurs since cell cannot metabolize [38].	Nucleus penetration increases the probability of DNA damage (may lower dosing requirements). T/N ratios >4.	Only 1 boron atom per molecule.
Porphryins	Accumulates via endosomal accumulation [67] or from leaky tumor vasculature [66].	Boron levels determined by spectrophotofluorimetric analysis [68]. Water soluble, minimally cytotoxic, dual-modality agent. High percent boron composition.	Intracellular boron levels did not achieve 20 ppm threshold for effective BNCT [67].
Antibody-Dendrimer Conjugates	Tumor epitope recognition [69].	Potential for high T/N selectivity based on tumor epitope expression.	Tumor and brain tissue uptake limited by BBB [72].
Cationic Polymers	EPR effect and/or targeting group (i.e., cationic moiety) [73].	Delivers a high boron payload. Polymer ratio can be fine tuned for ideal pharmacokinetic profile.	Large polymer size may lead to undesirable accumulation in other organs.
Cell-Membrane Penetrating Peptides	Facilitates transmembrane transport via macropinocytosis [74].	CPP improves uptake of agents with high percent boron composition but poor intracellular accumulation.	CPP may cause nonspecific uptake in other organs, which is undesirable during neutron irradiation.
Liposomes	Leaky tumor vasculature and EPR effect [37, 76, 77]; cationic liposomes recognize membrane negative charge [79].	Stable, minimally cytotoxic vehicle [78]. Uptake ratios superior than BPA alone; insoluble drugs may be encapsulated for delivery.	Liposome size >40 nm will likely not penetrate BBB [82]. Liposomes >100 nm may be cleared by macrophages.
Nanoparticles	Enhanced permeability and retention effect of NPs [76, 77]. BPO_4 NPs have increased selectivity from folic acid receptor upregulation [83].	Facile synthesis, stable NPs. Targeting moieties can be used. Versatile incorporation of boron agents.	Systemic NPs distribution increases off-target cell death during BNCT. Some NPs too large to cross BBB.

may lower dosing requirements for effective BNCT. Additionally, the Warburg effect indicates that most tumors will have increased uptake of carbohydrates compared to surrounding healthy tissue [61]. However, one disadvantage of these agents is that their intracellular trapping is likely mediated through human thymidine kinase-1 phosphorylation; since human thymidine kinase-1 activity is cell cycle dependent, the treatment response may also be cell cycle dependent [6, 58].

Unnatural Amino Acids

It has been observed that boron derivatives of cyclic amino acids preferentially accumulate in GBM and metastatic melanoma tumors compared to BPA [62]. While decreasing the ring size (6-, 5-, and 4-membered) of cyclic amino acids has been associated with increased tumor selectivity, the overall mechanism for tumor selectivity of unnatural amino acids is still largely unknown [63]. The most promising candidate, 1-amino-3-boronocyclopentanecarboxylic acid (ABCPC, Fig. 2), has been shown to achieve a T/B ratio of 8, T/N ratio of 21, and achieved intracellular boron accumulation in cancer cells of 28 ± 7 ppm in a B-16 melanoma mouse model [38, 62]. Additionally, ABCPC is capable of penetrating the nucleus and delivering twice as much boron to T98G human glioblastoma cells compared to BPA [62]. Similarly, treatment of F98 glioma bearing rats with ABCPC achieved a T/N ratio of 5 between infiltrating tumor cells and contiguous normal brain, and this level of selectivity is significant since previous studies with BPA report infiltrating tumor/normal brain tissue ratios of 1.5–2.0:1 [38].

The advantage of the unnatural amino acids is that their ability to penetrate the nucleus may lower dosing requirements for effective BNCT and provide a high T/N ratio; however the disadvantage is that it delivers only a single boron atom per molecule.

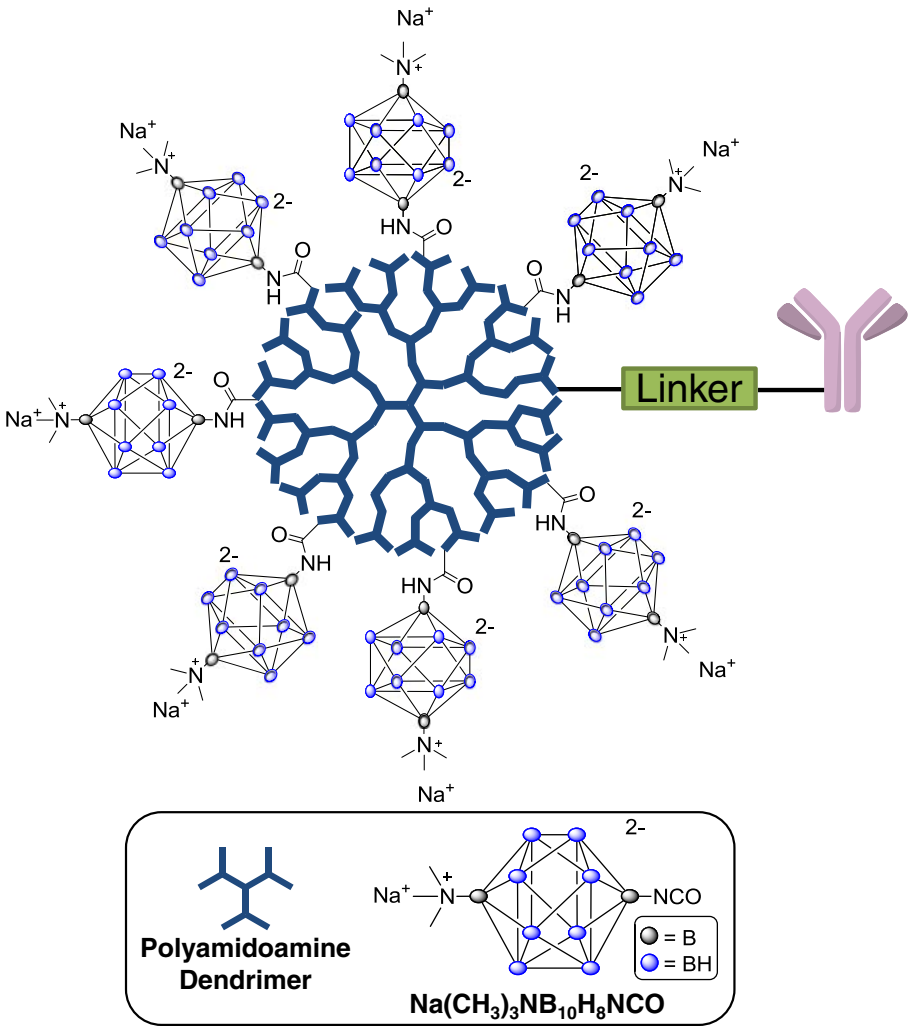

Fig. 3 Monoclonal antibody (Cetuximab or L8A4) conjugated to a boronated polyamidoamine dendrimer.

Porphyrins

In addition to their application in photodynamic therapy as a photosensitizer, porphyrins have been conjugated to boron-rich moieties for BNCT applications [64]. One such porphyrin, tetra-(4-nido-carboranylphenyl) porphyrin (H_2TCP), contains 36 boron atoms per molecule (Fig. 2) [65]. H_2TCP accumulates in tumors via leaky vasculature [66] and has an endosomal pattern of distribution [67]. Using a B16F1 melanoma mouse model, H_2TCP treatment has been evaluated against BPA-fructose [68]. Intratumoral injection of H_2TCP achieved a T/N ratio of approximately 6 with an associated tumor boron level of ~60 ppm. In comparison, *i.v.* administration of H_2TCP resulted in a T/N ratio slightly above 1 with tumor boron levels of 6 ppm. For either route of H_2TCP administration (intratumoral or *i.v.*), subsequent neutron irradiation resulted in a 5–6 day delay in tumor growth, but the most significant growth delay was observed in the BPA-fructose treatment arm.

Porphryins offer the advantages of high water solubility, minimal cytotoxicity, a high percent boron composition, and their concentration levels in biological systems can be determined by relatively simple spectrophotofluorimetric analysis [68]. However, one disadvantage is that porphyrins administered via an *i.v.* route may not be able to achieve intracellular boron levels >20 ppm for effective BNCT. In addition to BNCT, H_2TCP has demonstrated efficacy in photodynamic therapy and thus this agent should be investigated further as a potential BNCT/photodynamic therapy combination treatment regimen.

Antibody-Dendrimer Conjugates

Monoclonal antibodies (mAbs) specific for epidermal growth factor receptor (EGFR) have been investigated for the treatment of GBM due to the upregulation of EGFR in human GBM cancer cells. Two EGFR mAbs were utilized for a BNCT study: Cetuximab binds to the extracellular domain of human EGFR,

thereby competitively inhibiting epidermal growth factor from binding, whereas mAbs L8A4 specifically recognizes the oncogenic variant EGFRvIII (and not wildtype EGFR) [69]. For BNCT, each of these mAbs were conjugated to a boronated dendrimer, which was formed by conjugating methylisocyanato polyhedral borane anion $Na(CH_3)_3NB_{10}H_8NCO$ to the terminal amino groups of a polyamidoamine dendrimer (Fig. 3) [70]. The boronated antibodies Cetuximab and L8A4 were administered via convection-enhanced delivery to F98 rat gliomas (a 1:1 composition of EGFR wildtype and EGFRvIII expressing glioma cells were used to reflect patient tumor heterogeneity). Co-administration of both Cetuximab and L8A4 achieved a T/N ratio of 9.9 and boron levels of 24.4 μg/g in tumor tissue [69, 71]. Equally important, boron levels were undetectable in the blood (<0.5 μg/g). Rats with a composite tumor ($F98_{EGFR}$ + $F98_{EGFRvIII}$) that received both mAbs and subsequent neutron irradiation had a median survival time of 55 days; in contrast, rats that received only Cetuximab or L8A4 had median survival times of 38 and 36 days, respectively. This illustrates the importance of combination therapy with both antibodies for a heterogeneous glioma expressing both EGFR and EGFRvIII.

The advantages of boron-mAb conjugates are their high T/N selectivity and T/B ratios. However, one limitation is that systemic administration of mAbs will result in poor brain tumor uptake due to their limited ability to cross the BBB [72].

Cationic Polymers

Boronated cationic polymers have been used to target colon cancer polyps in a rat model. The polymers were administered locally by direct perfusion of the polymer solution into the colon lumen [73]. The boronated copolymer (Fig. 4) was constructed from three simple monomeric subunits: acrylamide forms the backbone of the polymer and was used to improve the aqueous solubility, N-acryloyl-diaminoethane served as the cationic moiety and allowed the polymer to accumulate in the negative cell surface space of the polyp, while aminophenylboronic acid was the boron source.

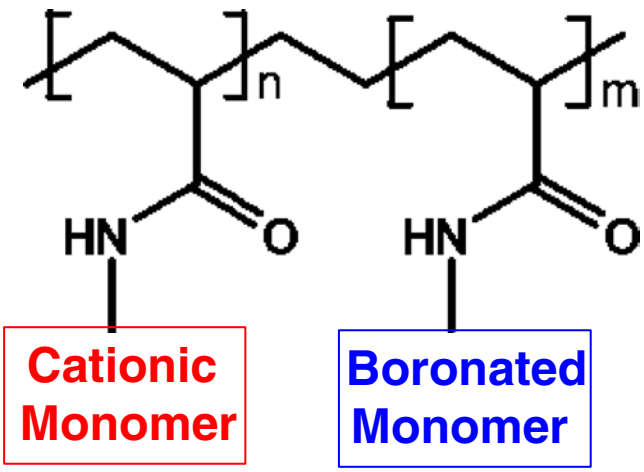

Fig. 4 Example of a boronated polymer as a potential BNCT agent: the cationic copolymer contains a ratio of acrylamide (backbone), aminophenylboronic acid (boronated monomer), and N-acryloyl-diaminoethane (cationic monomer).

The copolymer achieved polyp to surrounding tissue boron ratios of 6.57 ± 2.05, corresponding to boron levels of 88.5 ± 15.1 ppm in polyp tissue. In contrast, administration of free aminophenylboronic acid had a poor T/N selectivity of 1.23 ± 0.82. An equally important finding was that boron levels detected in the blood, lymph nodes, kidney, liver and spleen were significantly lower after copolymer administration compared to free aminophenylboronic acid, due to the low non-specific uptake of polymer in these tissues. Such a boron delivery system easily satisfies the tumor selectivity and intracellular boron level requirements for successful BNCT therapy.

Polymers have multiple advantages that make them a suitable boron delivery system for BNCT. First, copolymers can deliver a high boron load to the tumor, while having lower non-specific systemic distribution when administered directly to the intestinal tract. Second, the pharmacokinetics of a copolymer can be finely tuned by adjusting the ratio of the monomers that make up the copolymer. Additionally, copolymers may be linked to various targeting moieties to help improve their T/N ratio selectivity. The main disadvantage of polymers is that with systemic administration polymers may accumulate in filtrating organs and have low penetration across the BBB in the case of treating glioma.

Cell-Membrane Penetrating Peptides

Although agents like BSH are desirable for BNCT due to their high percent boron composition, BSH does not readily cross the cell membrane [74]. To overcome the poor intracellular accumulation of BSH, a cell-membrane penetrating peptide (CPP) was tethered to a peptide dendrimer containing BSH molecules (BSH-dendrimer-CPP, Fig. 5) [74]. *In vitro* studies using U87 glioma cells showed that treatment with BSH alone yielded intracellular boron levels of 15.9 $ng^{10}B/10^6$ cells. In contrast, treatment with the BSH-dendrimer-CPP reached boron levels of 5623.7 $ng^{10}B/10^6$ cells, a drastic increase in intracellular accumulation of BSH. This improved cellular uptake is in part attributed to the positively charged arginine rich portion of the CPP (11 arginine residues long) which is thought to promote intracellular accumulation of anionic BSH. Additionally, *in vivo* studies using U87 glioma cells injected into the striatum of nude mice followed by BSH-dendrimer-CPP *i.v.* injection in the tail vein showed preferential accumulation of BSH only in the tumor center and edge; BSH-dendrimer-CPP was not detected in the normal brain area on high magnification by confocal microscopy [74].

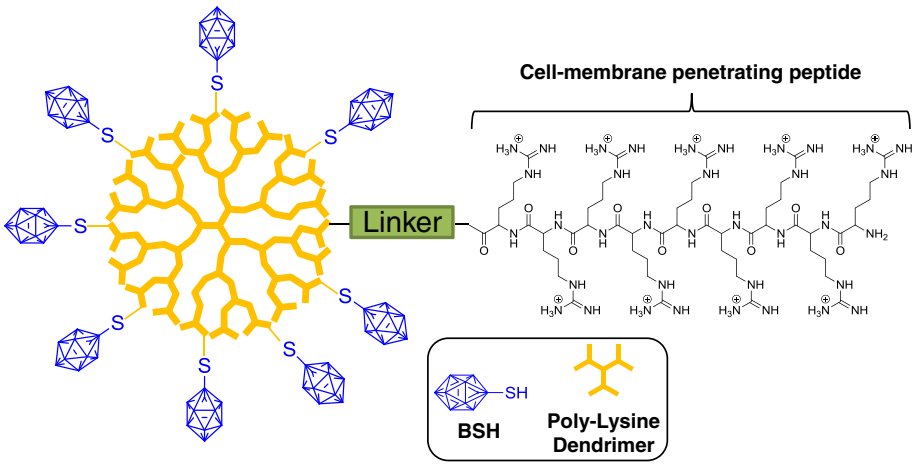

Fig. 5 Schematic representation of BSH containing dendrimer with a cell-membrane penetrating peptide.

The advantage of a CPP is that it improves the uptake of agents with a high percent boron composition that typically have poor intracellular accumulation; however, a CPP may cause nonspecific uptake in other organs, which is undesirable during neutron irradiation.

Liposomes

Liposomes have been investigated as potential delivery vehicles for BNCT. Liposomes are closed phospholipid bilayers that can encapsulate a drug of interest (Fig. 6) [75]. Even without cell targeting, liposomes can improve drug delivery and reduce cytotoxicity of select agents (i.e., Doxorubicin) [75]. Like nanoparticles, the liposome surface can also be modified to include targeting moieties. Liposomes are believed to accumulate within tumors because of local vasculature leakage known as the enhanced permeability and retention (EPR) effect [37, 75]. A majority of solid tumors have a defective blood vessel architecture coupled to an extensive production of vascular permeability factors [76, 77]. This combination contributes to the EPR effect and facilitates the transport of macromolecules into the tumor. Specifically, macromolecules greater than 40 kDa can selectively leak out from tumor vessels and thereby are retained in the tumor tissue [76].

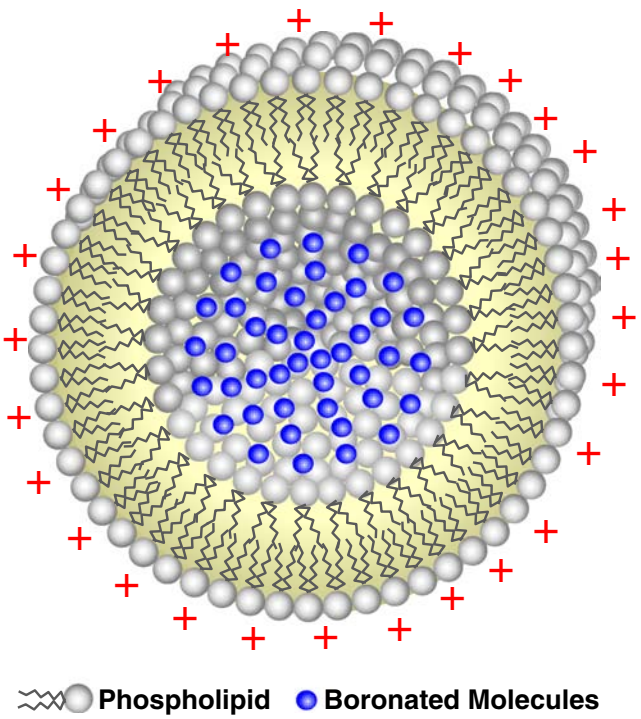

Fig. 6 Positively charged liposomes loaded with boron containing molecules.

Intratumoral liposomal accumulation relies significantly on the EPR effect. Phosphatidylcholine and dimyristoylphosphatidylcholine liposomes have recently been investigated for delivering the bis-*nido*-carborane dequalinium salt ($B_{18}C_{34}N_4H_{64}$) [78]. This carborane is a delocalized lipophilic cation, and it is reported to selectively accumulate in the mitochondria of a tumor cell. These liposomes exhibited suitable stability (zeta potentials of −10 mV) and encapsulation of the bis-*nido*-carborane in a liposome significantly reduced its cytotoxicity compared to its free administration in U87 glioma cells.

Liposomal efficacy of delivering the carborane agents *o*-closocarboranyl β-lactoside (LCOB) and 1-methyl-*o*-closocarboranyl-2-hexylthioporphyrazine (H_2PzCOB) with varying liposomal compositions using cationic, anionic, and zwitterionic lipid formulations has also been investigated [79]. Boron accumulation in DHD/K12/TRb rat colon carcinoma and B16-F10 murine melanoma cells was assessed by alpha spectrometry compared to BPA. While BPA treatment alone of DHD and B16-F10 cells showed uptake ratios of 0.07 and 0.2 respectively, cationic liposomes loaded with LCOB had uptake ratios of 4 and 20, respectively. Furthermore, cationic liposomes containing H_2PzCOB had an uptake ratio near 10 in DHD cells, indicating improved uptake compared to cationic LCOB liposomes in this cell line. Cationic

liposomes had superior uptake compared to their anionic and zwitterionic counterparts, presumably due to their preferable interaction with a negatively charged mammalian membrane.

While liposome encapsulation can improve the delivery of BNCT agents, only a limited amount of boron can be contained within the liposome interior. To increase the potential boron payload to tumors, Hawthorne *et al.* have designed a liposome system containing boron not only in the aqueous core but also in the bilayer membrane [80]. The ammonio derivative $Na_3[1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8]$ is encapsulated into the aqueous core, while the liposome bilayer contains the lipophilic agent $K[nido\text{-}7\text{-}CH_3(CH_2)_{15}\text{-}7,8\text{-}C_2B_9H_{11}]$. After *i.v.* administration (2 injections 24 h apart) of these liposomes into BALC/c mice containing EMT6 tumors (mouse mammary adenocarcinoma), a T/B ratio of 5.68 with a tumor boron concentration of 43 ppm was obtained 96 h post-injection. These liposomes have also shown promising potential in a hamster cheek pouch oral cancer model [81].

Liposomal encapsulation of BNCT agents offers several potential advantages. Liposomes are able to improve the uptake and selectivity of delivering boron to tumors; additionally, drugs with poor water solubility can be more suitable for administration within a liposome formulation. However, liposomes may have limited clinical potential for brain tumors because of size constraints. Only liposomes with diameters less than approximately 40 nm are able to penetrate the BBB adequately; liposomes larger than 100 nm may be taken up by macrophages and may be trapped in filtrating organs [82].

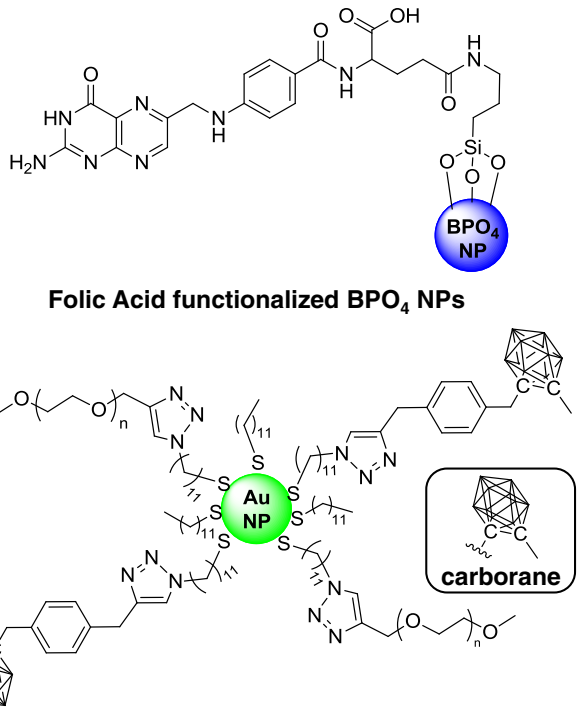

Fig. 7 Several nanoparticles investigated for BNCT: Boron phosphate nanoparticles functionalized with folic acid and gold NPs functionalized with PEG and carborane.

Nanoparticles

Recent studies have investigated using nanoparticles (NPs) as a boron delivery system. Boron agents can be readily incorporated into nanoparticles via surface adsorption, encapsulation or direct covalent linkage [83]. Analogous to liposomes, NPs rely significantly on the EPR effect for tumor accumulation. NPs may accumulate and be utilized to treat GBM and other brain tumors due to the compromised integrity of the BBB [84].

Boron phosphate NPs linked to folic acid have been proposed as a novel strategy for boron delivery (Fig. 7). Non-functionalized boron phosphate NPs induced erythrocyte hemolysis and platelet aggregation, while these same NPs functionalized with folic acid did not exemplify significant hemolysis or platelet aggregation, suggesting these NPs can be a suitable boron delivery system [83]. Additionally, the cytotoxicity of boron phosphate NPs containing folic acid was compared to BPA in both DHD rat colon adenocarcinoma and UMR rat osteosarcoma. It was determined that these NPs had comparable cytotoxicity to BPA and thus should be strongly considered as a new carrier for BNCT [83].

Carboranes linked to polyethylene glycol coated gold NPs may benefit from polyethylene glycol's enhanced permeability and retention effects [85, 86]. Gold NPs were assembled starting with azido-terminated gold NPs followed by "click" chemistry with the corresponding PEG-alkyne (2000 MW) and carborane-alkyne (Fig. 7) [85]. The aforementioned NPs have hydrodynamic diameters ranging between 10 and 16 nm, thereby satisfying therapeutic size requirements; typically NPs less than 10 nm in diameter are freely filtered by the glomerulus, whereas NPs >100 nm are removed by macrophages [85, 86]. The aforementioned NPs strategies have promise as potential BNCT agents.

Similar to liposomes, the advantage of NPs loaded with boron is improved intracellular uptake and selectivity to tumors; however, NPs may have limited clinical potential for brain tumors because they are unable to penetrate the BBB adequately, and also they may be trapped in filtrating organs.

One of the main barriers facing drug delivery with particulate delivery vehicles such as liposomes and NPs is uptake by the reticuloendothelial system (RES). Uptake of particles by the RES is in part mediated by the liver, spleen and lungs [87]. Increasing particle hydrophobicity or size is often correlated with increased uptake by the RES. Marcophage mediated clearance of particles can be minimized by creating particles with a hydrophilic surface and a diameter less than 100 nm [88]. Coating particles with hydrophilic polymers and regulating particle size are some strategies used to overcome uptake by the RES.

CONCLUSION

BNCT agents have made considerable advances since the initial BNCT studies utilizing boric acid as the boron carrier. Although BPA and BSH are approved agents for BNCT clinical trials, their modest T/N ratios encourage the development of more selective agents. Improving the T/N ratio not only indicates a more selective agent, but this critical factor should minimize off target tissue damage and translate into prolonged patient survival. Boron-containing liposomes, polymers, monoclonal antibodies and nanoparticles are just a few of the presented strategies that can be used to improve BNCT efficacy. Patient populations must be carefully selected for BNCT trials, and it is vital that the pharmacokinetics of the boron agent are well known on a patient-to-patient basis. To insure maximal therapeutic response, neutron irradiation should occur during the peak T/N ratio. To assist this goal, agents that are readily detectable in patients with noninvasive methods (i.e., imaging modalities) may play a prominent role in future BNCT studies. With the current precedent of using co-administration of BPA and BSH in clinical trials, one must consider this principle and apply it to our next generation agents. Most importantly, BNCT may achieve the best clinical results in combination with surgical resection and/or chemotherapy. BNCT remains a viable treatment modality that warrants further investigation to help provide answers for those affected by cancers with no answer.

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