The delivery of carboranes in the BNCT of cancers

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Presentation Overview

- Boron Neutron Capture Therapy (BNCT)
- Applications
- The Chemistry of BNCT
- Requirements
- Limitations
  - Cancer selectivity
  - Formulation
Background

BNCT:

- Targeted chemo-radiotherapeutic technique.

- Mainly used for the treatment of cancers in scarcely accessible sites of the body, e.g. brain, but also neck, liver, skin.

- Main application is treatment of Glioblastoma Multiforme (highly invasive, low survival rate 6/12 months)

- Early-stage clinical results (1940s-50s) were a disaster.

- Some successes have later been observed.

- Currently requiring input at all levels for development.
Birth of NCT

- 1932/1934 - J. Chadwick discovers the neutron and E. Fermi observes that some nuclides (such as $^{10}$B and $^{6}$Li) are able to absorb thermal or slow neutrons.

- 1936 - Locher laid the foundations for the development of BNCT by proposing the “use of strong neutron absorbers into the regions where it is desired to liberate ionization energy”

- 1940s - Linear Energy Transfer (LET) concept is introduced: number of ionizations per unit distance (densely ionising radiation can cause genetic damage). Radiation is often described as low LET or as high LET, the former being more effective at killing cells.
Why Boron?

- “Neutron-capture cross section” ($\sigma_{th}$) is the probability of capturing a neutron.

- Nuclides with large $\sigma_{th}$ values may be suitable for use in NCT.

- Boron has been widely studied because represent a good compromise between $\sigma_{th}$, toxicity and ease of chemistry.

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>$\sigma_{th}$ (b=barns)</th>
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<tbody>
<tr>
<td>$^1$H</td>
<td>0.333</td>
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<tr>
<td>$^6$Li</td>
<td>941</td>
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<tr>
<td>$^{10}$B</td>
<td>3838</td>
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<tr>
<td>$^{12}$C</td>
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<td>$^{14}$N</td>
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<tr>
<td>$^{16}$O</td>
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<tr>
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<td>254000</td>
</tr>
<tr>
<td>$^{199}$Hg</td>
<td>2150</td>
</tr>
<tr>
<td>$^{235}$U*</td>
<td>681</td>
</tr>
</tbody>
</table>

Taylor HJ, *et al.* *Nature* 1935; **135**: 341-8
Capture Reaction

\[ {^{10}\text{B}} + {^1\text{n}} \rightarrow [^ {11}\text{B}] \rightarrow ^4\text{He} + {^7\text{Li}} + 2.79\text{MeV (6.3\%)} \]

\[ ^4\text{He} + {^7\text{Li}} + {\gamma}\text{0.48MeV} + 2.31\text{MeV (93.7\%)} \]

▶ Energetic alpha particles produced by the interaction of $^{10}{\text{B}}$ with neutrons have high LET
BNCT

- Healthy cell
- Tumour cell
- $^{10}\text{B}$ = Boronated drug

$^{10}\text{B}$ Selective delivery → $^{10}\text{B}$ Irradiation → $^{1n}$ Tissue repair

- Non-toxic
- Selectively cytocidal
- Short path length (~1 cell diameter)**


**Schiffer D, Pilkington GJ. Mikkelsen/Liss, editors. 1998; pp. 161-84.
During Irradiation

- During Irradiation, $^7$Li nuclei and α particles are produced.
- It takes only a few α particles to kill a malignant cell.*
- Inevitable capture reactions that involve $^1$H and $^{14}$N from normal tissue produce γ rays and protons, respectively, but the $\sigma_{th}$ for these nuclei are too small to be of concern in cancer therapy.**
- $^{10}$B accumulated in the cell nucleus has little effect on neighbouring cells and it is much more efficient in cell killing (2.5 times the dose) than the same amount of boron distributed uniformly within the whole cell.***

Requirements

The drug should:

- be systemically non-toxic;
- be selective in targeting tumour cells in the presence of normal ones;
- persist intra-cellularly, especially during the course of neutron radiation;
- deliver an amount of $\sim10^9 \, ^{10}\text{B}$ atoms/cell;
- achieve tumour/normal tissues ratios higher than 3;
- be able to penetrate biological barriers, such as the BBB.

1950s: BNCT trials

- 1951: Brookhaven Graphite Research Reactor
- 1951: W. Sweet, Chief of Neurosurgery at the Massachusetts General Hospital initiates BNCT clinical trial
- 1959: Brookhaven Medical Research Reactor built within the National Research Center and nearby Hospital

Reasons for failure:
- Poor penetration of thermal neutrons in tissue.
- Boron levels in blood higher than those in tumor.
- Excessive damage to healthy tissues.
1950s: BNCT Trials - chemistry

- Sodium borate
- Sodium pentaborate

- considerable toxicity
- considerable side effects
- no selectivity
- charge makes difficult to cross BBB
In the early 1980s Mishima taking advantage of BPA’s structural similarity to melanin precursors (tyrosine analogue), started a program of research on the applications of BPA in the treatment of melanomas. By the early 1990s, BPA was used for the treatment of brain tumours.

BPA

- low toxicity
- used as fructose complex (BPA-F)
- low side effects
- good pharmacokinetic profiles
- BPA concentrates in tumor to levels 3.5 - 4 times higher than blood or brain
- but the most important advantage is that it is able to cross the BBB at some extent

BPA: Biodistribution

- BPA is characterised by advantageous tumour/blood ratios
- One $^{10}$B atom per molecule
- To achieve suitable $^{10}$B dose, high doses of BPA are required
- It is ideal to have more $^{10}$B atoms per molecule = reduced dose = reduced side effects

BPA: Clinical Trials

- BNCT clinical trial for GBM in Sweden evaluating 6-hour BPA infusions.
- MIT clinical trials now open for GBM or melanoma metastatic to the brain and cutaneous melanoma.
- Other BNCT clinical trials: Finland, Japan, The Netherlands, Czech Republic.

- BNCT for recurrent GBM
- Combinations:
  - BPA-F + another boron compound (GB-10, BSH, CuTCPH, BOPP)
  - BPA-F + radiosensitizer
  - BPA-F + photons or radiosurgery
Requirements

The drug should*:

- be systemically non-toxic;
- be selective in targeting tumour cells in the presence of normal ones;
- persist intra-cellularly, especially during the course of neutron radiation;
- deliver an amount of $\sim 10^9$ $^{10}$B atoms/cell;
- achieve tumour/normal tissues ratios higher than 3;
- be able to penetrate biological barriers, such as the BBB.

High Boron Content
-Polyhedral boranes-

Fairly low toxicity
- 12 atoms of $^{10}$B per molecule (reduce dose)
- Inert to metabolism
- Do not cross the BBB (requires formulation)


dimercaptooctachlorodecaborate and mercaptoundecahydrododecaborate (BSH) anions
BSH: Clinical Trials

- Associated with Prof. H. Hatanaka at the Hitachi Training Reactor (HTR) in Japan from 1968 to 1985.
- Craniotomy followed by slow infusion of BSH into the residual tumour. Half a day later, the residual tumour was irradiated by thermal neutrons at a nuclear reactor.
- 2 years survival rate was 11.4%, 5 years survival rate was 10.4%, and 10 years survival rate was 5.7% among the treated grade-4 GBM patients.*
- Hatanaka’s clinical research resulted in a successful treatment of grade III and IV gliomas, with several long-term survivors, including a man who survived for more than 24 years.**


BSH: Clinical Trials

Nakagawa treated 130 grade-4 GBM patients by using similar processes to Hatanaka at Musashi Institute of Technology Reactor (MuITR) and Japan Atomic Energy Research Institute Reactor (JRR).*

They reported the results of 21 months median survival time of patients after the treatment, which was better than Hatanaka's results.

In 2004, the Japan Atomic Energy Agency (JAEA) treated 7 GBM patients.

They used BSH and epithermal neutron beams.**

The median survival time was 20.7 months.


BSH + BPA: Clinical Trials

- Intracarotid injection of BPA (250 mg/Kg) + BSH (30 mg/Kg) has been shown to result in a two-fold increase in tumour boron concentration, as compared with intravenous administration.

- This, together with mannitol-induced BBB disruption, has been shown to effect a fourfold increase in tumour boron concentration in F98 glioma rats.

- This resulted in a twofold increase in mean survival time (MST) in rats, compared with a standard mannitol-free control.

- With this technique a 25 % cure rate at one year was observed!

Clinical $^{10}$B Agents

BPA

BSH
High Boron Content

-Carboranes-

Low toxicity
10 atoms of $^{10}$B per molecule (reduce dose)
Presence of carbon atoms (easy chemistry)
Inert to metabolism
Do not cross the BBB (requires formulation)

Anionic *nido*-Carboranes

- Easy to produce
- Charged
- Allows change of physicochemical properties
- Improved solubility in water
Carborane Derivatives

- o-Carboranyl maltoside
- o-Carboranyl thiouracil

- Nucleosides
- Porphyrins
- Phthalocyanines
- Epidermal growth factors
- CNS depressants
Requirements

The drug should*:

- be systemically non-toxic; ✔
- be selective in targeting tumour cells in the presence of normal ones; ✔
- persist intra-cellularly, especially during the course of neutron radiation;
- deliver an amount of \( \sim 10^9 \) \(^{10}\)B atoms/cell; ✔
- achieve tumour/normal tissues ratios higher than 3; ✔
- be able to penetrate biological barriers, such as the BBB.

What to Target?
-Mitochondria-

- Cell respiration
- Apoptosis (programmed cell death)
- Alterations are causative of cancer

Differences observed: increased trans-membrane potential (60 mV) in cancer cells (negative inside)

Mitochondrial Targeting

- Differences observed: increased trans-membrane potential (60 mV) in cancer cells (negative inside)

- Cations are driven to mitochondria according to the Nernst equation:

  \[ \Delta \Psi \text{ (mV)} = 61.5 \log_{10} \left( \frac{[\text{cation}]_{\text{in}}}{[\text{cation}]_{\text{out}}} \right) \]

- As a consequence every increase in membrane potential of ca. 61.5 mV will cause a ten-fold increase in the accumulation of the lipophilic cations within the mitochondria

Delocalized Lipophilic Cations (DLC)

- Lipophilic
- Delocalization of the positive charge over a large area
- Reduced free energy change while crossing lipid membranes
Previously

- Dequalinium-B derivative* in vitro showed tumour uptake and toxicology similar to those of its non-boronated analogue.

- Rhodamine B-phenyl boronic acid** - developed for colon cancer diagnosis purposes.

Boronated DLCs

Preliminary *in vitro* tests

Uptake: $^{10}\text{B atoms/cell >2.5}\times10^{11}$

Uptake efficiency: >93%

Preliminary *in vitro* tests

- PC3 prostate cancer cells treated with compound R
- PNT2 immortalized prostate cells treated with compound R

Currently

This compound is being tested on human brain cells at UoP

- Low molecular weight compounds containing carborane are quickly removed from cancer tissue and blood

- Hence, the need of formulating them
BNCT Limitations (so far)

- Toxicity associated with $^{10}$B-containing pharmacophores
- The need for target-tissue specificity
- Challenges imposed by biological barriers

The optimization of the transport and the delivery of boronated agents to the site of action is a challenge that has to be addressed by formulation

Interest:
- Liposomes
- Polymeric nanoparticles
- Single walled carbon nanotubes (SWCNTs)
Liposomes

Encapsulation

Incorporation

= Phospholipid  = Boron lipid  = Boronated drug

Liposomes Targeting Mitochondria

- Designer lipids for target recognition
- Mitochondrial targeting properties are not established

Small perturbation of the membrane

SWCNTs

- Innovative materials
- Resistant to metabolism
- Toxicity issues
- Cell penetration mechanism not fully elucidated

- Diameter of 1-2nm
- High surface area
- Thermal and chemical stabilities
- Covalent and non-covalent conjugation to various therapeutic biomolecules

Low dispersibility in aqueous media

Hydrophobicity of graphene sidewalls and strong $\pi - \pi$ interactions between CNTs resulting in inter-tubular Van der Waals forces

Functionalization can help bundling

Two types of functionalization:
- Covalent: demands the disruption of the $\pi$ cloud through rehybridization of some $sp^2$ carbon atoms to $sp^3$, impacting on stability.
- Non-covalent: by means of polymer- or lipid-assisted physisorption

Covalent

1. SWCNTs, solvent
2. NaOH, EtOH

- nido-Carborane presence makes them water soluble
- In vivo distribution of $^{10}$B in tumour-bearing mice showed 2 days retention, considerable specificity towards tumour cells (concentrations as high as 28 µg $^{10}$B/g tumour cells) and maximal tumour/blood ratios of 6

Polymer Coating

- SWCNTs coated with *N*-octyl-*O*-sulphate chitosan
- Polymer suspends SWCNTs 20 times more than other chitosan derivatives
- Polymer stabilizes SWCNTs
- Anticoagulation properties

- No carboranyl polymers have been so far tested for the purpose

Lipid Coating - 1

- SWCNTs coated with lyso-phosphatidylcholine (LPC)
- *ortho*-Carborane is physisorbed onto the external lipid coating
- Characterized by:
  - $\zeta$–potential
  - Atomic force microscopy (AFM)
  - Raman spectroscopy
  - UV
  - inductively coupled plasma-mass spectrometry (ICP-MS)

Lipid Coating - 2

Results indicated that:

- ultrasonication of SWCNTs, carborane and LPC affords a water dispersion
- preferential dispersion of smaller-diameter LPC/carborane/SWCNTs systems in PBS buffer
- increasing the relative amount of o-carborane in the mixture initially effects a steep increase in the number of particles in dispersion
- there is a ceiling value of carborane for its capacity of facilitating an increase in the proportion of LPC/SWCNTs in dispersion

Thank you

Any questions