BORON NEUTRON CAPTURE THERAPY: DELIVERY AGENTS USED IN BORON ADMINISTRATION

Galeş N. Laurentia¹, Anghel M. Rodica²

¹ University of Medicine and Pharmacy “Carol Davila” Bucharest

Abstract. Boron neutron capture theory (BNCT) is a binary therapy applied in malignant tumours, resistant to other treatment methods, namely chemotherapy and radiotherapy. One of the main components is Bor-10, a stable isotope that can be inserted in the tumour cells by using specific transporters. Through the use of adequate Boron compounds, that preferably are located in the tumour cells and not in the healthy tissue, Boron neutron capture therapy provides a higher curative potential with minimal toxicity, at the level of normal tissues. In order to obtain a proper tumour control with minimum side effects, 10B increased concentrations should be obtained by selective cumulation within the tumour cells, with an adequate ion gradient between the normal and tumour tissues. Therapeutic effectiveness can be reached by a minimal boron concentration of 20-30 ppm inside the tumour and a tumour/blood and tumour/normal tissues boron concentration gradient of approximately 3. So far, clinical practice has used two main boron delivery agents: sodium borocaptate (Na₂B₄H₁₁SH) (BSH) and a phenylalanine dihydroxy-boryl derivative, known as Boronophenylalanine (BPA). During the past 20 years, along with the development of new techniques of chemical synthesis and improved knowledge of biological and biochemical requirements for the effectiveness of an agent and ways of boron delivery, a new number of boron delivery agents have appeared in response to the agents up to now. They include: aminoacids, nucleic acid precursors, DNA-binding molecules, porphyrin derivatives, high-molecular-weight delivery agents, macromolecules or nanovehicles such as monoclonal antibodies or monoclonal antibody fragments that bind to tumour epitopes- EGF, VEGF or liposomes, dendrimers, dextran, polylysine, avidin, folic acid, etc. Next to the identification and study of new boron compounds or new compound combinations, other delivery methods are currently under research seeking to improve the classical intravenous administration and the selective intratumoral binding process as well as the therapeutic index (intraarterial, intratumoral administration, etc). Despite a large number of ongoing studies, a boron compound that fits all the ideal BNCT compound criteria has not yet been identified, especially in terms of tumour selectivity, the ratio of concentrations between the concentration in the tumour cells and the one in the normal cells, the delivery of a proper number of boron atoms in the tumour cells, hydrophilicity and lipophilicity, homogeneous intra-tumour distribution. The development of BNCT boron delivery agents has been initiated approximately 50 years ago and continues to represent at the same time a priority and a difficulty. Several bor-10 boron based pharmaceutical products have been prepared in view of their potential use in BNCT.

Key words: Radiotherapy, BNCT, boron compounds.

Introduction

Boron neutron capture theory (BNCT) is a binary therapy assessed for the cases of resistant malignant tumours to other treatment methods, namely chemotherapy and radiotherapy. Neither of the two therapeutic compounds, taken separately, has consistent effects on the tumour but their combination triggers a lethal effect for the tumour cells with a minimal damage on the healthy tissue. One of the compounds - Bor-10 - a stable isotope can be concentrated in the tumour cells by applying a specific set of transporters. The other compound is a beam of low-energy neutrons. The capture of 10B neutrons produces alpha energy particles, with a linear energy transfer at the level of the tissue. Hence, this generates effective cell destruction and a relatively higher biological efficiency than the
photons. By using the adequate Boron compounds that preferably bind to the tumour cells and not to the healthy tissue, Boron neutron capture theory provides a better curative potential with minimal toxicity for the normal tissues. [1, 2]

In order to obtain effective tumour control with reduced collateral effects, $^{10}$B increased concentration must be reached by accumulating it selectively within the tumour cells, with a sufficient ion gradient between the normal and tumour tissues. Subsequently, the patient undergoes radiation therapy with adequate energy and intensity neutron beams so that the maximum density of the thermal or epithermal neutrons be reached within the proximity of the tumour.

A productive therapy relies on a minimum boron concentration of 20-30 ppm inside the tumour and a normal boron concentration in the tumour/blood and tumour/normal tissues of approximately 3.

The substances used in BNCT boron administration must meet the following essential criteria:[1, 3, 4]

- An increased boron content (the natural abundance is around 20%);
- No systemic toxicity;
- Selective caption at the level of the tumour. The selectivity conditions the tumour/normal tissue therapeutic index; tumour concentrations ratio: the blood must be 3-4:1;
- Efficient boron transporters;
- The absolute boron content is essential while boron tumour concentration should be approximately $\sim 20 \mu g$ $^{10}$B/g per tumour. Together with the thermal neutrons flow, boron content establishes the necessary exposure time targeted to reach the prescribed physical dose and ratio from the total dose delivered to the boron compound. The compound must have a rapid blood and tumour clearance and should persist, nonetheless, in the tumour cell throughout BNCT;
- Homogeneously captured by the target.

Currently not a single boron delivery agent fulfils all the above mentioned criteria.

$[^{10}B]$ absolute value in the tumour, healthy tissue and blood are critical elements for BNCT.

For similar tumour/tissue or tumour/blood ratios, higher boron concentrations are more beneficial due to their permissiveness in terms of a lower exposure time, hence a reduced background irradiation associated dose.

An agent can hardly trigger all the tumour cells or at least, the largest part of them.

Combined administration of different boron compounds with specific properties and complementary absorption mechanisms would increase BNCT’s therapeutic advantages by merging tumour control mechanisms that aim distinct tumour populations and the reduction of cell toxicity that limits the dose.

The first association of this sort has been detected between the first two used agents, namely BPA and BSH which led to promising results in what concerned the improvement in the tumour/normal tissue boron index [5].

Another studied association was BPA with GB-10. BPA is selectively captured in the tumour but presents an intra-tumor inhomogeneity capture in viable/necrotic areas of $1.46/1$. GB-10 ($Na_{10}B_{12}H_{12}SH$) cannot cross the intact blood-brain barrier, but it will however deposit itself, homogeneously, inside the tumour with a 0.97/1 viable/necrotic areas ratio.

The concern with these cases of association is the difficult dosimetry, especially if a high weight and low weight molecular compound conflate.

**Boron delivery agents:**

So far, clinical practice has relied on two main boron based drugs: sodium borocaptate ($Na_{10}B_{12}H_{12}SH$) (BSH) and a phenylalanine dihydroxy-boryl derivative, known as Boronophenylalanine (BPA) [6, 7, 8, 9, 10, 11], a third one GB-10 ($Na_{10}B_{12}H_{12}SH$), being recently approved by FDA. [12]

During the past 20 years, along with the development of new techniques of chemical synthesis and improved knowledge of biological and biochemical requirements for the effectiveness of an agent and ways of boron delivery, a new number of boron delivery agents has appeared in response to the agents used so far (examples in Table I). [13]

They include: aminoacids, nucleic acid precursors, DNA-binding molecules, porphyrin derivatives, high-molecular-weight delivery agents, macromolecules or nanovehicles such as monoclonal antibodies or monoclonal antibody fragments that bind to tumour epitopes- EGF, VEGF or liposomes, dendrimers, dextran, polylysine, avidin, folic acid, etc. [14, 15].

<table>
<thead>
<tr>
<th><em>Boronophenylalanine</em> (&quot;BPA&quot;)</th>
<th>* Sodium borocaptate (&quot;BSH&quot;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecaborate cluster lipids and cholesterol derivatives</td>
<td>Carbamoryl nucleosides</td>
</tr>
<tr>
<td>&quot;GB10&quot; ($Na_{10}B_{12}H_{12}SH$)±</td>
<td>Carbamoryl porphyrins</td>
</tr>
<tr>
<td>Cholesteryl ester mimics</td>
<td>Boronated EGF(epidermal growth factor) and anti-EGFR mAbs</td>
</tr>
<tr>
<td>Boronated DNA metallo-intercalators</td>
<td>Boron-containing nanoparticles</td>
</tr>
<tr>
<td>Transferrin–polyethylene glycol (TF–PEG) liposomes</td>
<td>Carbamoryl porphrazines</td>
</tr>
<tr>
<td>Unnatural amino acids</td>
<td>Boronated cyclic peptides</td>
</tr>
<tr>
<td>Dodecahydro-closododecaborate clusters</td>
<td>Boron carbide particles</td>
</tr>
</tbody>
</table>

* The only boron delivery agents used by clinical practice. 
+ FDA approval

Tabel I. Boron delivery agents with low and high molecular weight that are currently used or under process of approval [16]
The chemical structure of the main boron delivery agents that are under assessment is presented in figure 1:

Presently the medical community use BPA (compound 1) and BSH (compound 2).

GB-10 (compound 3) recorded promising results on animal tests as well as nucleosides derivatives β-5-o-carboranyl-2’-deoxyuridine (D-CDU compound 4) and N5-2OH (compound 5). Compound 6, a trimethoxyindole derivative has been under research in vitro while compound 7, H2DCP, a porphyrin derivative, has shown tumour selectivity. Maltose derivative, compound 8, has demonstrated at the in vitro experiments, reduced cytotoxicity and tumour capture; compound 9, a biphosphonate, presented tumour selectivity and dequalinium-B derivative (DEQ-B, compound 10) has registered promising results at the in vitro studies. [1, 17, 12, 18, 19]

Next to the identification and study of new boron compounds or new compound combinations, other delivery methods are currently under research in order to improve the classical intravenous administration and the selective intratumoral binding process as well as the therapeutic index (intraarterial, intratumoral administration, etc). [14]

We will now proceed to briefly describe the main agents and ways of boron delivery.

**First and second generation boron delivery agents**
In the 1950s and early 1960s the first boron agents used in BNCT were boric acid and some of its derivatives. However, they were non selective agents, presenting poor tumour retention and a reduced tumour/tissue therapeutic index. [20, 21] During the 1960’s, second generation agents entered in trial, one that was aryl boronic acid based, BPA [22] and another one, based on polyphedral boranes and sodium mercaptoundecahydro-closo-dodecaborate, known as BSH [23]. Second generation compounds have low toxicity, persist longer inside the tumour tissue and have a supraunitary tumour/brain and tumour/blood therapeutic index. [24, 25] BPA has been improved with 10B and mixed with fructose in order to obtain better hydrosolubility and, along with BSH, has been used for both brain tumours as well as for other tumours. [26].

**Third generation boron delivery agents**
The third generation compounds consist mainly of a group of stable boron compounds attached to a high tumour affinity fragment through a hydrolitic stabilised bond (low weight molecular biomolecules or monoclonal antibodies). In this sense, EGFR targeting and the mutant EGFRvIII isoform overexpressed in brain tumours and squamos cell carcinoma of the head and neck actually represented such an approach. [27] Tumour cell nucleus and DNA are attractive targets due to the fact that the necessary boron amount to produce a lethal effect can be substantially reduced if it is localised inside or close to the nucleus. [28] Organelles (endoplasmic reticulum, mitochondria, lysosome, etc) are also a potential target. Hydrosolubility is an important property in the systemic administration, while lipophilia allows the crossing of blood-brain barrier and intratumoral diffusion making amphiphilic products the most studied within their class. Another possibility to cut across the blood-brain barrier is the incorporation of delivery agents such as liposomes which would make amphiphile unnecessary. Molecular weight is another factor that could determine the intratumoral diffusion rate.

**a. Low molecular weight agents**
Boron aminoacids and polyphedral boranes: several boron aminoacids have been assessed—cysteine, tyrosine, aspartic acid, alanine, methionine and glycine as well as non-natural amino acids [29-34]. The advantage is that compared to BPA they have increased boron content, reported at their weight, thus being able to deliver more intratumoral boron without causing consecutive increase in toxicity. One of the non-natural amino acids, 1-amino-3-borono-cyclopentanecarboxylic acid (ABCPC) is worth mentioning because it has registered blood/tumour concentrations of 8 and tumour/normal tissue of 21 on melanoma murine models, a highly advantageous ratio compared to the usual boron compounds. [35] Several polyphedral boranes were also evaluated one of them being: decahydro --closo decaborate -- dianon B10H102. The simple sodium salt in this compound, the one known as GB-10 has reduced systemic toxicity and tumour selectivity which enabled FDA to approve its use in glioblastomas and melanomas [12]. Other polyphedral boranes, although with a proper boron content, have reduced tumour selectivity which qualifies them for liposomal encapsulation. [36, 37] Biochemical precursors and DNA binding agents have been tested on animal models: nucleic acid analogues, purine, pyrimidine, nucleosides, nucleotides,
of boron atoms for each immunoconjugates molecule (up to 1000 boron atoms). The first compounds proved to have high affinity especially for the liver and spleen. [65, 66, 69, 83]

An alternative to binding of boron enriched dendrimers is their connection with receptor ligands, as for example EGF, with the clear benefit of a lower molecular weight compared to the antibody bioconjugation. [83, 92]

Merging dendrimers with anti-folate receptor

The folate receptor (FR) is overexpressed in various cancers: ovarian, pulmonary, mammary, endometrial and renal cancers [93-95]. Folic acid (FA) is a vitamin that is transported to the cell by FR mediated endocytosis. Binding of folic acid to other molecules by grouping carboxil does not have an impact on endocytosis. [96]. Folic acid was studies as a intracellular transporter vector for a series of substances: cytostatics, toxins, enhnacing agents, liposomes, immunotherapeutic agents, etc. Coupling with a boron-enriched dendrimer can also render it attractive for BNCT [97].

An alternative method to deliver born compounds through dendrimers is to incorporate Carboron boron sites within the dendrimers, this resulting in a hydrosoluble compound that has the ability to deliver a sufficient number of boron atoms. [98]

**Liposome incorporated delivery agents**

Liposomes are non-toxic, biodegradable vesicles that have been used to supply both hydrophilic and hydrophobic agents [99]. Boron liposomes stand as another possibility for boron delivery [100-103]. Due to their incapacity to break the blood-brain barrier as a consequence of their dimensions- >50 nm they are less attractive for brain tumours and more beneficial for liver tumours [102, 104, 105]. Rapid growth tumours due to structural deficiencies in the neoformation vessels are the pre-elected target of liposomes or other nanoparticles. PEGylation or the attachment to antibodies increases the tutor selectivity as well as the circulation time. [106]. Liposomal encapsulation extends the time for elimination by the reticuloendothelial system, thus maintaining sufficient intratumoral quantities- from 3 to 6 hours even to 24 hours from the systemic administration. Hence, BNCT administration is rendered easier, especially if irradiation is performed remotely. [108, 109]

Polyphedral boranes and carbonades can also be encapsulated into the liposome. This generates compounds with multiple boron atoms that are resistant to metabolic degradation and are hypophilic, thus enabling a simpler penetration of the tutor cell membrane [110].

The attachment of molecules with affinity for certain cell epitopes that engenders immunoliposomes facilitates a selective delivery of the boron to the tumour. Similar to dendrimers, liposomes may couple with monoclonal antibodies (anti CEA [111, 112] or anti HER-2 [113] as an example), with EGF [114-116], or FR [117]

**Dextran based delivery agents**

Dextrans represent glucose polymers used as carriers for various drugs or proteins to increase their
circulation time. Considering that their chemical structure has been modified, dextrans can now be applied for targeting the tumour cells. [118, 119]. In order to bind boron to dextrans B-decachloro-o-carborane derivatives were developed, where one of the carbon atoms was substituted with a -CH₂CHOHCH₂-O-CH₂CH₂CH₂ group, thus reaching a boron content of 4.3% [120, 121]. Subsequently, the modified dextran is coupled with specific anti-tumour antibodies. [121-124] However, bioconjugates intratumoral capture is reduced in the systemic administration, intratumoral application being the considered option. [27]

In the case of liver tumours, the association of a classical boron delivery agent, BPA or BSH with lipiodol has been studied with the conclusion that there is sufficient intratumoral boron capture for BNCT application. Intra-hepatic artery administration scaled up following this selectivity [125].

Other molecules used for BNCT boron delivery were polylysine (also coupled with antitumor antibodies), unimolecular nanovehicles (e.g. dodecahydro-closododecaborate clusters), etc [68, 126-128].

Conclusions

Although BNCT is an innovative and promising treatment method, still several aspects continue to be critical which prevents it from being applied on a larger scale. BNCT’s success depends on the boron delivery compound, the quality of the neutrons’ beam, dosimetry and the experience of the medical staff. The first problematic aspect regards the boron delivery compound. Despite numerous ongoing studies, so far, a boron compound that fits all criteria for the ideal BNCT compound is yet to be identified, especially in the areas of tumour selectivity, ratio concentration between tumour and normal cells, the delivery of a sufficient number of boron atoms to the tumour cells, hydrophilicity and lipophilicity, homogeneous intratumor distribution. The development of boron delivery agents for BNCT has begun 50 years ago and continues to be a difficult issue but a priority, nonetheless. Several bor-10 boron-based pharmaceutical products have been prepared in view of their potential application in BNCT. [1, 17]

References

3. Junjie Huang - Boron Neutron Capture Therapy for Cancer Treatments - A dissertation submitted to the Department of Physics, University of Surrey, in partial fulfillment of the degree of Master of Radiation and Environmental Protection - Department of Physics, Faculty of Electronics & Physical Sciences, University of Surrey - July 2009, p 1-25.
23. Soloway AH, Hatanaka H, Davis MA. Penetration of brain and brain tumor. VII. Tumor-


1. Therapeutics, Pharmacology and Clinical Toxicology


5. 2000, 357, 107-17.


17. 2003, 29, 361-70.


34. 1990, 126, 95-102.


37. 2000, 357, 107-17.