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REVIEW

Boron-Containing Bioactive Molecules: An Approach to Boron Neutron Capture Therapy

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ABSTRACT

Boron Neutron Capture Therapy (BNCT) is a binary therapeutic modality for cancer treatment that involves the irradiation of ^{10}B-localized tumours with low-energy neutrons, which results in the production of highly cytotoxic particles ($^{4}He^{2+}$ and $^{7}Li^{3+}$). The vast potential of this therapeutic modality has resulted in worldwide efforts aimed at the development of agents for selective localization of ^{10}B in tumour cells at concentrations of 10-30 ppm, which have been predicted to afford a significant therapeutic advantage.

Clinical considerations include historical viewpoints and the latest applications. As part of these clinical uses are boron neutron capture synovectomy (BNCS), *in vivo* imaging of the neutron capture therapy agent BSH using ^{10}B MRI, SIMS imaging of amino acids, and liposomes. A biodistribution of boron compounds in an animal model of human undifferentiated thyroid cancer for BNCT have been recently reported. Polyhedral borate anions and carboranes are presented as prominent substructures of the boron delivery agents. Current development of the following BNCT agents are shown: corticosteroid-carborane esters, oligomeric phosphate diesters (OPDS), ADP derivatives, *o*-carboranes carrying 1,3,5-triazine units, amines and polyamines, platinum (II)-amine complexes, porphyrins, phenanthridinium derivatives, benzimidazoles, amino acids, peptides, the antibacterial protein avidin, boron derivatives of harmane, nucleosides, and carbohydrates.

Key words: Boron Neutron Capture Therapy

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INTRODUCTION

Boron Neutron Capture Therapy (BNCT) is a binary form of cancer treatment which uses a boron-10 (^{10}B)-enriched compound that preferentially concentrates in tumour cells. The tumour site is then irradiated by a low-energy neutron beam (Soloway *et al*., 1998). The thermal neutrons in the beam interact with the boron in the tumour to cause the boron atom to split into an *alpha* particle and lithium nucleus. Then, under these conditions highly energetic particles ($^{7}Li^{3+}$,$^{4}He^{2+}$, sharing *ca*. 2.8 MeV of kinetic energy) are formed which destroy cancer cells in the immediate vicinity (Barth *et al*., 1990a,b, 1999). Both of these particles have a very short range (about one cellular diameter) and cause significant damage to the cell in which it is contained. Consequently, damage is done to the tumour cell, while largely sparing healthy tissue.

For potentially effective BNCT, tumour boron concentrations from a new agent should be greater than 30 ppm and tumour/blood and tumour/normal tissue boron concentration ratios should be greater than 5/1 without causing significant toxicity. The major obstacle to clinically viable BNCT is that effective therapy requires the selective localization of 5-30 ppm ^{10}B in tumour (Fairchild and Bond, 1985). A variety of ^{10}B-enriched compounds have been prepared as well as boronated antibodies. Tumour-directed antibodies or their immunoreactive fragments are attractive candidates for the selective delivery of ^{10}B, provided that *ca*. 1000 ^{10}B atoms can be attached to each immunoreactive protein without significantly altering its biological properties (Chen *et al*., 1994). It has been calculated that antibodies bearing this quantity of ^{10}B could deliver therapeutic amounts of ^{10}B to tumour.

Previous studies have revealed problems associated with randomly conjugating whole monoclonal antibodies (Mabs) with large numbers of small boron-containing compounds (Mizusawa *et al*., 1982, 1985; Goldenberg *et al*., 1984) or with limited numbers of heterogeneous (Alam *et al*., 1989; Pettersson *et al*., 1989) or homogeneous boron-rich polymers (Varadarajan and Hawthorne, 1991; Paxton *et al*., 1992). Hawthorne (1991) reported an approach to this problem based upon the synthesis of a homogeneous boron-rich 'trailer' compound and its conjugation to a specific site of a tumour directed antibody fragment (Fab-SH).

The chemistry of boron analogues of biomolecules (Morin, 1994), as well as the pharmaceutical development and medical applications of porphyrin-type macrocycles have been reviewed (Mody, 2000).

THE BORON NEUTRON CAPTURE REACTION

Boron compounds used for BNCT are usually not tissue specific. These compounds might accumulate in tumours at somewhat higher concentrations due to higher metabolic rates within tumours, but they are generally not specifically targeted towards the tumour.

The stable isotope of boron ^{10}B (19.8% natural abundance) captures neutrons, while the ^{11}B isotope does not. Biomolecules and drugs containing ^{10}B-enriched carborane and borane substituents (which preferentially localize in tumour cells and rapidly clear from normal cells) can thus be used for cancer therapy.

When the ^{10}B nuclei are bombarded by thermal or epithermal neutrons, on absorption of a neutron the $_{5}^{10}$B atom undergoes a fission reaction that produces high energy particles, that is high Linear Energy Transfer (LET) $_{2}^{4}$He (*alpha* particles) and $_{3}^{7}$Li nuclei that are confined to a radius comparable to the dimension of a cell. These nuclei deposit their energy within a short range (10 μm), which accounts for *ca.* one cell diameter. Moreover, a *gamma* photon of 0.48 MeV is emitted which can be used to image the ongoing neutron capture. Since these α particles travel only about 10 μm or less, they selectively destroy cancer cells where the ^{10}B nuclei are localized. This process is known as BNCT.

If ^{10}B is sequestered in the cell nucleus, a maximum effect of BNCT is to be expected.

The BNC reaction is based on the ^{10}B(n, α)^{7}Li reaction (Fig. 1), which is induced when boron-10, which has a large capture cross section relative to the most abundant endogenous nuclei (^{1}H, ^{12}C, ^{31}P, ^{14}N), is exposed to thermal neutrons. BNCT is referred to as a binary therapy because each component, the boron and the neutrons, is required for the treatment to be efficacious. BNCT has the potential to be a selective therapy because the highly energetic daughters of the BNC reaction, an *alpha* particle and the lithium ion, traverse a distance which is less than the diameter of a typical cell, thereby depositing their substantive energies in a confined area.

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Epithermal neutrons beam from reactor → Thermal neutrons → Boron(n, *alpha*) reactions in tumour cells (10 μm):

$$^{10}B \rightarrow {}^{11}B^{*}\text{----}t = 10^{-12}\ \text{secs} \rightarrow E_{^{7}Li^{3+}} = 0.84\ \text{MeV} + E_{alpha} = 1.47\ \text{MeV} + E_{gamma} = 0.48\ \text{MeV}\ (94\%)$$

Figure 1. Boron Neutron Capture Reaction.

An epithermal beam of neutrons is directed towards a patient's head, during their passage through tissue these neutrons rapidly lose energy by elastic scattering (a process called 'thermalization') until they end up as thermal neutrons (Fig. 1). The thermal neutrons thus formed, are captured by the ^{10}B atoms which become ^{11}B atoms in the excited state for a very short time (*ca*. 10^{-12} seconds). The excited ^{11}B atoms then fission producing *alpha* particles, ^{7}Li recoil nuclei and in 94% of the reactions, *gamma* rays. Tumour cells are killed selectively by the energetic *alpha* particles and ^{7}Li fission products.

BNCT has been shown to significantly prolong the lifespan of patients with brain tumours, and a number of BNCT reagents are currently in Phase I and II clinical trials.

CANCER THERAPY AND NEUTRON CAPTURE THERAPY

An ideal therapy for cancer would be one whereby all tumour cells can be selectively destroyed without damaging normal tissues. Most of the cancer cells should be destroyed, either by the treatment itself or by the body's immune system, otherwise the tumour may reestablish. Although today's standard treatments, *e.g.*, surgery, radiation therapy and chemotherapy, have successfully cured many kinds of cancers, there are still many treatment failures. The promise of a new experimental cancer therapy with some indication of its potential efficacy has led scientists from around the world to work on this approach (Brownell *et al*., 1978; Barth *et al*., 1990a).

Four years after the discovery of neutrons in 1932 by J. Chadwick of Cambridge University, a biophysicist, G.L. Locher of the Franklin Institute at Pennsylvania introduced the concept of Neutron Capture Therapy (NCT) (Locher, 1936). The physical principle of NCT consists of a binary radiation therapy modality that brings together two components that when kept separate have only minor effects on cells. The first component is a stable isotope of boron (^{10}B) that suffers a nuclear reaction when irradiated with the second component, a beam of low-energy neutrons (Fig. 1).

Although other nuclides have shown higher thermal neutron capture cross sections than ^{10}B, NC by such nuclides resulted in the emission of highly penetrating *gamma* rays. However, gadolinium-157 (^{157}Gd) n-*gamma* reaction was also accompanied by some internal conversion and, by implication, Auger electron emission. Irradiation of Gd^{3+}-DNA complexes with thermal neutrons resulted in the induction of DNA double-strand (ds) breaks, but the effect was largely abrogated in the presence of EDTA. Thus, by analogy with the effects of decay of Auger electron-emitting isotopes such as ^{125}I, the Gd NC event must have taken place in the close proximity of DNA in order to induce a DNA ds break. It has been proposed that ^{157}Gd-DNA ligands therefore have potential in NCT. The thermal neutron capture cross section of ^{157}Gd, a nonradioactive isotope, was more than 50 times that of ^{10}B (Martin *et al.*, 1989). Induction of DNA double-strand breaks by ^{157}Gd neutron capture carried out in the Peter MacCallum Cancer Institute, Melbourne, Australia, has been reviewed (Martin *et al*., 1989).

Then, there are a number of nuclides that have a high tendency for absorbing low energy or thermal neutrons. Of the various nuclides that have high neutron capture cross-sections, ^{10}B is the most attractive for the following reasons: (a) it is non radioactive and readily available, comprising approximately 20% of naturally occurring boron; (b) the particles emitted by the capture reaction ^{10}B(n, [[*alpha*]])^{7} Li are largely high LET, $\delta E/\delta x$; (c) their combined path lengths are *ca*. one cell diameter; *e.g*., about 12 microns, theoretically limiting the radiation effect to those tumour cells that have taken up a sufficient amount of ^{10}B, and simultaneously sparing normal cells; (d) the well understood chemistry of boron allows it to be readily incorporated into a variety of chemical structures.

Although the neutron capture cross-sections for the elements in normal tissue are several orders of magnitude lower than for ^{10} B, two of these, hydrogen and nitrogen, are present in such high concentrations that their neutron capture contributes significantly to the total absorbed dose. In order to reduce this 'background' dose it is essential that the tumour attain high ^{10}B concentrations so that the neutron flow delivered (neutrons/cm^2) can be held to a minimum, thereby minimizing the (n,p) reaction with nitrogen

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[^{14}N(n,p)^{14}C] and the neutron-*gamma* (n, *gamma*) reaction with hydrogen [^{1}H (n,*gamma*) ^{2}H] and maximizing the ^{10}B(n,*alpha*)^{7}Li reaction in the tumour cells.

Alpha particles and lithium ions, from the ^{10}B(n,*alpha*) ^{7}Li reaction, give rise to closely spaced ionizing events. They have a combined path length of *ca.* 12 μm, and have high LET (Coderre and Morris, 1999). There is, therefore, little if any cellular repair from the induced radiation injury. Since the ^{10}B(n, *alpha*)^{7}Li reaction will produce a significant radiobiological effect only when there is a sufficient flow of thermal neutrons and a sufficient amount of ^{10}B near to, on, or within the cell. Selectivity is simultaneously one of the advantages and disadvantages of BNCT, since it requires delivery of ^{10}B to tumour cells in greater amounts than normal cells. In contrast to the ionizing radiation produced by radionuclides, little or no radiation is delivered to surrounding cells, which have no ^{10}B, if the ^{10}B is selectively localized on or within the tumour cells.

Alpha particles, with high LET, have other biological advantages. Unlike some forms of ionizing radiation, such as X-rays, *alpha* particles do not require oxygen to enhance their biological effectiveness. In a rapidly expanding tumour, some regions receive less oxygen than normal tissues do. As a result of this oxygen depletion, the tumour can be more resistant to the effects of photon or electron (*e.g.*, low LET) radiation therapy. However, tumour sensitivity to *alpha* particles is retained, even when the tumour has limited oxygen supply.

Another advantage of *alpha* particles and lithium ions is that they can kill dividing and non dividing tumour cells alike, what is important because tumours are known to have a large number of viable but inactive cells. Other forms of radiation treatment and chemotherapy tend to work best only on the cells that are dividing (Barth *et al*., 1990a).

A major advantage of a binary system is that each component can be manipulated independently of the other. With BNCT one can adjust the interval between administration of the capture agent and neutron irradiation to an optimum time when there is the highest differential ^{10}B concentrations between normal tissues and the tumour. Furthermore, the neutron beam itself can be collimated so that normal tissues with high ^{10}B concentration can be excluded from the treatment. Protection of normal tissues near and within the treatment volume is achieved by selective targeting of ^{10}B to the tumour.

HISTORICAL CONSIDERATIONS

BNCT has undergone most developments since Locher introduced the concept of NCT in 1936 and the development of nuclear energy during World War II. The Cold War expanded the new field of polyhedral borane chemistry, rapid advances in nuclear reactor technology and the increase in the number to reactors potentially available for BNCT (Hawthorne and Lee, 2003). Clinical BNCT studies have been performed in USA during the 1950s and 1960s for the treatment of malignant brain tumours.

In 1951, Sweet first suggested that BNCT might be useful for the diagnosis and treatment of brain tumours, and in particular, the treatment of the most highly malignant and therapeutically persistent of all brain tumours, glioblastoma multiforme (GBM).

Sweet and Javid in 1952 first showed that certain boron compounds would concentrate in human brain tumour relative to normal brain tissue. A clinical trial of BNCT was performed at Brookhaven National Laboratory during 1951 and 1952 (Farr *et al*., 1954; Godwin *et al*., 1955) and at the Massachusetts Institute of Technology (MIT) research reactor MITR-I (Asbury *et al*., 1972) during 1961 and 1962, using a thermal neutron beam and sodium tetraborate, borax ($Na_2B_4O_7.10H_2O$), as the capture agents.

Unfortunately, these trials failed to show any evidence of therapeutic efficacy because of: (1) thermal neutrons were attenuated rapidly in tissue due to absorption and scattering, and then, the depth of penetration for BNCTwas limited to 3-4 cm. Therefore, only superficial tumours have been destroyed by that ^{10}B capture reaction (Choi *et al*., 1989; Harling *et al*., 1989), and (2) the boron compounds that were used were freely diffusible, low molecular weight substances that did not achieve selective localization in the tumour, affecting especially those which had high blood values. This was the reason of so much radiation delivered to adjacent normal brain.

In fact, the first trial, conducted in 1951 to 1961 in the USA to test BNCT on patients suffering of glioblastoma was a failure, essentially because ^{10}B was located in the cerebral capillaries rather than in the tumour cells (Pignol and Chauvel, 1995).

Since 1968, 183 patients with different kinds of brain tumours were treated in Japan by BNCT, and this clinical experience has been recently reviewed (Nakagawa *et al*., 2003). Furthermore, beginning in 1972, Mishima and colleagues have achieved useful concentrations of ^{10}B-boronophenylalanine (BPA), an analogue of the melanin precursor tyrosine, for BNCT of melanomas (Sweet, 1997).

However, BNCT of malignant brain tumours has been efficiently performed since March 1977. In 1981, the Hopital Tenon group and the Orleans neutron therapy team in France started a collaborative study for the treatment of grade IV astrocytomas

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using a combination of photons (30 Gy total brain) followed by a neutron boost (7 Gy) (Breteau *et al*., 1996). Doses were progressively increased from 6 to 7 Gy and later up to 8 Gy. Since October 1994, a neutron boost of 7.5 Gy has been delivered. At the time of evaluation, 294 patients had a minimum follow-up of 12 months. Univariate analysis indicated that clinical status, tumour location and photon fractionation scheme had no significant influence on survival. On the contrary, age, surgical procedure and neutron dose were found to be prognostic factors. In a multivariate analysis, the prognostic value of the surgical procedure disappeared and the only remaining independent prognostic factors up to 11 months after treatment (P = 0.001) were age and the neutron dose. As far as neutron dose was concerned, survival increased with dose from 6 to 7 Gy up to 15 months. However, after 15 months, there was no longer any benefit in survival for the patients treated with 8 Gy, and complications related to overdosage began to appear. There was a long-term survival group: 55 patients were alive 18 months after treatment (18%). The median survival was 26.7 months. The best survival was observed for patients treated with a neutron boost of 7 Gy in eight fractions over 11 days (25 *vs* 18%). A possible benefit when combining external fast neutrontherapy with BNCT could reasonably be expected (Breteau *et al*., 1996).

Owing to encouraging clinical studies done in Japan by Hatanaka *et al*. in 1986 (Hatanaka *et al.*, 1986) for the treatment of malignant gliomas and those of Mishima *et al*. in 1989 (Mishima *et al*., 1989; Mishima, 1996) for melanoma, there has been renewed interest for BNCT. Moreover, tolerance of normal human brain to BNCT has been recently analysed (Coderre *et al*., 2004). Furthermore, the first human case of malignant melanoma was successfully treated on July 1987 in the japanese reactor (Musashi reactor, TRIGA-II, 100 kW). To obtain both good irradiation field characteristics and a better irradiation facility, some tests and developments have been continued in aggreement with the study of medical and biological irradiations. The results of these evaluations and the status of the medical irradiation facility at the reactor of the Musashi Institute of Technology in Japan have been reviewed (Matsumoto *et al*., 1989).

The development of BNCT as well as the design and dosimetry of an intermediate energy neutron beam, developed at the Harwell Laboratory, Oxfordshire, UK, has been reviewed (Perks *et al*., 1988). Early trials have required extensive neurosurgery to exposure the tumour to the thermal neutrons and were unsuccessful. It was thought in UK that intermediate-energy neutrons will overcome many of the problems encountered in the early trials, because they have greater penetration prior to thermalization, so that surgery will not be required. Therefore, an intermediate-energy neutron beam has been developed at the Harwell Laboratory for research into BNCT. Neutrons from the core of a high-flux nuclear reactor were filtered with a combination of iron, aluminium and sulphur. Dosimetry measurements have been made to determine the neutron and *gamma*-ray characteristics of this beam, and to monitor them throughout the four cycles used for BNCT research. The beam was of high intensity (*ca*. 2 x 10^7 neutrons/s.cm^2, equivalent to a neutron kerma rate in water of 205 mGy/h) and nearly monoenergetic (93% of the neutrons had energies *ca*. 24 keV, corresponding to 79% of the neutron kerma rate) (Perks *et al*., 1988).

Ionizing radiation has demonstrated clinical value for a lot of CNS tumours. Correlation of radiation dose with effect on cranial soft tissues, normal brain, and tumour has been effective in improving survival and decreasing complications (Marks, 1989). By using different physical modalities to change the distribution of radiation dose, it was possible to increase the dose to the tumour and reduce the dose to the normal tissues (Gahbauer *et al*., 1998).

The search of therapeutic gain using hyperbaric oxygen, neutrons, radiation sensitizers, chemotherapeutic agents, and BNCT has met with limited success. Both neoplastic and normal cells were affected simultaneously by all modalities of treatment, including ionizing radiation. In the case of radiation, it was the brain that limited delivery of curative doses, and in the case of chemical additives, other organ systems, *e.g*., bone marrow, liver, lung, kidneys, and peripheral nerves. Thus, the major obstacle in the treatment of malignant gliomas was the inability to preferentially affect the tumour with the modalities available. Until it was possible to directly target the neoplastic cell without affecting so many of the adjacent normal cells. Concepts and strategies of radiation treatment of brain tumours have been reviewed (Marks, 1989).

In fact, the history of BNCT is linked to GBM, which is a cancer of the glial supportive tissues of the Central Nervous System (CNS) (Díaz *et al*., 2000). Glial cells provide the environment, in the form of chemical and physical support, which sustains the neurons. Ninety percent of the cells of the CNS are glial cells.

Macroscopically, in GBM, the evidence of anaplasia (*e.g.*, a reversion of the cells or tissue to more primitive, embryonic or undifferentiated form often with increase of capacity for multiplication) is shown where the smooth, homogeneous texture and

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color of normal tissue is replaced with a more friable granular gray tumour tissue with areas of necrosis and edema. A contrast-enhanced Computerized Tomography (CT) scan of a patient with high-grade glioblastoma can show tumour and edema well-distinguished regions.

Microscopically, as the name 'multiforme' suggests the prominent feature is the variety of cell forms. The anaplastic areas vary within a wide range, but collectively make up the familiar picture of the GBM. The important features of the malignant process are increase cellularity, obvious polymorphism of the tumour cells associated with mitosis, alterations in the architectural arrangements of the cells and a variety of secondary changes. These features together with the confirmation of several macroscopic features, often make the diagnosis clear even with a low-power optical microscope.

Standard radiotherapy for the treatment of high-grade brain tumours following a biopsy or subtotal resection was to give external beam radiation with high-energy X-rays (4 - 6 MeV) to a dose of *ca.* 60 Gy in fractions of 1.8 or 2.0 Gy daily, five days a week (Madoc-Jones *et al*., 1989). There have been a number of series reported (Chun *et al*., 1989; Leibel *et al*., 1989) in which tumour doses up to 160 Gy were used (mostly due to interstitial radiation). Unfortunately, despite these high doses, there has been yet no evident therapeutic benefit.

Average results of conventional treatment showed that the median survival for GBM ranged from eight to fourteen months, and untreated GBM resulted in a median survival of *ca.* three months (Levin *et al*., 1989).

The continued apparent success of BNCT in Japan since 1968, led indirectly to the re-start of clinical trials on BNCT in 1994 at both Brookhaven and MIT, in USA. Similar trials started soon at Petten, The Netherlands, in Europe. Worldwide, many neutron beam designs have been proposed with either thermal or epithermal neutrons, emanating predominantly from nuclear research reactors (Moss *et al*., 1997).

These early results indicated that BNCT appeared to be as effective as conventional therapy for GBM and it was clearly a therapy which did not require as great an investment in time by the patient as conventional radiotherapy. Results from BNCT trials of melanoma have shown complete or partial tumour control in several cases. Both the F98 and 9L rat glioma models were used to evaluate the effectiveness of BNCT of brain tumours (Barth *et al*., 2003). In both models, glioma cells were implanted intracerebrally into syngeneic Fischer rats and *ca.* 10-14 days later BNCT was started at the Brookhaven National Laboratory Medical Research Reactor. Two low molecular weight (M_r < 210 Da) ^{10}B-containing drugs, BPA and/or sodium borocaptate (BSH) were used as capture agents, either alone or in combination with each other (Barth *et al*., 2003). The 9L gliosarcoma, which has been difficult to cure by means of either chemo- or radiotherapy alone, was readily curable by BNCT. The best survival data were obtained using BPA at a dose of 1200 mg/kg (64.8mg ^{10}B), administered i.p., with a 100% survival rate at 8 months. Molecular targeting of the epidermal growth factor receptor (EGFR) has been also investigated using F98 glioma cells, which had been transfected with the gene encoding EGFR and, intratumoural injection of boronated EGF as the delivery agent, followed by BNCT. These studies demonstrated that there was specific targeting of EGFR and provided proof of principle for the use of high molecular weight, receptor targeting-boron delivery agents (Barth *et al*., 2003).

In fact, clinical interest has focused primarily on the treatment of high-grade gliomas and either cutaneous primaries or cerebral metastases of melanoma, ocular melanoma (Pignol *et al*., 1994) as well as head and neck carcinoma (Clasen, 1990), and liver cancer. There is growing interest in using BNCT in combination with surgery to treat patients with primary, and possibly metastatic brain tumours (Soloway *et al*., 1997). Non-malignant diseases such as rheumatoid arthritis offer additional opportunities for BNCT (Hawthorne, 1998).

Today, with great improvement in the boronated compounds which show an uptake preferentially inside the cells, the quality of neutron beams, and the knowledge of the microdosimetry, BNCT may be clinically used to increase the local control of radioresistant tumours, like the high grade gliomas, cutaneous or uveal melanoma, and perhaps soft tissue sarcomas (Pignol and Chauvel, 1995; Barth *et al*., 1996).

Neutron therapy has shown to be clinically useful in cases of advanced, slow-growing radioresistant head and neck carcinoma (Clasen, 1990). Therapeutic effects might have been based on direct DNA damaging and thus immediate cell-killing, on the generation of free oxygen radicals and, also, on the fact that heavy particle radiation was said to be less dependent on the presence of oxygen than *gamma* rays, i.e. on a lower oxygen enhancement ratio (OER). The smaller difference in reaction between oxygenated and nonoxygenated cells could entail advantages as well as disadvantages, depending on the characteristics of the tumour cell population and of the normal tissue. It was therefore essential to select patients and tumours with an expectedly high therapeutic gain factor. Fission neutrons for tumour

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therapy have been evaluated by several *in vitro* and *in vivo* studies (11/13) and the biological efficiency of the RENT (Reactor Neutron Therapy) beam in Munich. For a single dose range between 2 and 8 Gy the biological efficiency for chronic radiation damage was relatively small. Consequently, patients with recurrent or metastatic carcinomas of the head and neck were treated with a single dose of 200-250 cGy after previous surgery and/or combined radiochemotherapy. The main limitation of fission neutrons was the small penetration depth. Possibilities of clinical implementation of BNCT in otorhinolaryngology were evaluated. In near surface tumours it was possible to administer high doses of ^{10}boron not selectively. Animal experiments with intratumoural injection of ^{10}boron glycine have shown a strong effect on tumour growth delay (Clasen, 1990).

BNCT is currently undergoing clinical trials in Japan, Europe, and the US with patients afflicted with deadly brain cancer (GBM) or melanoma (Hawthorne, 1998). Treatment of patients has consisted first of surgery to remove as much of the tumour as possible, followed by BNCT at varying times after surgery. Barth *et al*. (1992) have reviewed the radiobiologic considerations on which BNCT is based, including a brief discussion of microdosimetry and normal tissue tolerance.

The most critical and difficult step in an efficient BNCT is the tumour targeting. It is today possible to synthesize a large number of boron compounds and conjugate them to tumour-seeking macromolecules, such as monoclonal antibodies or different polypeptides (Carlsson *et al*., 1992). The boron-containing compounds considered for therapy are the sulfhydryl-containing polyhedral borane sodium borocaptate ($Na_2B_{12}H_{11}SH$) or sulfhydryl boron hydride (BSH), and *p*-boronophenylalanine (BPA), which are currently in clinical use for the treatment of gliomas and malignant melanomas, respectively. The distribution patterns and radiobiological characteristics of BPA and BSH have been evaluated in a range of normal tissues and tumour types (Coderre and Morris, 1999). Over the past 20 years, other boron delivery agents, which show potentials as targeting molecules have been designed and synthesized, *e.g.*, boronoporphyrins, carboranyl uridines (CBU), boron-containing amino acids, biochemical precursors of nucleic acids, DNA-binding molecules, nucleosides, and polyamines (Barth *et al*., 1996, 1999, 2005).

Conjugation of boron compounds to macromolecules, *e.g.*, monoclonal antibodies (MoAbs or MAb), bispecific antibodies, epidermal growth factor (EGF) and dextran is also employed for active or passive tumour targeting (Barth *et al*., 2005). Boron delivery *via* microparticulate carriers such as liposomes, high density lipoproteins and microcapsules is also attractive for its potential application in BNCT (Mehta and Lu, 1996).

To increase the selective uptake of boron by tumour cells, would be necessary to exploit tumour transformation related cellular changes such as over-expression of growth factor receptors (Carlsson *et al*., 2003). However, the number of receptors varies from small to large and the uptake of large amounts of boron for each receptor interaction is necessary in order to deliver sufficient amounts of boron. Since each targeting moiety must deliver large number of boron atoms, receptor-targeting ligand liposomes should be used, containing large number of boron atoms. Studies of boron containing liposomes, with or without ligand, have been recently reviewed (Carlsson *et al.,* 2003). Two recent examples from the literature are ligand liposomes targeting either folate or EGF receptors on tumour cells. Other potential receptors on gliomas include PDGFR and EGFRvIII. Besides the appropriate choice of target receptor, it is also important to consider delivery of the ligand liposomes, their pharmacodynamics and pharmacokinetics and cellular processing (Carlsson *et al*., 2003).

A number of potentially useful boron agents are known which have not been biologically evaluated beyond a cursory examination and only three boron-10 enriched target species are approved for human use following their Investigational New Drug classification by the US Food and Drug Administration (FDA): BSH, BPA and GB-10. All ongoing clinical trials with GBM and melanoma have been performed with one of these three species and most often with BPA (Hawthorne and Lee, 2003). BSH-mediated BNCT elicited proportionately less damage to normal tissue than did BNCT mediated with BPA. However, BPA showed superior *in vivo* tumour targeting and has proved much more effective in the treatment of brain tumours in rats (Coderre and Morris, 1999).

Methodology has been developed to heavily boronate MAb using a precision macromolecule, a 'starburst' dendrimer, which can be linked to MAb by means of heterobifunctional reagents (Barth and Soloway, 1994). Although the resulting immunoconjugates retain their *in vitro* immunoreactivity, they lose their *in vivo* tumour localizing properties and accumulate in the liver. In order to overcome this problem, bispecific Mab were produced, which can simultaneously recognize a tumour-associated antigen and a boronated macromolecule (Barth and Soloway, 1994). Other

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boronated compounds considered are ligands for receptor-amplified tumour cells, antibodies for tumour cells with specific antigens and thioureas for treatment of melanotic melanomas.

The required boron concentration has been given by the relative dose due to neutron capture in ^{10}B and that of the competing capture reactions in nitrogen and hydrogen. Capture in nitrogen has produced protons with a range of about 10-11 microns and this gave a radiation dose to all cells in the neutron activated area. Calculations showed that the local concentration of ^{10}B near the critical radiation target, DNA, had to be higher than 10 ppm (10 micrograms/g). Increased emphasis has been put on the development of combinations of treatments that fulfil the requirements for attacking the microscopic spread of the tumour (Carlsson *et al*., 1992).

Nuclear reactors are the exclusive source of neutrons for BNCT, and the fission process within the core produces a mixture of low-energy thermal and epithermal neutrons, fast or high (> 10,000 eV) energy neutrons, and *gamma* rays. These nuclear reactors are available in the US, Japan, several European countries, and Argentina. Low-energy (0.025 eV) thermal neutrons and higher-energy (1-10,000 eV) epithermal beams have been used, but beam optimization, and possible alternative neutron sources (accelerators) have been also considered. These accelerators are being developed in several countries, but none are currently being used for BNCT. Further studies in Japan, Europe and the United States concerning the treatment of glioblastomas and melanomas by BNCT have been performed. All these points of view have been reviewed (Barth *et al*., 1992, 1996, 1999; Barth, 2003).

Although thermal neutron beams have been used clinically in Japan to treat patients with brain tumours and cutaneous melanomas, epithermal neutron beams are being used in the United States and Europe because of their superior tissue-penetrating properties (Barth *et al*., 1996, 1999).

The radiobiological and clinical data concerning the alteration of the blood-brain barrier (BBB) after cerebral irradiation have been reviewed (Gregoire *et al*., 1993). Before starting BNCT clinical applications, it became necessary to assess the integrity of the BBB after different dose ranges and fractionation of radiotherapy, and after different time intervals following irradiation. Extrapolation of the available radiobiological and clinical data suggested that for rather small hydrophilic compounds, such as BSH or *L*-BPA, an early increase in transport through the BBB might be foreseen after single photon dose larger than 10 Gy or after a full standard radiotherapy regimen. However, there was no evidence that the first fractions of a BNCT application (typically 2 to 4 Gy equivalent per fraction) would increase the permeability of the BBB sufficiently to permit transport of large boronated compounds such as porphyrins or antibodies, or even of smaller hydrophilic compounds, *e.g.*, BSH and *L*-BPA. The dose selectivity of BNCT is unlikely to be compromised by early alteration of the BBB due to the first fractions of a typical BNCT fractionated regimen (Gregoire *et al*., 1993).

At present, there are several research groups working on BNCT. Much of the complexity has been overcome through a combination of preclinical experimentation and clinical dose escalation experience (Coderre *et al.,* 2003). Over 350 patients have been treated in a number of different facilities worldwide. The individual components and methodologies required for effect BNCT have been recently reviewed (Coderre *et al*., 2003): the boron delivery agents, the analytical techniques, the neutron beams, the dosimetry and radiation biology measurements, and how these components have been integrated into a series of clinical studies. According to these authors (Coderre *et al.,* 2003) of the US Nuclear Engineering Department, MIT, USA, the most important disadvantage of BNCT at the present time is non-uniform delivery of boron into all tumour cells. It is necessary to improve boron delivery agents, boron administration protocols, and also to combine BNCT with other modalities.

The MIT/Harvard group makes use of a fission converter based epithermal neutron beam at the MITR-II Research Reactor that is filtered by aluminium, teflon, cadmium, and lead (Harling *et al*., 2002; Riley *et al*., 2004a,b). Fission reactor neutron sources for neutron capture therapy have been reviewed (Harling and Riley, 2003).

This arrangement provides a broad spectrum epithermal beam with low incident *gamma* and fast neutron contamination while maintaining an incident neutron flux of *ca*. 5 x 10^9 neutron/ cm^2.sec. This beam allows irradiations for clinical trials to be conducted in 1 - 4 fractions in 10 minutes or less (Riley *et al*., 2003; Binns *et al*., 2004a,b). A critical examination of the results from the Harvard-MIT NCT program phase I clinical trial of neutron capture therapy for intracranial disease has been reported (Palmer *et al*., 2002; Busse *et al*., 2003).

Furthermore, a clinical review of the Japanese experience with BNCT and a proposed strategy using epithermal neutron beams have been recently reported (Nakagawa *et al*., 2003). For a retrospective study in the Department of Neurosurgery of the National Kagawa Children's Hospital of Japan, 105 patients

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with glial tumours who were treated in Japan between 1978 and 1997 were selected. In the analysis of side effects due to radiation, all the 159 patients treated between 1977 and 2001 were included. With respect to the radiation dose (i.e. physical dose of boron n-*alpha* reaction), the new protocol prescribed a minimum tumour volume dose of 15 Gy or, alternatively, a minimum target volume dose of 18 Gy. The maximum vascular dose should not exceed 15 Gy (physical dose of boron n-*alpha* reaction) and the total amount of *gamma* rays should remain below 10 Gy, including core *gamma* rays from the reactor and capture *gamma* in brain tissue. The outcomes for 10 patients who were treated by the new protocol using a new mode composed of thermal and epithermal neutrons have been reported (Nakagawa *et al*., 2003).

The present status of BNCT for malignant glioma has been reviewed by a japanese group (Kageji *et al*., 2005), from the Department of Pharmacy of the University Medical Center, Amsterdam (van Rij *et al*., 2005), and from the Department of Pathology of The Ohio State University, Columbus, Ohio, USA (Barth *et al*., 2005).

Recently, scientists at the Department of Organic Chemistry at the Georg-August-University of Göttingen, Germany, have been developing a new compound class for the use in BNCT (www.mbm.uni-goettingen.de/Projekte/). This new class of substances allows using BNCT in a tissue and tumour specific manner. This way, radiation dose and side-effects are reduced while enhancing efficacy. A patent application covering this compound class and its use was filed.

a) Computational dosimetry and treatment planning for BNCT. Calculation of neutron field quality for accelerator-based neutron source.

In neutron beam development, a variety of optimization parameters have been used by the research groups resulting in beams being quite different from each other. Then, the design, development, testing, patient pharmacokinetics and the evaluation of the results from these studies differ widely (Gupta *et al.,* 2003). Also, the clinical trials involving patient treatments vary in their dose escalation strategies, treatment planning methodologies, and the reporting of data. Therefore, it is necessary to standardize each aspect of the design, implementation, and reporting of clinical trials (Gupta *et al*., 2003).

A calculation model of dosage in BNCT (Rassow *et al*., 1993), dose modification factors (Allen, 1993), and radiation oncology, biology, and physics perspective of BNCT (Dom, 1994; Gabel, 1994) have been reviewed. Different aspects have been applied to dosage in BNCT compared to that in the case of normal radiotherapy with photons, electrons or heavy particles such as neutrons. Complex geometrical calculations have been required with respect to ranges of the heavy particles smaller than a cell. Apart from the direct effects of radiation without ^{10}B, the dosage therefore depended on thermal neutron fluence, ^{10}B concentration, its extreme inhomogeneous macroscopic distribution in the tumour tissue, the cellular localization of the ^{10}B atoms in the large intercellular space, the cell membrane, within cytoplasm or the cell nucleus, the geometrical probability of hitting the cell nucleus, and that such a hit finally resulted in a cell killing, and a Poisson statistical enhancement factor, which described the dose-effect relation for cell survival. The required calculations were demonstrated in the case of a normal and a tumour cell type, each with representative cell diameter and nucleus size (Rassow *et al*., 1993). Obviously, the microscopic distribution of ^{10}B atoms was considered one of the most critical parameters.

The role of various microscopic dose modification factors can be of critical importance in the evaluation of normal tissue tolerance levels. These factors are important in designing BNCT experiments and the selection of appropriate boron compounds. These factors have been defined and applied to the case of brain tumours with particular attention to capillary endothelial cells and oligodendrocytes (Allen, 1993).

Computational dosimetry and treatment planning for BNCT have been reviewed (Nigg *et al*., 1997). Because of the more complex nature of the problem, the computational methods used for treatment planning in photon radiotherapy can not be applicable to BNCT. The required methods have been developed and have been successfully used both for research applications as well as human trials. Computational geometry for BNCT applications have been constructed directly from tomographic medical imagery and computed radiation dose distributions have been shown in formats that are familiar to the radiotherapy community (Nigg *et al*., 1997).

The American and European studies are Phase I trials using BPA and BSH, respectively, as capture agents, and the Japanese trial is a Phase II study. Boron compound and neutron dose escalation studies have been planned, and these could lead to Phase II and possibly to randomized Phase III clinical trials that should provide data regarding therapeutic efficacy (Barth *et al*., 1999).

Quality assurance for performance and safety characteristics of the facility for BNCTin Petten, The Netherlands, have been compared with medical

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electron accelerators (Rassow *et al*., 2001, Sauerwein, 2001). Preliminary results of *in vivo* measurements done with a set of ^{55}Mn, ^{63}Cu and ^{197}Au activation foils for all single fields for the four fractions at all 15 treated patients showed with < ± 4% up to now a worse reproducibility than the used dose monitoring systems (± 1.5%) caused by influence of hair position on the foil-skull distance.

BNCT can be regulated according to the principles of quality assurance procedures for therapy with medical electron accelerators. The reproducibility of applied neutron fluence (proportional to absorbed doses) and the main safety aspects were equal for all teletherapy methods including BNCT (Rassow *et al*., 2001).

Low-energy light ion accelerator-based neutron sources (ABNSs) for the treatment of brain tumours through an intact scalp and skull using BNCT have been developed (Blue and Yanch, 2003). A major advantage of an ABNS for BNCTover reactor-based neutron sources is the potential for siting within a hospital. An ABNS for BNCT is composed of: (1) the accelerator hardware for producing a high current charged particle beam, (2) an appropriate neutron-producing target and target heat removal system (HRS), and (3) a moderator/reflector assembly to render the flux energy spectrum of neutrons produced in the target suitable for patient irradiation. Progress has been made on the design, manufacture, and testing of these three components.

Both electrostatic and radio frequency linear accelerators of reasonable cost (*ca.* 1.5 M dollars) appear to be able to produce charged particle beams, with combinations of accelerated particle energy (a few MeV) and beam currents (*ca.* 10 mA) that are suitable for a hospital-based ABNS for BNCT. The specific accelerator performance requirements depend upon the charged particle reaction by which neutrons are produced in the target and the clinical requirements for neutron field quality and intensity. The accelerator performance requirements are more demanding for beryllium than for lithium as a target. However, beryllium targets are more easily cooled. Target HRSs that are based on submerged-jet impingement and the use of microchannels have emerged as viable target cooling options. Neutron fields for reactor-based neutron sources provide an obvious basis of comparison for ABNS field quality (Blue and Yanch, 2003).

Monte Carlo calculations of neutron field quality for an ABNS and an idealized standard reactor neutron field (ISRNF) have been compared (Blue and Yanch, 2003). The comparison showed that with lithium as a target, an ABNS can create a neutron field with a field quality that is significantly better (by a factor of *ca.* 1.2, as judged by the relative biological effectiveness-dose that can be delivered to a tumour at a depth of 6 cm) than that for the ISRNF. Also, for a beam current of 10 mA, the treatment time is calculated to be reasonable (*ca.* 30 min) for the boron concentrations that have been assumed (Blue and Yanch, 2003).

b) Fast Neutron Radiotherapy.

BNCT uses a thermal/epithermal neutron beam for irradiation, while boron neutron capture potentiation uses the addition of the captures in a fast neutron irradiation.

The fields of BNCT and fast neutron radiotherapy have been reviewed (Laramore, 1997). Design and construct of non-reactor-based epithermal neutron sources have been developed to deploy this technology to major medical centers if the clinical research proves successful.

Fast neutron radiotherapy is a mature field with selected clinical indications for locally advanced salivary gland tumours and inoperable sarcomas of bone and soft tissue. Clinical trials for locally advanced prostate cancer and other tumours have been reviewed (Laramore, 1997). A clinical trial for BNC enhancement of fast neutron for nonremoved glioblastomas has been analysed in the Department of Radiotherapy of the Hopital du Hasenrain at Mulhouse, France (Pignol *et al*., 1999).

Research is in progress about the development of advanced boron agents and neutron sources, other than nuclear reactors, for the treatment of a variety of cancer types using novel ^{10}B delivery methods (Hawthorne, 1998).

Studies have been carried out in both normal and neoplastic tissues to characterize the relative biological effectiveness of each radiation component. In terms of fractionation effects, BNC irradiation modalities are comparable with other high-LET radiation modalities such as fast-neutron therapy (Coderre and Morris, 1999). There was no appreciable advantage in increasing the number of daily fractions of thermal neutrons beyond two with regard to sparing of normal tissue in the rat spinal cord model.

c) Boron Neutron Capture Synovectomy (BNCS) .

Radiation synovectomy, the destruction of inflamed synovial tissue using radioactive substances, has been shown to be an effective approach for the treatment of severe cases of rheumatoid arthritis (Valliant *et al*., 2000).

The limited effectiveness of pharmaceutical and surgical methods led to the evaluation of BNCT as an alternative treatment technique for rheumatoid

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arthritis. This approach, which is referred to as boron neutron capture synovectomy (BNCS), involves using the daughters of the boron neutron capture reaction to ablate arthritic tissue, thereby preventing further damage to surrounding structures (cartilage, bone, etc.).

The advantages of BNCS over radiation synovectomy is that the ionizing events can be made to be highly localized (through the use of a highly selective targeting agent) and, because the boron-10 delivery vehicles are stable (*e.g.*, not radioactive) both before and after irradiation, they will minimize damage to healthy tissue if they leak from the treatment zone (Valliant *et al*., 2000). Furthermore, the non-radioactive boron compounds pose no contamination hazard, thereby implifying administration of the treatment.

d) *In vivo* imaging of the neutron capture therapy agent BSH using ^{10}B MRI.

In vivo imaging of the BNCT agent BSH in mice using ^{10}B Magnetic Resonance Imaging (MRI) has been reported (Bendel *et al*., 2001). Thus, ^{10}B-enriched BSH was injected into the tail vein of mice with implanted M2R melanoma xenografts and the first *in vivo* images using 3D gradient echo ^{10}B MRI were obtained.

^{10}B NMR spectroscopy, localized mainly to the tumour by virtue of the use of a small surface coil, was applied to measure the T_1 (2.9 ± 0.3 ms) and T_2 (1.75 ± 0.25 ms) values of the ^{10}B signal (Bendel *et al*., 2001). The MRI experiments detected levels of about 20 ppm (microg boron/g tissue) at 6 x 6 x 6 mm spatial resolution in a total scan time of 16 min (Bendel *et al*., 2001).

e) Detection and identification of molecules used for BNCT by ^{10}B and ^{11}B NMR.

Bendel (2005) from the Chemical Research Support Department of The Weizmann Institute of Science of Rehovot, Israel, has reviewed the detection and investigation of molecules used for BNCT by ^{10}B and ^{11}B NMR.

NMR research efforts have been applied in two directions: (1) to investigate the metabolism and pharmacokinetics of BNCT agents *in vivo*, and (2) to use localized NMR spectroscopy and/or MRI for non-invasive mapping of the administered molecules in treated animals or patients (Bendel, 2005). While the first standpoint can be pursued using ^{11}B NMR for natural-abundance samples (80% ^{11}B / 20% ^{10}B), molecules used in the actual treatment are > 95% enriched in ^{10}B, and must therefore be detected by ^{10}B NMR. Both ^{10}B (spin 3) and ^{11}B (spin 3/2) are quadrupolar nuclei, and their typical relaxation times, in common BNCT agents in biological environments, are rather short.

The first attempts at ^{11}B NMR and MRI detection of BNCT agents in biological tissue were performed over a decade ago. Since then, results from ^{11}B MRI in laboratory animals and in humans have been reported, and ^{11}B NMR spectroscopy provided interesting and unique information about the metabolism of some BNCT agents in cultured cells. ^{10}B NMR has been applied either 'indirectly' (in double-resonance experiments involving coupled protons), but also by direct ^{10}B MRI in mice. However, no results involving the NMR detection of ^{10}B-enriched compounds in treated patients have been reported yet (Bendel, 2005).

f) SIMS imaging of amino acids.

Ion microscopy (IM), a mass spectrometry based isotopic imaging technique, is uniquely suited for ion transport related problems in biological systems. IM can image the transport and distribution of both major and minor elements (isotopes) at subcellular resolutions. The images of major elements such as K, Na, Cl, etc., can be viewed directly and recorded in real-time from the microchannel plate-fluorescent screen detector of the instrument. The low concentration physiologically important elements, such as Ca, require about one minute of integration for good quality imaging. The isotopic imaging capability of IM provides a unique approach for the use of stable isotopes as tracers. Thus, one can image both the endogenous and the transported isotopes independently.

Strict cryogenic sample preparations are essential for ion transport studies. Correlative imaging of the same cell with laser scanning confocal microscopy and IM can positively identify smaller cytoplasmic compartments such as the Golgi apparatus in calcium images. Morrison *et al*. (1994) have identified the Golgi apparatus as a calcium storing organelle.

Another unique application of IM is the imaging of boron from boronated drugs used in BNCT of cancer. IM is capable of rapid screening of potential drugs for BNCT (Morrison *et al*., 1994).

Accordingly, IM a potentially powerful technique for localization of isotopically labeled molecules. Recently, Chandra (2004) carried out a SIMS feasibility study, the double labeled (^{13}C and ^{15}N) amino acids *L*-arginine and phenylalanine being used for subcellular localization in cryogenically prepared cells.

The ability to localize isotopically labeled molecules in subcellular compartments, *via* the detection of the isotopic label is one of the most underused features of dynamic SIMS in biology and

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medicine (Hindie *et al*., 1992; Chandra and Morrison, 1995). This feature can be used in studying many aspects of the transport and metabolism characteristics of a broad variety of molecules including amino acids, sugars, lipids, and therapeutic agents. For both dynamic and static SIMS studies, it has now become clear that any subcellular localization studies of molecules must be made in cryogenically prepared reliable samples in order to avoid the artifactual relocation of molecules to high chemical affinity sites (Colliver *et al*., 1997; Chandra *et al*., 2000).

Since nitrogen does not have a radionuclide tracer isotope, SIMS techniques can become powerful tools for the detection of nitrogen-containing molecules. This can be a valuable contribution of SIMS, since proteomics currently dominates the field of mass spectrometry. The correlative optical imaging with SIMS can be a useful approach in the recognition of smaller structures, such as the nucleolus.

Amino acids labeled with stable isotopes can be used as tracers for studying their transport and metabolism in distinct subcellular compartments with SIMS. Further studies of phenylalanine uptake in human glioblastoma cells may have special significance in BNCT as a boron analogue of phenylalanine because BPA is a clinical approved compound for the treatment of brain tumours (Chandra, 2004).

g) Endothelial cells, and liposomes.

Targeting liposomes to tumour endothelial cells for BNCT proved to be efficient.

The growth of solid tumours strongly depends on the growth of new blood vessels in the process of angiogenesis (Griffioen and Molema, 2000). Inhibition of angiogenesis has shown to be useful for the inhibition of tumour growth. Moreover, specific targeting of angiogenic endothelium for the induction of damage to tumour endothelial cells or the induction of blood clotting has resulted in strong anti-tumour activity (Huang *et al*., 1997; Arap *et al*., 1998; Schiffelers *et al.*, 2003). Recent studies pointed towards a critical role of endothelial cell apoptosis on the response of the total tumour on radiotherapy (Folkman and Camphausen, 2001; Garcia-Barros *et al*., 2003), indicating that tumour endothelial cells are sensitive to radiation-induced damage. The latter was true at least *in vitro* for treatment with *beta*-particles that induced a cytostatic effect on endothelial cells (Fareh *et al*., 1999). Somatostatin-mediated targeting of ^{111}In to angiogenic endothelium strongly inhibited angiogenesis in an *in vitro* angiogenesis assay and represents one of the first angiogenesis targeted radiotherapy approaches (Gulec *et al.*, 2001).

The advantages of targeting radiotherapy to angiogenic endothelium are: (1) Endothelial cells are accessible for circulating drugs or drug carriers. (2) Endothelial cells are genetically stable and are not proned to develop resistance to drugs or radiation treatment. (3) At angiogenic sites endothelial cells express several cell surface proteins that allow for specific recognition of these tumour blood vessel endothelial cells (Griffioen and Molema, 2000). (4) Damage to a few endothelial cells or infarction of a single tumour blood vessel may result in a strong growth inhibitory effect on the surrounding tumour cells that depend on that particular blood vessel for their supply of nutrients and oxygen.

Liposomal targeting devices (Mastrobattista *et al*., 1999; Koning *et al*., 1999, 2002) have been used to improve tumour specificity and to deposit the required compounds into endothelial cells of tumour vasculature (Schiffelers *et al.*, 2003).

In fact, vascular endothelial cells present an attractive new target cell type for BNCT of solid tumours. Recently, Koning *et al.* (2004) directed BNCT compounds specifically to tumour endothelial cells for the growth inhibition of angiogenic endothelium, the induction of damage to tumour blood vessels and at last, tumour regression.

These authors demonstrated that proliferating endothelial cells of human origin showed considerable sensitivity towards radiation treatment. Therefore, they studied the interaction of endothelial cell targeted ^{10}B-containing RGD-liposomes (Kok *et al*., 2002; Schiffelers *et al.*, 2003) with human umbilical vein endothelial cells, and tested the therapeutic activity after neutron irradiation. These *in vivo* studies showed that RGD-liposomes are an attractive carrier for the delivery of BNCT compounds to tumour vasculature.

h) Distribution of BPA and metabolic assessment in glioblastoma patients during BNCT treatment: a microdialysis study.

As it is known, BNCT is dependent on the selective accumulation of ^{10}B in tumour cells, so that the neutrons should be delivered when the ratio between the boron concentration in tumour cells to that in normal tissues reaches a maximum.

Pharmacokinetic modeling for BPA-fructose mediated BNCT has been performed, including ^{10}B concentration predictions and dosimetric consequences (Kiger III *et al*., 2003). Preliminary treatment planning and dosimetry for a clinical trial of NCT using fission converter epithermal neutron beam have been recenly analysed (Kiger III *et al*., 2004).

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THERAPY REQUIREMENTS

BNCT is based on the delivery of an stable isotope, ^{10}B, to the tumour and the subsequent induction of radioactivity by local irradiation with a neutron beam. BNCT has the advantage that it uses non-toxic isotopes that only locally, in the tumour area, are activated to produce radiation that is able to cause cell death by inducing double strand breaks in the cellular DNA.

Several requirements must be taken into account for this therapy to be effective: (1) A concentration of 20–30 μg $_5{}^{10}B$ atoms/g of tumour must be achieved, provided the ^{10}B concentration in surrounding normal tissue is significantly lower (< 5 μg of ^{10}B/g of cells); (2) a tumour/normal tissue ratio of the boron delivery agent greater than 1 is required; (3) the boron drug should be of low toxicity (Fairchild and Bond, 1985; Zamenhof *et al.*, 1992; Sauerwein *et al*., 2002); (4) the ideal drug for BNCT should be stable under physiological conditions.

In fact, considerably less boron concentration than 20-30 μg ^{10}B atoms/g of tumour is required for effective cell damage when it localizes in close proximity to the cell nucleus (Gabel *et al*., 1987; Hartman and Carlsson, 1994; Ye, 1999; Hartman *et al*., 2000).

In the period between 1994 and 1999, BNCT researchers at Harvard-MIT carried out clinical studies involving patients with glioblastoma, melanoma metastatic to the brain, or subcutaneous melanoma of the extremities (Palmer *et al.*, 2002; Busse *et al*., 2003). Two patients receiving treatment for brain tumours developed a fatal acute respiratory distress syndrome (ARDS); one other patient developed an acute pneumonitis, but recovered after intensive supportive care. It was not clear whether the ARDS was due to the radiation dose to the lung. This would have implied that at least some part of the lung received a total dose nearly equal to a single photon dose of 8 Gy (Van Dyk *et al*., 1981).

In tissue, BNC irradiation produces a mixture of radiation qualities that differ in their LET characteristics and hence in their biological effectiveness. To express the total BNCT dose in photon-equivalent units it is necessary the experimental determination of weighting factors for each of the high-LET components (Coderre and Morris, 1999). Thus, the total, weighted BNCT dose is

$$D_w = w\gamma D\gamma + w_T D_T + w_F D_F + w_B D_B$$

where $w\gamma$, w_T, w_F and w_B are weighting factors for the photon, thermal neutron, fast neutron and the ^{10}B absorbed doses, respectively. In lung, the biological effectiveness weighting factors are critical to estimation of the total weighted dose to the lung.

Effects of BNC irradiation on the normal lung of rats have been recently reported (Kiger *et al*., 2004). The whole lung of rats was irradiated with X-rays, thermal neutrons, or thermal neutrons in the presence of BPA. Preliminary data indicated the biological effectiveness factor for BPA in the lung is *ca*. 1.5.

X-ray doses of 12 Gy to the whole rat lung have been reported to produce 100% response using the breathing rate assay. A beam delimiter was designed to deliver an adequate thermal neutron flux to the lung region while shielding the nearby radiosensitive tissues. The dose contribution from the $^{10}B(n,\alpha)^7Li$ reaction was calculated using the relationship: Dose (cGy) = 8.66×10^{-6} F Φ, where F is the weight fraction of ^{10}B in the tissue, and Φ is the thermal (2200 m/s) neutron flow (n/cm^2) determined by subtracting the bare and cadmium covered foil activities.

Simulations with the MITR-II thermal neutron beam showed that the collateral regions could be shielded effectively, and the approach taken to deliver a more uniform thermal neutron flow to the lung volume (≈ 50 cm^3) was to irradiate using parallel opposed fids. This approach delivered the specific dose to the lung volume with a dose variation of *ca*. ± 10%. Measurements of changes in breathing rate indicated that the biological effectiveness of the MITR-II thermal neutron beam was 1.2. This indicated that the biological effectiveness of the high-LET component of the dose (primarily thermal neutron capture in nitrogen) was *ca.* 2.2, assuming a photon weighting factor of 1.0. If the initial dose effect curve for thermal neutrons in the presence of BPA was extrapolated, parallel to the fitted curves, the preliminary estimate of the ED_{50} would be 7.5 Gy. This would indicate that the CBE factor for BPA in the lung was *ca*. 1.5. This value was similar to the value of 1.3 calculated for the CNS and considerably lower than the values calculated for BPA of 3.7 in the skin and 4.9 in the oral mucosa (Coderre and Morris, 1999). These data suggested that the BPA boron distribution in the lung was more similar to that in brain than to skin or oral mucosa, leading to the speculation that the radiation damage in lung could be due to damage to the lung vasculature.

THERAPEUTIC FIGURES

The performance of BNCT beams can be described by three figures of merit, which were developed by the MIT/Harvard BNCT group (Harling *et al.*, 1989; Zamenhof *et al.*, 1975, 1989). First is the **advantage depth** (**AD**) which provides a measure of the maximum useful depth for therapeutic benefit.

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Advantage depth is defined as the depth in tissue at which the total therapeutic dose is equal to the maximum total background dose. The total therapeutic dose is the sum of the total background dose and the $^{10}B(n,alpha)^{7}Li$ dose. A maximum advantage depth (AD_{max}) occurs when the boron dose ratio between the tumour and the healthy tissue/blood is infinite. However, more realistically this ratio is 3/1 to 4/1. Moreover, it has been shown that there is a geometrical dose absorption factor of about three to one for those boron reactions that are started in the microvasculature of the brain (Rydin *et al.*, 1976).

By using an *effective* tumour/blood ratio of 10/1, one can represent a reasonable boron dose partition between tumour and normal tissue, for boron compounds which have a tumour/blood ratio of 10/3 and negligible concentration in normal tissue surrounding the capillaries. The currently accepted figure of merit is simply AD. Then, AD is the depth at which the maximum dose to normal tissue equals the dose to tumour. The dose to normal tissue includes an estimate of dose from B-10 in normal tissue.

The second figure of merit in characterizing a BNCT beam, is the concept of **advantage ratio** (**AR**). The AR gives a measure of a particular treatment beam's ability to minimize integral dose to normal brain when a tumouricidal dose is delivered to brain tumour. The one-dimensional AR is defined as the integral dose that would be delivered to tumour tissue if it were uniformly distributed within the brain, divided by the integral dose that would be delivered to normal brain, along a particular one-dimensional axis through the brain. Normally, the axis of greatest interest corresponds to the central axis of the treatment beam.

The third figure of merit is the **advantage depth dose rate** (**ADDR**), which is the RBE dose rate to tumour defined at the advantage depth. From the previous definition of AD, the ADDR is the maximum RBE dose rate to normal tissue. The ADDR was developed primarily as a clinically meaningful neutron beam intensity criterion for epithermal neutron beam design studies.

These three figures of merit provide a method for comparing and evaluating the neutron beams for BNCT.

Targeted delivery of boron to tumours is a critical prerequisite for successful BNCT. Strategies that involve synthetic chemical approaches and biochemical and biophysical approaches are used to meet this requirement:

DEVELOPMENT OF BNCT AGENTS

Boron Neutron Capture Therapy (BNCT) is a bimodal cancer treatment based on the selective accumulation of ^{10}B in tumours and concurrent irradiation with thermalized neutrons. The short-range, high-LET radiation produced by the capture of neutrons by ^{10}B could potentially control tumour while sparing normal tissue if the boron compound targets tumour selectively within the treatment volume.

Extensive research has been carried out to develop potential BNCT agents. Their properties should ideally include high selectivity for and retention in malignant cells, low systemic toxicity, and sufficient bioavailability for tumour cell targeting (Soloway *et al.*, 1998).

The agents used in BNCT are supposed to have the following advantages over many conventional chemotherapeutics: (1) When irradiated with thermal neutrons, an unstable isotope ^{11}B is formed whose rapid decay yields local radioactivity and a thermal effect; (2) because the free path of the released particles is close to the cell diameter, the tissues outside the tumour should gain less damage; (3) local radioactivity and heat should be harmful for cells that, in the course of their natural history, acquired the determinants of altered response to many toxic stimuli.

Novel BNCT agents should have the following properties: (1) a high boron content, and (2) target specific receptors found only on tumours.

A higher specificity of damage would be achieved if the drugs accumulate mostly in cancer cells rather than in non-malignant counterparts. Therefore, optimization of agents for BNCT presumes the design of chemicals with improved accumulation/retention in cancer cells.

Historically, only two boron compounds, BSH and a BPA were allowed by the U.S. Food and Drug Administration (FDA) for the BNCT clinical trials in the treatment of GBM. However, both compounds have been recently found to be non-tumour specific for GBM (Sauerwein *et al.*, 2002). Research and development searching for new BNCT agents have been extensively performed from boron-10 enriched boric acid to the most recent BNCT drugs (Hosmane *et al.*, 2002).

Over the past 20 years, other classes of boron-containing compounds have been designed and synthesized that include the carborane containing amino acids (Radel and Kahl, 1996; Nakamura *et al*., 1998; Malan and Morin, 1998), carbohydrates (Sneath *et al.*, 1976; Giovenzana *et al.*, 1999), nucleic acid bases (Goudgaon *et al.*, 1994), nucleosides and nucleotides (Hawthorne, 1993; Li *et al.*, 1996; Liu *et*

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al., 1996; Su *et al*., 1997) , and peptides but also porphyrins and DNA intercalators/binders (Soloway *et al.*, 1998). High molecular weight delivery agents include monoclonal antibodies and their fragments, which can recognize a tumour-associated epitope, such as epidermal growth factor, and liposomes.

The rationale for their synthesis was that they may interact in a similar way with biological material as the naturally occurring compounds and are selectively incorporated in malignant cells.

POLYHEDRAL BORATE ANIONS AND CARBORANES

BNCT reagents are usually based on 10- to 12-vertex borane cages. A new synthetic route to boron-10 enriched pentaborane(9) from boric acid and its conversion to *iso*-$^{10}B_{18}H_{22}$ has been developed (Adams *et al*., 2002).

Pentaborane(9) is an important synthon for a number of higher polyhedral borane cages, including $[B_9H_{14}]^-$ (Savory and Wallbridge, 1973), $[B_{11}H_{14}]^-$ (Hosmane *et al*., 1987), $[B_{12}H_{12}]^{-2}$ and other cage expanded borane anions (Lawrence *et al*., 1986), and the neutral decaborane, $B_{10}H_{14}$ (Toft *et al*., 1982). The corresponding ^{10}B-enriched species are the precursors for a number of potential boron drugs for use in the clinical trials using BNCT.

The small-cage C_2B_4 carborane systems were studied mainly due to the available supply of the pentaborane(9) (B_5H_9) from US-government, which could then be reacted with a suitable alkyne to form the carborane. At present, since that source is no longer so available a new synthesis of pentaborane(9) was developed, e.g., a one-pot method.

Adams *et al.* (2002) explored alternative routes to ^{10}B-enriched polyhedral boranes starting from boric acid, $H_3{}^{10}BO_3$. A new preparation of boron-10 enriched pentaborane(9) and its one-pot conversion to cage-fused neutral *anti*-$^{10}B_{18}H_{22}$, a precursor in BNCT research, was reported.

Thus, the boron-10-enriched boric acid, $H_3{}^{10}BO_3$, was converted to the corresponding sodium borohydride, $Na^{10}BH_4$, in quantitative yields, *via* butyl borate, $(n\text{-}OBu)_3{}^{10}B$, first and then reacting it with NaH in mineral oil at 250°C. The subsequent oxidation reaction of $Na^{10}BH_4$ with I_2 in diglyme, followed by the addition of dioxane, gave the dioxane-complexed sodium salt of octahydrotriborate (-1), $Na[^{10}B_3H_8].3(C_4H_8O_2)$, in almost quantitative yields (Fig. 2).

$$H_3{}^{10}BO_3 + 3\ n\text{-BuOH} \rightarrow (n\text{-OBu})_3{}^{10}B + 3\ H_2O$$
$$4\ NaH + (n\text{-OBu})_3{}^{10}B \rightarrow Na^{10}BH_4 + 3\ NaOBu$$
$$Na^{10}BH_4 + I_2\ (\text{in diglyme/dioxane}) \rightarrow Na[^{10}B_3H_8].3(C_4H_8O_2) + 2\ H_2 + 2\ NaI$$

Figure 2. Preparation of dioxane-complexed sodium salt of octahydrotriborate (-1).

These synthetic routes were established in the early 1950s and 1970s, and are still the best available methods for these species.

Treatment of $Na[^{10}B_3H_8].3(C_4H_8O_2)$ with $NiCl_2$ in anhydrous benzene or heavy mineral oil at 110°C gave the corresponding ^{10}B-enriched pentaborane(9), $^{10}B_5H_9$ (Fig. 3).

$$2\ Na[^{10}B_3H_8].3(C_4H_8O_2) + NiCl_2 \xrightarrow[110^oC/12\ h]{\text{- benzene or heavy mineral oil}} {}^{10}B_5H_9 + 2\ H_2 + C_4H_8O_2.{}^{10}BH_3$$
$$\text{- } 2\ NaCl/\text{-}Ni^o$$
$$\text{- } 2\ C_4H_8O_2$$

Figure 3. Preparation of pentaborane(9).

The reaction of natural pentaborane(9) has been used for the syntheses of a number of cage-expanded boron hydrides including the $[B_9H_{14}]^-$ ion. Therefore, the ^{10}B-enriched pentaborane(9) was converted to lithium or sodium salt of the corresponding $[^{10}B_9H_{14}]^-$ *in situ* and was reacted further with anhydrous $NiCl_2$ in 2:1 molar ratio to produce the neutral fused borane, *anti*-$^{10}B_{18}H_{22}$, in 42% yield as a single pure isomer. The natural analogue of this species, along with its *syn*-isomer as a mixture, has been synthesized by the oxidation reaction of the [*closo*-$B_{10}H_{10}]^{2-}$ ion, derived from decaborane.

Since the biomolecules carrying large-cage borane moieties have the potential to deliver more ^{10}B atoms

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to the specific tumour cells for an effective BNCT in cancer treatment (Larsson *et al.*, 1997; Soloway *et al.*, 1998), the synthetic route is of special interest in that its ^{10}B-enriched species can be prepared in sufficient quantities in the laboratory as a precursor to large-cage boron analogues including those of the fused-cage $[^{10}B_{22}H_{22}]^{2-}$ ion (Hosmane *et al.*, 1998; Volkov *et al.*, 1999).

A synthesis of a $[B_{22}H_{22}]^{2-}$ polyhedral cage from $NaBH_4$ (or ^{10}B-enriched $NaBH_4$) was reported (Adams *et al.*, 2002). This structure may be considered a hybrid of decaborane ($B_{10}H_{14}$) and the dodecaborane cage ($[B_{12}H_{12}]^{2-}$), the two principal precursors for many BNCT agents. Since an estimated 10^{18} atoms (10-30 μg ^{10}B/tumour) is required for tumour destruction, polyhedral derivatives of the $[B_{22}H_{22}]^{2-}$ cluster, which has nearly doubled the boron content of normal carboranes, may be useful as analogues of promising carborane-derivatized amino acids, nucleosides, nucleotides, porphyrins, antibodies, etc.

One of the advantages in using carboranes over other more common ligands is that they can be conveniently generated from the stable ($C_2B_9H_{12}^-$) ions in alkaline solutions and they can be prepared having a wide range of different functional groups.

Ortho, *meta* and *para* isomers of dicarba-*closo*-dodecaboranes are known. These isomers differ in the relative positions of the carbon atoms in the cluster. The structures of the three isomers and the IUPAC numbering for *ortho*-carborane are shown in Fig. 4. The clusters have nearly icosahedral geometry in which each of the carbon and boron atoms are hexacoordinated.

Ortho-carboranes are prepared by the reaction of acetylenes, including both mono and disubstituted alkynes, with $B_{10}H_{12}L_2$, which is generated, often *in situ*, from decaborane ($B_{10}H_{14}$) and a weak Lewis base (L = CH_3CN, RSR, R_3N). The reaction of $B_{10}H_{12}L_2$ with acetylenes can be performed in the presence of a wide range of functional groups, *e.g.*, esters, halides, carbamates, ethers, nitro, and other groups.

The *meta* and *para*-carborane isomers are prepared by thermal isomerization of *ortho*-carborane under an inert atmosphere. At 400-500°C *ortho*-carborane is converted to the *meta*-isomer, which in turn rearranges to the *para*-isomer between 600°C-700°C. All three carborane isomers and decaborane are commercially available.

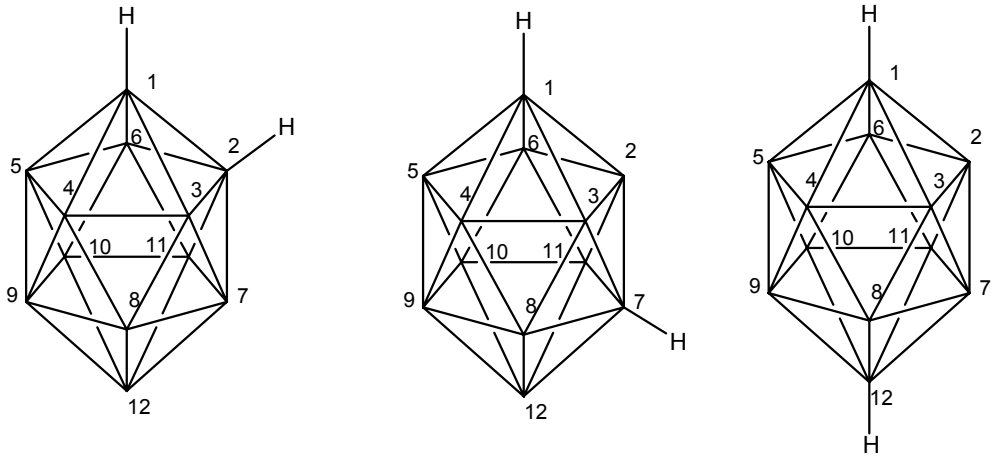

Figure 4. Structures of *ortho*, *meta* and *para* isomers of dicarba-*closo*-dodecaboranes, and IUPAC numbering.

Alkoxide bases react with the B-3/B-6 and B-2/B-3 atoms of *ortho*- and *meta*-carboranes respectively yielding the more hydrophilic dicarbaundecaborate (1-) ions. The 7,8- and 7,9-*nido*-carboranes can also be formed using amines, such as pyrrolidine, and fluoride ion. These conditions are suitable for converting *closo*-carboranes to the more water-soluble *nido* clusters in the presence of alkoxide sensitive functional groups (Fig. 5).

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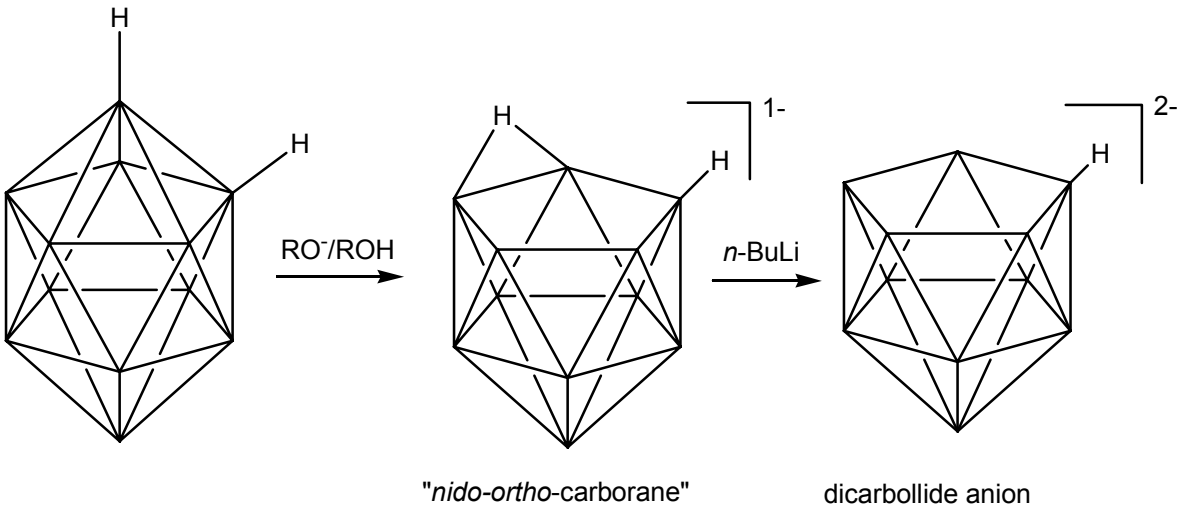

Figure 5. Conversion of *closo-ortho*-carborane to *nido-ortho*-carborane.

There has been interest in the syntheses of non-toxic, boron compounds for pharmacological applications (Vyakaranam *et al*., 2001a,b). As it is known, a number of amineborane adducts have shown promising activity for anticancer (Sood *et al*., 1992a,b; Spielvogel *et al.*, 1994), anti-inflammatory (Rajendran *et al.*, 1994), and antiosteoporotic drugs (Spielvogel *et al.*, 1979). These amineborane adducts can also be precursors for potential boron delivery agents to tumour cells in the treatment of cancer by BNCT (Spielvogel *et al.*, 1991; Tjarks *et al.*, 1992; Spielvogel *et al*., 1993; Malmquist and Sjöberg, 1996; Ghaneolhosseini *et al*., 1997a). Since several boron analogues of the phosphonoacetates are effective hypolipidaemic agents (Hall *et al.*, 1992), further studies about the corresponding phosphineboranes have been carried out.

Substituted-borano-phosphate nucleosides have been synthesized (Vyakaranam *et al.*, 2002a). Moreover, a convenient one-pot synthesis of triphenylphosphinecarbomethoxyborane has been reported (Vyakaranam *et al.*, 2002b), together with antitumour activity and structural investigation. The molecular geometry of this triphenylphosphinecarbomethoxyborane was unambiguously determined by single crystal X-ray analysis (Vyakaranam *et al.*, 2002b).

Therefore, the reaction of equimolar quantities of triphenylphosphine and trimethylaminecarbomethoxyborane (Spielvogel *et al.*, 1989), in anhydrous 1,2-dimethoxyethane (monoglyme), followed by extraction in ethyl acetate and purification by recrystallization using dichloromethane:pentane (8:2) solution led to triphenylphosphinecarbomethoxyborane (Fig. 6) in 63% yield, *via* a Lewis-base exchange reaction. The structure of the product showed that the triphenylphosphine moiety coordinated to the boron atom with the $COOCH_3$ group intact. The elemental analysis, IR spectra, and ^1H and ^{11}B NMR spectra (Wisian-Neilson *et al*., 1981) were all consistent with the formulation of $PPh_3BH_2COOCH_3$. For example, the ^{11}B NMR chemical shift at δ = -29:5 ppm was similar to a value of δ = -31.7 ppm found for Ph_3PBH_2CN.

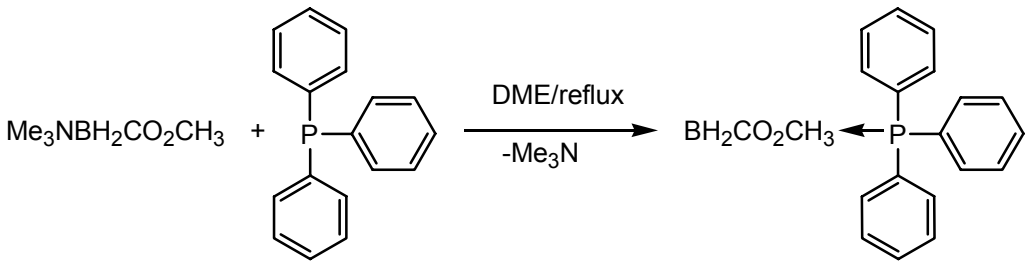

Figure 6. Synthesis of triphenylphosphinecarbomethoxyborane.

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Preliminary *in vivo* antitumour screens (Ehrlich Ascites in mice) of triphenylphosphinecarbomethoxyborane have been carried out at the US National Cancer Institute (NCI). Dosages of 20 mg/kg per day into the CF_1 male mice resulted in inhibition of tumour growth of 94% and the LD_{50} value was 963 mg/kg which proved that triphenylphosphine-carbomethoxyborane is relatively non-toxic.

Owing to this significant antitumour activity, syntheses of a number of triphenylphosphine-substituted borane adducts ($Ph_3P.\ BH_2X$, X = CN, COOH, CONHEt), using the general procedure outlined in Fig. 6 were carried out. Carboranyl derivatives of amineboranes and boron analogues of esters have been also synthesized (Rana *et al.*, 2003).

Recently, cyanuric chloride has been used as a scaffold for the synthesis of potential agents for BNCT containing a carborane cluster, a sugar moiety as hydrophilic arm and an amino acid for the conjugation with bioactive molecules. This approach is, in principle, particularly suitable for a combinatorial approach (Ronchi *et al.*, 2004).

New boron-rich building blocks have been synthesized for BNCT or energy-filtering transmission electron microscopy (TEM) (Raddatz *et al.*, 2004). Therefore, a new *ortho*-carborane acidic ketone, $[(XCH_2)_2CH]_2C{:}O$ (X = 1,2-dicarbadodecaboran-1-yl), has been prepared from a tetraalkynylated ketone by the addition of decaborane (Raddatz *et al.*, 2004). The keto group was then easily modified to yield two glycosides, which contained glucose or galactose, respectively, and a nucleotide.The cyclic ketone 2,2,6,6-$(XCH_2)_4$-1,4-cyclohexanedione 4-ethylene ketal was also synthesized (Raddatz *et al.*, 2004). X-ray diffraction analysis of the acyclic ketone indicated the presence of two toluene guest molecules per molecule of the host compound. These compounds can be used as building blocks for new biomolecules containing high-density carborane clusters (Raddatz *et al.*, 2004).

CORTICOSTEROID-CARBORANE ESTERS

It is worth to mention that steroid-carborane conjugates were synthesized to selectively deliver boron to arthritic tissue. These corticosteroid-carborane esters have been designed for the treatment of rheumatoid arthritis *via* boron neutron capture synovectomy (BNCS) (Valliant *et al.*, 2000). The key to BNCS, like BNCT, is the development of agents, which selectively target a sufficient quantity of boron to the target cells (inflamed synovial tissue in this case).

A carborane derivative of the corticosteroid hydrocortisone has been synthesized through the use of a BOP-Cl promoted esterification. (Fig. 7), and its crystal structure was determined (Valliant *et al.*, 2000).

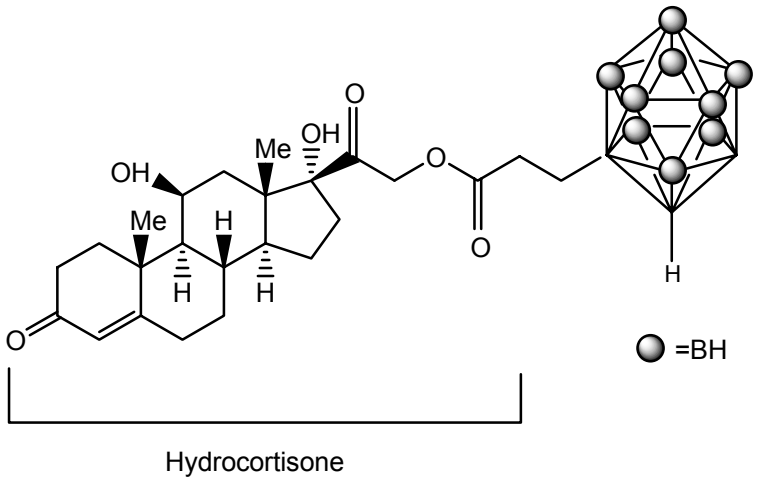

Figure 7. Carborane derivative of the corticosteroid hydrocortisone.

OLIGOMERIC PHOSPHATE DIESTERS (OPDS)

One approach to ^{10}B localization is based upon tumour-selective antibodies conjugated to boron-rich macromolecules ('trailers'). Boron-rich trailer molecules could also be used in conjunction with other tumour-targeting agents such as bioregulatory peptides or sex hormones or as boron-rich reagents in as bispecific antibody-mediated approach to localization (Hawthorne, 1991). Both structurally

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heterogeneous boron-rich trailers (Alam *et al.*, 1989; Pettersson *et al.*, 1989), and functionalized homogeneous trailers of precisely defined structure have been reported (Rana *et al.*, 2003).

Boron-rich oligomeric phosphate diesters (OPDs) are boron delivery vehicles for use in BNCT, which are obtained from selected carborane-containing dihydroxy precursors and assembled using phosphoramidite coupling chemistry. In addition, boron-rich OPDs are inherently hydrophilic or amphiphilic according to their anionic phosphate backbone.

Kane *et al.* (1993a) described the use of a conventional automated DNA synthesizer for the synthesis of large functionalized boron-rich OPDs trailers that are homogeneous and extremely hydrophilic. Solution-phase synthesis of oligophosphates containing up to 30 boron atoms was also reported (Kane *et al*., 1993b).

A homogeneous boron-rich 'trailer' compound has been synthesized, and its conjugation to a specific site of a tumour-directed antibody fragment (Fab-SH) has been described (Hawthorne, 1991).

Kane *et al*. (1993b) reported the stepwise solution-phase synthesis of short boron-rich oligophosphates, -7,8-$C_2B_9H_{11}]^-$ cage fragment. which may be useful as intermediates in the assembly of tumour-localizing boron-rich compounds for BNCT.

The use of derivatives of *o*-carborane (**1**) is pervasive in BNCT research, since these relatively stable boron-rich compounds can be readily functionalized. Furthermore, lipophilic *closo*-carborane derivatives can be converted under mild conditions to stable anionic *nido*- carborane derivatives (**2**; Fig. 8) which show high hydrophilicity. The oligophosphates are derived from the *o*-carborane diol (**3**), which is prepared by the condensation of dilithio-*o*-carborane with an excess of trimethylene oxide (90%). Yield refers to material that is homogeneous by NMR, TLC, and/or HPLC. All compounds have been appropriately characterized (HRMS, multinuclear NMR, etc).

Treatment of diol (**3**) afforded (**4**) (48%, Fig. 9) after chromatographic separation of the mixture of mono- and diprotected products and unreacted diol.

Closo-carborane, *o*-carborane, or carboranyl refer to derivatives of the *closo*-1,2-$C_2B_{10}H_{12}$ cage, while *nido*-carborane refers to derivatives of the anionic [*nido*

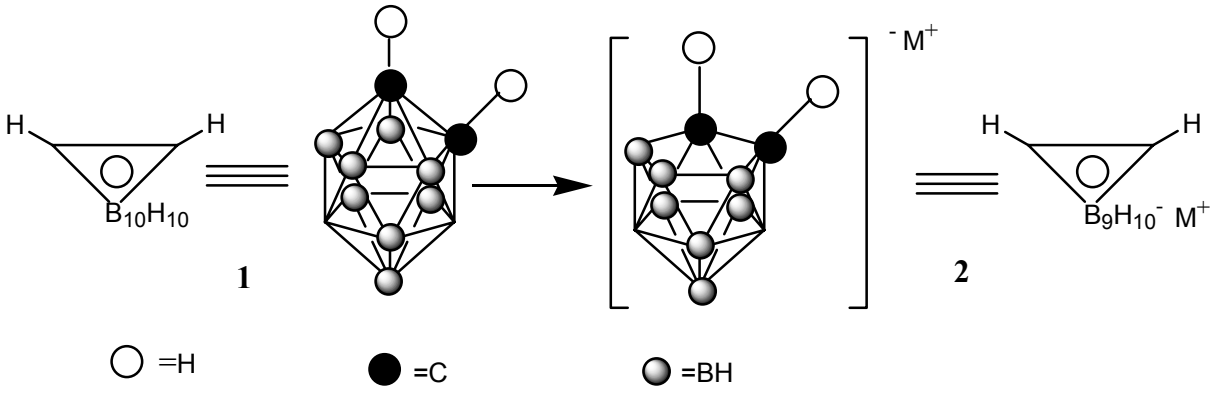

Figure 8. Conversion of *closo*-carborane derivatives to anionic *nido*-carborane derivatives.

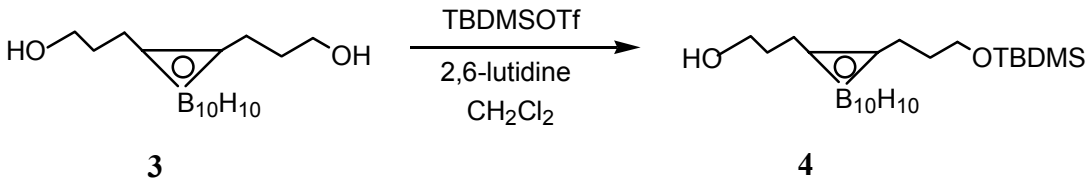

Figure 9. Protection of one hydroxyl group of the *o*-carborane diol.

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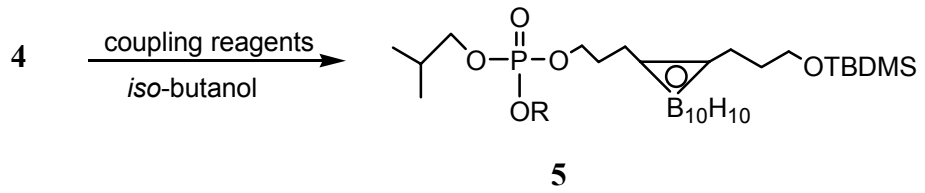

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Figure 10. Conversion of monoprotected *o*-carboranyl diol to phosphotriesters.

Monoprotected *o*-carboranyl diol (**4**) was coupled with isobutyl alcohol under a variety of conditions, affording phosphotriesters (Fig. 10), *e.g.*, phosphotriester (**5**).

Kane *et al.* (1993b) studied the conversion of the hydrophobic phosphotriester (**5**) to anionic derivatives expected to be much more hydrophilic. Workup followed by cation exchange (Na^+ form resin) afforded sodium salt (**6**) in 87% yield (Fig. 11). Suspension of this anion in pyrrolidine at room temperature (1 h) resulted in the isolation (71% crude yield) of the desired anionic *nido*-carboranyl phosphate (**7**) together with a small amount of an alkene (elimination product **8**).

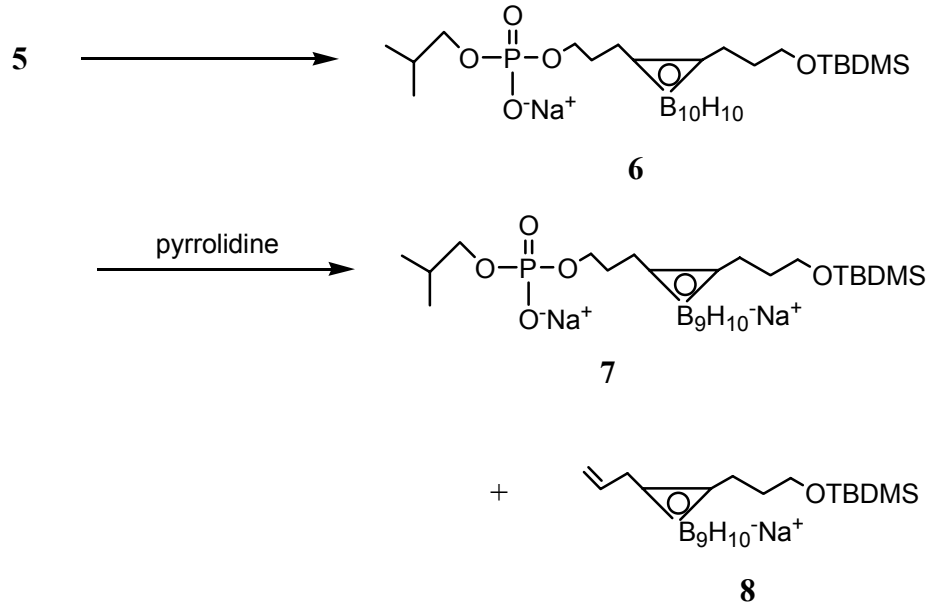

Figure 11. Conversion of the hydrophobic phosphotriester to hydrophilic anionic derivatives.

Thus, Kane *et al*. (1993b) have shown the viability of synthesizing small boron-rich oligophosphates in solution. This solution-phase strategy will be useful for the synthesis of large quantities of short boron-rich oligophosphates that can be further derivatized and as such affords a method for the production of highly hydrophilic boron-rich compounds for use in BNCT.

The most common method for automated DNA synthesis involved the stepwise coupling of 5'-*O*-(dimethoxytrityl)-3'-(*N*,*N*-diisopropylamino)-(*beta*-cyanoethyl)phophoramidite nucleoside derivatives (**9**). The boron-rich phosphoramidite (**10**), which was synthesized in three steps starting from *o*-carborane, was functionally equivalent to the nucleoside monomers in supporting oligophosphate synthesis (Fig. 12).

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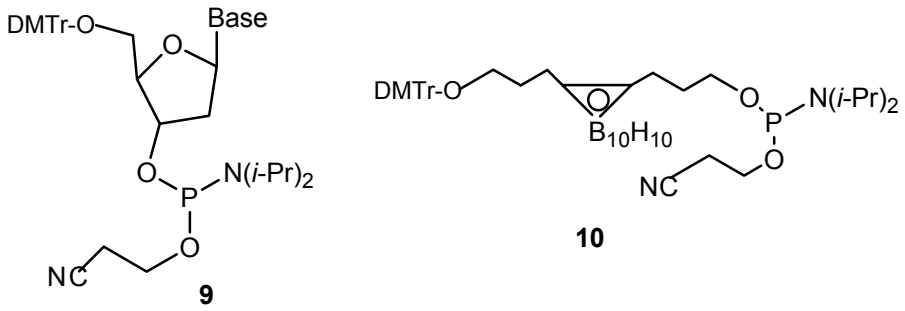

Figure 12. 5'-*O*-(Dimethoxytrityl)-3'-(*N*,*N*-diisopropylamino)-(*beta*-cyanoethyl) phophoramidite nucleoside derivatives (**9**) and the boron-rich phosphoramidite (**10**).

Boron-rich oligophosphates have been prepared on a μmol scale using (**10**) on a commercial DNA synthesizer and the standard coupling program (Kane *et al.*, 1993a). The flexibility of this approach to the synthesis of homogeneous functionalized boron-rich oligomers is evident in the varied compositions and sequences of oligomers.

After the completion of their synthesis, the boron-rich oligophosphates were removed from the CPG ('controlled pore glass') support by treatment with concentrated NH_4OH (*ca*. 5 min at room temperature), which also removed the protecting groups, affording free water-soluble boron-rich oligophosphates. ^{11}B NMR analysis of these oligomers revealed that the carborane residues retained their *closo* structure. Extended NH_4OH treatment (30 min at 80°C or 2.5 h at 66°C, conditions currently used for removing the base protecting groups in DNA synthesis), quantitatively converted the oligophosphates containing *closo*-carborane cages to their anionic *nido* derivatives. Thus, water-soluble oligophosphates containing either neutral *closo*-carboranes or anionic *nido*-carboranes were available, depending on the deprotection conditions used subsequent to oligomer synthesis. The oligomers were characterized in the *nido* form after ion exchange. The carboranes in these oligomers were typically > 95% *nido*, as shown by ^{11}B NMR.

Another useful method for oligomer characterization is polyacrylamide gel electrophoresis (20% acrylamide (3% bis), 7M urea). Moreover, several of these oligomers have been characterized by negative-ion electrospray mass spectrometry.

These authors have demonstrated a general method for the efficient synthesis of homogeneous boron-rich oligophosphates containing up to 400 boron atoms. It has been demonstrated the coupling of both amino and thiol functionalized oligomers to a free thiol group present on an antibody fragment (F(ab')) using suitable bifunctional cross-linking reagents. Biotin-functionalized oligomers have been also shown to bind to avidin *in vitro*.

The fast (< 5 min per coupling cycle), and efficient (> 98% coupling efficiency per step) automated methodology led to the synthesis of a vast library of designed boron-rich oligomers and others in which single strands of DNA or other biomolecules may be attached (Chen *et al.*, 1994; Guan *et al*., 1998).

Oligomers have been labeled with fluorescein and investigated *in vitro* by both microinjection of living cells (10^9 boron atoms ~ nominal BNCT dose) and in other experiments with permeabilized cells. TC7 cells, a subline of African green monkey kidney cells, were chosen because of their apparent resilience to the process of microinjection and the need to use a mammalian cell line. The objective of this study was to explore possible subcellular boron localization and distribution by both *closo*- and *nido*-OPDs in living cells using microscopy and a fluorescent marker (Nakanishi *et al.*, 1999).

ADP DERIVATIVES

The rationale for the design and synthesis of boron containing nucleosides is that such compounds may concentrate selectively in rapidly dividing tumour cells, and following their conversion to the corresponding nucleotides, may be trapped within the cell or, ideally, may be incorporated into nuclear DNA of tumour cells (Mishima, 1996). In the case of BNCT, such a nuclear localization of boron carrier, *e.g.*, boronated nucleoside, would be advantageous since the effect of neutron capture reaction in nucleus is 2-5 times greater than in cytoplasm (Hoshino *et al*., 1986).

The construction of a species involving direct P-$C_{(cage)}$-nucleotide linkages offers stability under physiological conditions required for any ideal drug for BNCT.

Boron-containing ADP and GDP analogues have been synthesized in good yields, *e.g.*, adenosine 5'-(Pa-boranodiphosphate) (ADPaB) and guanosine 5'-

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(Pa-boranodiphosphate) (GDPaB) (Lin *et al*., 2000). Their diastereoisomers were successfully separated by RP-HPLC, and chemical structures were established *via* spectroscopic methods. The isoelectronic substitution of borane (BH_3) for one of the non-bridging *O*-atoms in phosphate diesters should impart an increase in lipophilicity and change in polarity in ADPaB and GDPaB. The boronated nucleoside diphosphates could be employed for research of the stereochemical course and metal requirements of enzymic reactions involving ADP and GDP, and as carriers of ^{10}B in BNCT for the treatment of cancer (Lin *et al*., 2000).

The first carboranyl *bis*(adenosine diphosphate) (CBADP) has been recently synthesized (Vyakaranam *et al.*, 2003).

All new compounds were characterized by IR spectra, ^{1}H, ^{13}C, ^{11}B and ^{31}P NMR spectra and elemental analyses.

o-CARBORANES CARRYING 1,3,5-TRIAZINE UNITS

Substituted *o*-carboranes carrying 1,3,5-triazine units have been prepared as potential BNCT agents (Lee *et al.*, 2003). Symmetric 2,4,6-*tris*(2-R_1-*o*-carboran-1-yl)-1,3,5-triazines (R_1 = H, Me, or Ph) were prepared by reaction of cyanuric chloride with the corresponding lithiated *o*-carboranes (1:3 mol ratio) (Lee *et al.*, 2003). The same reaction in 1:1 and 1:2 mol ratios gave, 4,6-dichloro-2-(2-R_2-*o*-carboran-1-yl)-1,3,5-triazines and 6-chloro-2,4-*bis*(2-R_2-*o*-carboran-1-yl)-1,3,5-triazines (R_2 = H, Me). Prepared compounds were assayed for cytotoxicity and boron incorporation with B-16 melanoma cells.

OXONIUM DERIVATIVES

Tetramethylene oxonium derivatives of the dodecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ was a convenient precursor for the synthesis of functional compounds for BNCT (Sivaev *et al*., 2000).

Various derivatives of the $[B_{12}H_{12}]^{2-}$ anion containing hydroxyl, amine, acid, and amino acid functions were prepared by ring opening reactions of the tetramethylene oxonium derivative $[B_{12}H_{11}O(CH_2)_4]^{-}$ with different nucleophiles (Sivaev *et al*., 2000). This approach is suitable for the development of compounds to be used in tumour selective BNCT and as linkers for the attachment of radioactive halogen labels for radioimmunodetection and radioimmunotherapy.

AMINES AND POLYAMINES

Carboranyl polyamines for DNA targeting have been prepared. Therefore, three groups of internal and terminal *N*-substituted carboranyl spermidines/spermines were synthesized as potential BNCT agents for the treatment of malignant tumours (Cai and Soloway, 1996). *In vitro* studies have shown that these compounds retain the ability to displace ethidium bromide from calf thymus DNA, possess the ability for rapid uptake by F98 glioma cells, and have greater toxicity than spermidine/spermine, especially those with terminal *N*-substituted boron compounds (Cai and Soloway, 1996).

The preparation, *in vitro* toxicology and *in vivo* tissue distribution of other possible new compounds for BNCT have been recently reported (Bauer *et al*., 2002). The reaction of $B_9H_{13}SMe_2$ with primary amines yielded azanonaboranes, those of the type [3-(RNH_2)B_8H_{11}-5,6-μ-NHR] being water-soluble when hydrophilic groups R = $(CH_2)_3OH$ were incorporated. These groups were introduced by reaction of $B_9H_{13}SMe_2$ or its amino-derivatives with allylamines, followed by disiamylborane hydroboration-hydroxylation.

Five compounds with different numbers of hydroxypropyl groups were obtained: $[(HO(CH_2)_3NH_2)B_8H_{11}NHCH_3]$, $[(HO(CH_2)_3NH_2)B_8H_{11}NH(CH_2)_3OH]$, $[((HO(CH_2)_3)_2NH)B_8H_{11}NHCH_3]$, $[((HO(CH_2)_3)_2NH)B_8H_{11}NH(CH_2)_3OCH_3]$ and $[((HO(CH_2)_3)_2NH)B_8H_{11}NH(CH_2)_3OH]$ (Bauer *et al*., 2002).

New boron-containing polyamines were also synthesized: (aminoalkylamine)-*N*-(aminoalkyl)azanona- borane(11) derivatives $[H_2N(CH_2)_nH_2NB_8H_{11}NH(CH_2)_nNH_2]$, with n = 4-6 and 12, and $[H_2N(CH_2)_3H_2NB_8H_{11}NH(CH_2)_4NH_2]$, and their toxic effect was tested *in vitro* with V79 cells (El-Zaria *et al.*, 2002). (4-Aminobutylamine)-*N*-(4- aminobutyl)azanonaborane and (3-aminopropylamine)-*N*-(4-aminobutyl)-azanonaborane were less toxic *in vitro* (LD_{50} *ca.* 700 and *ca.* 1100 μM, respectively) than spermine, while (4-aminobutylamine)-*N*-isopropylazanonaborane with its hydrophobic *iso*-propyl group and those with n = 5, 6, and 12 were toxic under similar conditions (LD_{50} « 500 μM) (El-Zaria *et al.*, 2002).

PLATINUM (II)-AMINE COMPLEXES

Dinuclear platinum(II)-amine complexes of 1,7-dicarba-*closo*-dodecaborane(12) have been synthesized and their DNA-binding properties have been evaluated (Woodhouse and Rendina, 2001). Preliminary *in vitro* DNA-binding experiments indicated that the complexes are capable of targeting plasmid DNA.

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PORPHYRIN-MEDIATED BORON NEUTRON CAPTURE THERAPY

In the last decade several porphyrins have been proposed as highly promising boron delivery agents for the BNCT of tumours, in particular of malignant brain tumours (Barth *et al*., 1999; Vicente, 2001).

The accumulation of porphyrins and related compounds within tumour cells, being retained for long periods of time, along with their photophysical and photosensitizing properties led to their potential use in a variety of medical applications, *e.g.*, in photodynamic therapy (PDT) (Dougherty *et al.*, 1998), BNCT, radiation therapy (RT) and magnetic resonance imaging (MRI) (Vicente, 2001). PDT and BNCT are binary cancer therapies, which include activation of tissue-localized sensitizers with either light (PDT) or low-energy neutrons (BNCT). In both of these therapeutic methodologies, local tumour control with minimal side effects relative to other forms of cancer treatment (surgery, radiotherapy, chemotherapy) can be achieved. PDT involves the irradiation with light of a tumour-localized photosensitizer, which produces singlet oxygen and other cytotoxic species that cause irreversible photo-oxidative damage to tumour tissue (Rosenthal *et al*., 2001).

Photofrin, a porphyrin derivative, has been approved in the USA as a PDT drug by the U.S. Food and Drug Administration (FDA), and also in Japan, Canada and in eleven European countries. The FDA also approved Visudyne, another porphyrin derivative for the PDT treatment of the 'wet-form' of age-related macular degeneration. In addition to cancer treatment porphyrins are also under investigation for application in the treatment of a variety of other diseases (Vicente, 2001).

Some porphyrins and metalloporphyrins can interact with DNA and can induce DNA damage upon activation with light (Marzilli, 1990; Bustamante *et al*., 1994; Pasternack *et al*., 2001), *e.g.*, DNA damage has been reported to occur in PDT (Ramakrishnan *et al.*, 1989; Penning *et al.*, 1994). Porphyrins and their diamagnetic metal complexes can be easily detected in tumour tissues owing to their fluorescence (Mang *et al.*, 1993). Therefore, Vicente *et al*. (2002a) considered that boron-containing porphyrins can be designed and synthesized in order to be used for deliver of therapeutic amounts of boron to tumours not only for BNCT but also for PDT treatment of tumours.

nido-Carboranylporphyrins have been evaluated as potential sensitizing agents. Therefore, a tetra(4-*nido*-carboranylphenyl)porphyrin and its zinc(II) derivative were synthesized, evaluated their dark toxicity and the ability of the metal-free porphyrin to cause DNA photodamage *in vitro*.

The main porphyrin-DNA binding modes are intercalation and/or outside binding with or without self-stacking (Marzilli, 1990; Sari *et al*., 1990; Croke *et al*., 1993; Bustamante *et al*., 1994; Pasternack *et al*., 2001). Intercalation is usually favoured for positively charged porphyrin macrocycles, whereas a wide range of porphyrins have been observed to externally bind to DNA. It has been shown (Lauceri *et al*., 2001) that metal-free tetra(*nido*-carboranylphenyl)porphyrins of different basicity and with different distribution of the negatively charged *nido*-carborane groups at the porphyrin periphery, interact with DNA by self-aggregating to the outside. These types of porphyrins have shown to be phototoxic to cells *in vitro* and to be located intracellularly close to the cell nucleus (Vicente *et al*., 2002b). Vicente *et al*. (2002a) reported on the ability of a *nido*-carboranyl- porphyrin to induce DNA photodamage *in vitro*.

The conversion of porphyrins, such as **11**, into the corresponding water-soluble *nido*-carboranylporphyrins was accomplished with pyridine–piperidine (3:1 ratio) followed by piperidinium /K exchange (Vicente *et al*., 2000, 2002b).

The molecular structure of **11** (Fig. 13) exhibited a non-planar saddle conformation (Jentzen *et al*., 1997) of the porphyrin macrocycle. Zinc(II) porphyrins have shown to adopt domed, waved and planar conformations. The saddle conformation is particularly observed in sterically crowded β-substituted Zn(II)-*meso*-tetraphenyl porphyrins. The Zn-N and Zn-O bond lengths are within previously observed ranges for these values.

Both tetra(*nido*-carboranylphenyl)porphyrin and its zinc(II) complex were found to be relatively non-toxic in the concentration range 100–200 μM. Results were in agreement with those previously reported for carboranylated protoporphyrin IX derivatives (Woodburn *et al*., 1993; Hill *et al*., 1995). Lauceri *et al.* (2001) recently showed that tetra(*nido*-carboranylphenyl)porphyrin, despite its negative charge and the bulkiness of the carborane substituents, is able to interact with DNA by binding to its outside with self-aggregation. Vicente *et al.* (2002a) showed a light-catalyzed interaction of this porphyrin with DNA. The DNA damage determinations were based on the tail moments measured for K562 cells. These results were in agreement with those reported using boron-free porphyrin derivatives (McNair *et al*., 1997; Rousset *et al*., 2000).

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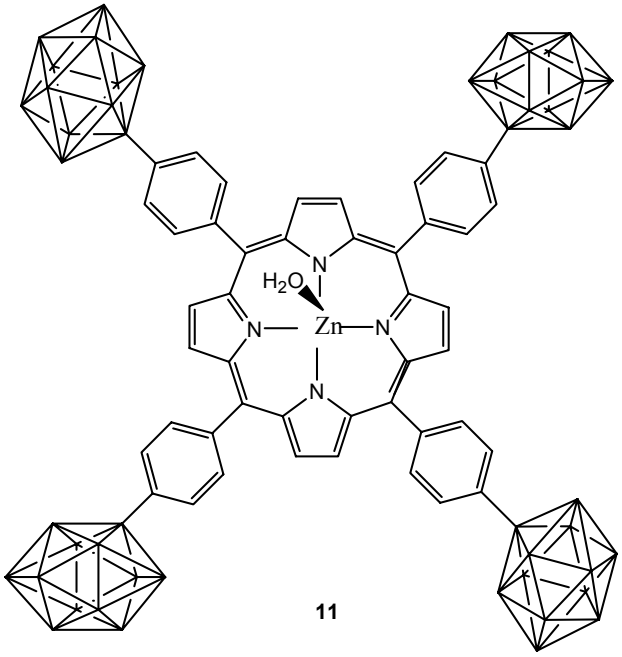

11

Figure 13. The molecular structure of Zn(II)-porphyrin (**11**) illustrating the saddle conformation of the porphyrin macrocycle, the axial H_2O ligand, and the four carborane–porphyrin linkages at the *para*-positions of the phenyl rings.

Carborane-containing porphyrins have been evaluated as tumour targeting agents for BNCT, being administered to mice bearing *s.c.* transplanted mammary carcinomas (Miura *et al*., 1998). The water-insoluble tetraphenylporphyrins (TTPs) were less toxic to mice, and delivered greater amounts of boron to tumour than those water-soluble TPPs and the heme analogues. Neither compound was toxic.

The boron delivered by each of the porphyrins tested remained in tumour tissue longer than did boron delivered by either BPA or BSH (Miura *et al*., 1998).

Plasma pharmacokinetics and tissue biodistribution of boron was performed after administration of a boronated porphyrin in dogs (Tibbitts *et al.*, 2000).

Copper and nickel chelates of these porphyrins were prepared and behaved identically *in vivo*, the former being suitable for imaging by ^{67}Cu-mediated single photon emission computed tomography (SPECT) to aid BNCT treatment planning (Miura *et al*., 1998).

BNCT with a new boron-porphyrin compound, STA-BX900, was tested in a rat brain tumour model (rat 9L glioma) (Shibata *et al*., 1998). The concentration of boron in the tumour was too low for a therapeutic effect, but relevant histopathological changes, such as necrosis, congestion and bleeding were observed in the tumours of the rats tested for BNCT (Shibata *et al*., 1998).

Chemistry and some medical applications of metal complexes as photo- and radiosensitizers have been reviewed (Ali and Van Lier, 1999), *e.g*., porphyrins, chlorins, bacteriochlorins, purpurins, benzochlorins, porphycenes, phthalocyanines, naphthalocyanines, and other compounds such as merocyanines.

The boronated porphyrin STA-BX909 was developed and evaluated *in vitro* and *in vivo* as a possible agent for BNCT (Matsumura *et al*., 1999). In the 9L rat brain tumour model, STA-BX909 achieved a higher boron tumour/blood ratio 24 h after injection in comparison to BSH. A boron concentration study in cultured glioma cell lines (U-251, U-87, 9L) demonstrated an increased boron concentration as a function of exposure time to STA-BX909, while the boron concentration remained stable with increasing exposure time to BSH (Matsumura *et al*., 1999). Use of a colony forming assay with thermal neutron irradiation revealed more cytotoxicity with STA-BX909 than BSH when the same concentration of ^{10}B was administered.

Several porphyrinoid-based drugs are being developed as phototherapeutic agents, X-ray radiation enhancers and boron neutron capture agents (Mody, 2000).

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Total syntheses of six *o*-carboranyl-containing *meso*-tetraphenylporphyrins bearing 33-44% boron by weight for potential application in BNCT of tumours, have been reported (Vicente *et al*., 2000). The synthesis of compounds containing polyhedral boron cages and porphyrin or phthalocyanine units connected covalently in one molecule has been reviewed (Bregadze *et al*., 2001). Current interest in the binding of polyhedral boron compounds to porphyrins and phthalocyanines is due to improved uptake and good persistence in tissues shown by these compounds.

BNCT of a murine mammary carcinoma using a lipophilic carboranyltetraphenylporphyrin has been reported (Miura *et al*., 2001). This is the first control of a malignant tumour *in vivo* by porphyrin-mediated BNCT. In mice bearing implanted EMT-6 mammary carcinomas, boron uptake using a single injection of either BPA or BSH was compared with either a single injection or multiple injections of the carboranylporphyrin CuTCPH (Miura *et al*., 2001). CuTCPH is a novel, non-toxic compound that may be advantageous in terms of selective and absolute delivery of boron to tumour tissues. For BNCT, boron concentrations averaged 85 mg ^{10}B/g in the tumour and 4 mg ^{10}B/g in blood 2 days after the last of six injections (over 32 h) that delivered a total of 190 mg CuTCPH/g bw. During a single 15, 20, 25 or 30 MW-min exposure to the thermalized neutron beam of the Brookhaven Medical Research Reactor, a tumour received averaged absorbed doses of *ca*. 39, 52, 66 or 79 Gy, respectively. A long-term (>200 days) tumour control rate of 71% was achieved at a dose of 66 Gy with minimal damage to the leg. Equivalent long-term tumour control by a single exposure to 42 Gy X rays was achieved, but with greater damage to the irradiated leg (Miura *et al*., 2001).

The boron-rich, water-soluble porphyrin-labeled carboranyl phosphate diester has been also synthesized by coupling of two carboranyl alcohols with 2-chlorophenoxy-phosphorus dichloride, followed by conjugation to an amine-functionalized tetraphenyl-porphyrin *via* an amide linkage (Maderna *et al*., 2002).

Optimization of agents for BNCT presumes the design of chemicals with improved accumulation/ retention in cancer cells. In particular, carboranyl-substituted porphyrins, the stable conjugates of macrocyclic porphyrins with complex boron-containing polyhedra, are considered good candidates for BNCT due to their uptake by cancer cells and high boron content. Moreover, the carboranylporphyrins for BNCT of cancer have been recently reviewed (Evstigneeva *et al.,* 2003). The proposed mechanisms of pharmacological effects of carboranyl-porphyrins make these compounds potentially appropriate for elimination of resistant tumour cells (Evstigneeva *et al*., 2003).

Biodistribution of a carborane-containing porphyrin as a targeting agent for BNCT of oral cancer in the hamster cheek pouch has been recently reported (Kreimann *et al.*, 2003). In previous studies, the hamster cheek pouch model of oral cancer for BNCT studies proved that absolute and relative uptake of the clinical employed boron compound BPA would be potentially therapeutic in this model and provided evidence of the efficacy of *in vivo* BPA-mediated BNCT to control hamster oral mucosa tumours with virtually no damage to normal tissue. Kreimann *et al.* (2003) reported the biodistribution and pharmacokinetics of a lipophilic, carborane-containing tetraphenylporphyrin (CuTCPH) in the hamster oral cancer model. CuTCPH was *i.p.*administered as a single dose of 32 μg/g bw (10 μg B/g bw) or as four doses of 32 μg/g bw over 2 days.

Various boronated porphyrins have been shown to preferentially target a variety of tumour types. Of the different porphyrins evaluated, copper tetra-phenyl-carboranyl porphyrin (CuTCPH) is a strong candidate for future preclinical evaluation. Recently, the responses of two critical normal tissues, skin and central nervous system (CNS), to BNC irradiation in the presence of CuTCPH were evaluated (Morris *et al.*, 2003). Standard models for the skin and spinal cord of adult male Fischer 344 rats were used. CuTCPH was administered by *i.v.* infusion at a dose of 200 mg/kg bw, over 48 h. Dose-response data were fitted using probit analysis and the doses required to produce a 50% incidence rate of early and late skin changes or myeloparesis (ED_{50} ± SE) were calculated from these curves

Biodistribution studies indicated very low levels of boron (< 3 μg/g) in the blood 3 days after the administration of CuTCPH (Morris *et al.*, 2003). Levels of boron in the CNS were also low (2.8 ± 0.6 μg/g) after 3 days. However, the concentration of boron in the skin was considerably higher at 22.7 ± 2.6 μg/g. This was primarily due to the very low blood boron levels (from CuTCPH). Analysis of the relevant dose-effect data gave compound biological effectiveness factors of about 1.8 for skin (moist desquamation) and about 4.4 for spinal cord (myeloparesis) for CuTCPH. These values were based on the BNC radiation doses to tissues calculated using the blood boron levels at the time of irradiation.

Then, CuTCPH-mediated BNC irradiation will not cause significant damage to skin and CNS at clinical

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relevant radiation doses provided that blood boron levels are low at the time of radiation exposure (Morris *et al.*, 2003).

meso-Substituted porphyrins carrying carboranes and oligo(ethylene glycol) units have been recently synthesized for potential applications in BNCT. Two series of carborane-carrying porphyrins were prepared, with additional functionality for attachment of uncharged potentially water-solubilizing polyethers (Frixa *et al.*, 2003). 3-(1,2-Dicarba-*closo*-dodecaboran(12)-1-ylmethoxy)benzaldehyde was prepared by protection of the aldehyde group of 3-(prop-2-ynyloxy)benzaldehyde as a dithioacetal, treatment with decaborane(14) and deprotection (Frixa *et al.*, 2003). Condensation with a 3-nitrophenyldipyrromethane gave a separable mixture of *meso*-(3-nitrophenyl)-*meso*-(3-carboranylmethoxyphenyl)porphyrins, resulting from extensive scrambling at the porphyrinogen stage. Likewise, condensation of 3-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)benzaldehyde with this dipyrromethane gave an analogous mixture of *meso*-(3-nitrophenyl)-*meso*-(3-carboranylphenyl)porphyrins. In this second series, the two regioisomeric *bis*(nitrophenyl)*bis*(carboranylphenyl)porphyrins could only be distinguished by X-ray crystallography, their NMR spectra being identical (Frixa *et al.*, 2003). The nitro groups of the mono(nitrophenyl)-porphyrins and the *bis*(nitrophenyl)-porphyrins were reduced to the corresponding amines with tin(II) chloride and the monoamines were coupled with a ω-methoxy poly(ethyleneglycol) chloroformate of mean molecular weight 600 to give the MeOPEGylated tricarboranyl porphyrins (Frixa *et al.*, 2003).

Recently, a highly water-soluble boronated porphyrin called TABP-1 was synthesized and *in vivo* evaluated as a possible BNCT agent in U-87 MG intracerebral human glioblastoma xenografts (Ozawa *et al.*, 2004). When the maximum tolerated dose (MTD: 15 mg/kg) of TABP-1was injected into the tail vein of athymic rats bearing intracerebral (ic) human glioblastoma U-87 MG xenografts, the compound accumulated preferentially in brain tumours compared to normal brain. However, the level of boron in the tumours was less than the 30 μg/g of tissue that is generally considered necessary for BNCT. Next, convection-enhanced delivery (CED) improved the boron distribution. CED produced relatively high tumour/normal brain ratios of *ca.* 5/1 for ipsilateral brain and *ca*. 26/1 for contralateral brain tissues at the 0.5 mg dose. Thus, therapeutic BNCT efficacy was achieved with minimal systemic toxicity or radiation-induced damage to normal tissue by administering TABP-1 using CED (Ozawa *et al.,* 2004).

Recently, synthesis and reactions of *meso*-(*p*-nitrophenyl)porphyrins have been also reported (Luguya *et al.,* 2004). The regioselective nitration of the phenyl groups of *meso*-tetraphenylporphyrin was achieved, using $NaNO_2$ and trifluoroacetic acid. The nitroporphyrins were further reduced to the corresponding aminoporphyrins under standard $SnCl_2$/HCl conditions. Reaction of 4,4',4''-(20-phenyl-21*H*,23*H*-porphine-5,10,15-triyl)*tris*-benzenamine with 1-formyl-*o*-carborane followed by reduction using $NaBH_4$ gave a novel *tri*carboranyl-porphyrin bearing amine linkages between the porphyrin and the carborane groups (Luguya *et al.,* 2004).

PHENANTHRIDINIUM DERIVATIVES

Boronated phenanthridinium derivatives have been synthesized for potential use in BNCT. Therefore, 3,8-diamino-5-[3-(12-(3-aminopropyl)-*p*-carboran-1-yl)propyl]-6-phenyl phenanthridinium chloride hydrochloride (**12**) was synthesized by reacting 3,8-diacetamido-6-phenyl- phenanthridine with 1-(3-*N*,*N*-*bis*(benzyloxycarbonyl)aminopropyl)-12-(3-iodopropyl)-1,12-dicarba- *closo*-dodecaborane in nitrobenzene at 120° for 4 days and subsequent removal of the protective groups using 33% HBr/AcOH (Ghaneolhosseini *et al.,* 1997b). Conversion to the chloride hydrochloride was accomplished by the action of HCl on the pseudo-base of **12**.

This boronated phenanthridinium derivative **12** was prepared to increase the hydrophilicity and decrease the non-specific cell-binding for potentially improved performance in BNCT (Ghaneolhosseini *et al.,* 1997b).

BENZIMIDAZOLES

2'-Carbaboranyl-2,5'-bi-1*H*-benzimidazoles containing ^{10}B-atoms and labeled with Se or the positron-emitting radionuclide ^{73}Se ($t_{1/2}$ = 7.1 h) have been synthesized for cancer treatment by BNCT and to compound-distribution measurements *in vivo* by positron-emission tomography (PET). Thus, 2,2'-{[2'-{4-[1,2-dicarba-*closo*-dodecaboran(12)-2-ylmethoxy]phenyl}-[2, 5'-bi-1*H*-benzimidazol]-5-yl]imino}*bis*[ethanol] (**13**) was obtained by the reaction of 2,2'-[(3,4-diaminophenyl)imino]*bis*[ethanol] with Et 2-{4-[1,2-dicarba-*closo*-dodecaboran(12)-2-ylmethoxy]phenyl}-1*H*-benz imidazole-5-carboximidate hydrochloride, as well as the analogues (**14**) (R = $CH_2C_2B_{10}H_{11}$-*o*) and (**15**) (R = $C_2B_{10}H_{11}$-*o*). *N*,*N*,*O*,*O*-tosylation of compound (**13**) gave 4

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regioisomers which, after selenation, produced 2'-{4-[1,2-dicarba-*closo*-dodecaboran(12)-2-ylmethoxy]phenyl}-5-(tetrahydro-2*H*-1,4-selenazin-4-yl)-2,5'-bi-1*H*-benzimidazole (**16**) in 42% yield (Dos Santos *et al.,* 2000).

AMINO ACIDS

One of the most challenging tasks continues to be the finding of suitable targeting strategies for the selective delivery of boron rich DNA-intercalator/alkylator to tumour cells. The amino acid BPA has clinical use, but other boronated amino acids might also be candidates for BNCT either free, as part of tumour-seeking peptides or conjugated to targeting macromolecules. A large number of boronated *L*- and *D*-amino acids with varying liphophicility and sterical requirements are now available for evaluation. Synthetic and biological studies of aromatic boronoamino acids, carboranylamino acids and carboranyl amines have been also reviewed (Sjöberg *et al*., 1997).

Amino acids of the polyhedral carboranes have potential applications in BNCT and in other areas of bioorganic chemistry.

The new potential BNCT agent, an unnatural amino acid 1-amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic acid, was prepared *via* the monoalkylation of *m*-carborane with 4-bromobutene to produce 4-*m*-carboranyl-1-butene, which was then subjected to a 2 + 2 cycloaddition using dichloroketene. The resultant boronated cyclobutanone was reductively dechlorinated *prior* to the formation of the corresponding hydantoin, which was hydrolysed to the final product, 1-amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]-cyclobutanecarboxylic acid, in excellent yield (Srivastava *et al.,* 1997).

A general method for synthesis of *C*-amino-*C*-carboxy derivatives of *o*-, *m*-, and *p*-carborane has been reported, starting from their respective monoacids and proceeding through nucleophilic attack by an alcohol on the intermediate *C*-isocyanates (Kasar *et al.,* 1999). Deprotection of the resulting carbamates provided a simple preparation of the *C*-amines. The *C*-isocyanates have been also isolated for further reactions. Carbonylation of the carbamates at the remaining carboranyl CH resulted in high-yield production of the carbamate-protected amino acid (Kasar *et al.,* 1999).

A water soluble boronated amino acid containing a cascade polyol, 1-amino-3-[2-(7-{3-[2-(2-hydroxymethyl-ethoxy)-1-(2-hydroxy-1-hydroxy methyl-ethoxymethyl)ethoxy]propyl}-1,7-di-carba-*closo*-dodecaboran-1-yl)ethyl]cyclobutanecarboxylic acid, has been also synthesized (Das *et al.,* 2000). The key step was the alkylation of 3-[2-(1,7-dicarba-*closo*-dodecarboran-1-yl)ethyl]cyclobutanone hemithioketal with toluene-4-sulfonic acid 3-[2-(2-benzyloxy-1-benzyloxymethyl-ethoxy)-1-(2-benzyloxy-1-benzyloxy methylethoxy-methyl)-ethoxy]propyl ester which gave the precursor ketone which was then converted to final amino acid product *via* a Bucherer-Strecker synthesis followed by hydrogenolysis to remove the benzyl protecting groups (Das *et al.,* 2000).

Enantioselective synthesis and absolute configurations of the enantiomers of *o*-carboranylalanine, 3-(1,2-dicarba-*closo*-dodecaborane(12)-1-yl)-2-aminopropanoic acid (**17**) have been reported (Lindstrom *et al.,* 2000), using the Fitzi-Seebach imidazolidinone and the Oppolzer-Lienard sultam procedure, respectively. Both methods gave high diastereoselectivity but some racemization of **17** (EP, 91-96%) was observed after the final hydrolysis step in the imidazolidine procedure (Lindstrom *et al.,* 2000). The Oppolzer procedure gave **17** with EP > 99%. The absolute configuration of the (-)-**17** (CH_3OH) was established as *S*. The preparation of (*S*)-Boc-**17** has been also reported (Lindstrom *et al.,* 2000). A spontaneous self-degradation of the zwitterionic form of **17** was observed in water and methanol solutions.

A novel water soluble amino acid, 1-amino-3-[2-[7-(6-deoxy-α/β-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecarboran(12)-1-yl]ethyl]cyclobutanecarboxylic acid (Das *et al.,* 2001) and a novel boronated 1-amino-cyclobutanecarboxylic acid (**18**) (Fig. 14) (Kabalka and Yao, 2003) have been also synthesized for potential use in BNCT.

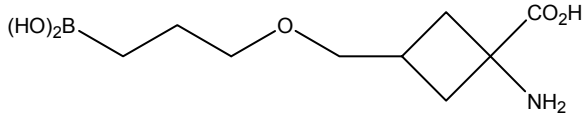

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Figure 14. A boronated 1-aminocyclobutanecarboxylic acid.

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1-Aminocyclobutanecarboxylic acid has shown high uptake in brain tumours (Kabalka and Yao, 2003).

Recently,*DL*-2-amino-2- methyl-3- (4-dihydroxyboryl phenyl)propionic acid (**19**) and 1-amino-3-(4-dihydroxyborylbenzyl) cyclobutanecarboxylic acid (**20**), which are *DL*-BPA analogues containing a quaternary center, were synthesized from 4-allylbromobenzene (Zaidlewicz *et al.*, 2004). The compound **19** was prepared from *DL*-alanine, the route being suitable for the synthesis of α-alkyl-BPA analogues.

PEPTIDES

4-Boronophenylalanine(BPA)-containing peptides for BNCT of cancer cells have been synthesized. In fact, the synthesis of several dipeptides containing *N*-terminal BPA has been developed, and the water solubility of the products was examined (Wakamiya *et al.*, 1999).

New functionalized water-soluble carboranyl anions have been also prepared from *ortho*-carborane through lithiation and subsequent derivatization. Thus, the reaction of $Li_2[1,2\text{-}C_2B_{10}H_{10}]$ with Me_3NBH_2X resulted in the carboranylborane dianions, $[1,2\text{-}(BH_2X)\text{-}1,2\text{-}C_2B_{10}H_{10}]^{2-}$ (X = H (**21**), CN (**22**), COOMe (**23**), COOH (**24**)), while the reaction of $[1\text{-R-}2\text{-Li-}C_2B_{10}H_{10}]^-$ with Me_3NBH_2X gave the monoanions $[1\text{-R-}2\text{-}BH_2X\text{-}C_2B_{10}H_{10}]^-$ (R = C_6H_5, X = H (**25**), CN (**26**), COOH (**27**), $COOCH_3$ (**28**), *L*-CONHCH(CH_2OH)COOMe (**29**), *L*-CONHCH($CHMe_2$)COOMe (**30**), *L*-CONHCH(4-$CH_2C_6H_4OH$)COOMe (**31**); R = Me, X = H (**32**), COOH (**33**)) (Spielvogel *et al.*, 2002). These water-soluble carboranylboranes are considered to be effective hydrophilic BNCT agents. The crystal structure of the peptide precursor Me_3NBH_2CONHCH(4-$CH_2C_6H_4OH$)COOMe (**34**), which is a promising BNCT agent, has been also described (Spielvogel *et al.*, 2002).

Recently, a Tyr_3-octreotate conjugated *closo*-carborane has been obtained *via* Fmoc (Fmoc = 9-fluorenyl- methyloxycarbonyl) solid phase peptide synthesis (Schirrmacher *et al.*, 2003). The boron cluster $[C_2B_{10}H_{11}]$ was introduced through the reaction of 6,9-*bis*(acetonitrile)decaborane and 5-hexynoic acid yielding a new *closo*-carborane conjugated carboxylic acid which was coupled subsequently with solid phase conjugated Tyr_3-octreotate (Schirrmacher *et al.*, 2003). The final boron-containing peptide was purified by preparative RP-HPLC and identified by MALDI-TOF mass spectrometry (Schirrmacher *et al.*, 2003).

A new peptide compound for a tissue/tumour specific BNCT approach has been recently developed by scientists at the Department of Organic Chemistry at the Georg-August-University of Göttingen, Germany (www.mbm.uni-goettingen.de). This approach should result in higher boron concentrations within the tumour and therefore in a higher BNCT efficacy (better killing effect/lower radiation levels). Therefore, a carborane was linked with a tetrapeptide which bound to the gastrin receptor. The three step-synthesis was straightforward and resulted in good yields (52%). These steps can be automated and have additional optimization potential. The resulting compound has a good stability and is soluble in DMSO or aqueous media. In cytotoxicity tests it showed a lower unspecific toxicity than other Boron compounds.

^{123}I and ^{111}In labelled gastrin peptides have been used successfully to target Gastrin-/CCK-B-receptor positive cells *in vivo*. Tumours with this receptor are: small cell lung carcinoma, astrocytoma, prostate carcinoma, ovarian carcinoma and medullary thyroid carcinoma. Several of these carcinomas have very bad predictions, *e.g.* 80% of all small cell lung cancer patients are suffering from metastases at discovery and formation of recidivists after chemotherapy is common. Therefore, the development of these new potential BNCT agents is very promising for this type of carcinomas.

THE ANTIBACTERIAL PROTEIN AVIDIN

It has been earlier shown that the antibacterial protein avidin self-associations with the boric acid gel polymer, and avidin-coated gel particles in the micrometer and submicrometer size ranges are affinity boron carriers of interest for BNCT (Bench *et al.*, 2004).

The avidin-coated gel particles eventually cross-linked formed a solid matrix and precipitated on a timescale measured on the order of an hour. At shorter exposure times rapid agglutination-like reactions were observed with biotinylated bovine albumin, suggesting that two-stage pretargeting of specific tissues should be possible with biotinylated antitumour antibodies (Bench *et al.*, 2004). However, avidin's interaction with the gel needs to be strengthened for use in BNCT, and all aryl-$B(OH)_2$ groups on the particle surfaces must be blocked, or otherwise the particles will interact strongly and nonspecifically with each other and with the carbohydrate groups present on most cell surfaces (Bench *et al.*, 2004). Glyceric acid delays the

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precipitation of the particle suspensions while most simple and complex carbohydrates accelerate it.

BORON DERIVATIVES OF HARMANE

[^{10}B]-8-Dihydroxyborylharmine has been synthesized in high yields (Sintas *et al.*, 2000a). Other ^{10}B derivatives of harmine alkaloids have been also prepared (Sintas *et al.*, 2000b) and have shown to be potential agents for BNCT in brain.

NUCLEOSIDES

Boron containing nucleosides are potential vehicles for incorporating boron compounds into nucleic acids of neoplastic cells. For this purpose, carboranyl uridines have been synthesized with the boron moiety on either the pyrimidine base or on the carbohydrate component (Barth and Soloway, 1994). Although such structures appear to be avidly taken up and retained by tumour cells *in vitro*, only the 5-carboranyl-nucleosides are converted biologically to the nucleotide. There is no evidence, however, that the latter are incorporated into nucleic acids. Other carboranyl nucleosides currently are being synthesized that may have better tumour localizing properties (Barth and Soloway, 1994).

Then , boronated nucleosides may be good candidates for BNCT because of their metabolic potential for incorporation into rapidly dividing cells (Tjarks *et al.*, 2000). Boronated thymidines, for example, may be converted to their corresponding 5'-monophosphates by cytosolic thymidine kinase 1 (TK1). This would occur primarily in proliferating tumour cells in which TK1 has elevated activity levels in contrast to quiescent cells (Arnér and Eriksson, 1995). Cellular efflux of such 5'-monophosphates should be retarded due to the negatively charged phosphate moiety allowing selective intracellular uptake in tumour cells. Further conversion to the di- and triphosphates and possible subsequent incorporation into tumour cell DNA could result in the relocation of boron in close proximity to DNA, the most critical target of the *alpha*-particles and lithium nuclei (Hartman and Carlsson, 1994). Consequently, boronated nucleosides are compounds of interest for development and evaluation (Hall *et al*., 1992; Sood *et al*., 1992b, 1994; Yamamoto *et al*., 1992; Burnham *et al*., 1993, 1995; Goudgaon *et al*., 1994; Burnham *et al*., 1995 ; Hall *et al.*, 1996; Kabalka *et al*., 1996 ; Imamura and Yamamoto, 1997; Graciet *et al*., 1998; Lesnikowski *et al*., 1999; Mourier *et al*., 1999a,b; Hurwitz *et al*., 2000; Schinazi *et al*., 2000).

Several *C*-5-substituted carboranyl 2'-deoxyuridine analogues and a small number of *N*-3-substituted carboranyl thymidines have been evaluated in phosphoryl transfer assays with recombinant human TK1 and recombinant human mitochondrial thymidine kinase (TK2) (Lunato *et al*., 1999), the only two thymidine phosphorylating enzymes in animal cells (Arnér and Eriksson, 1995). The results indicated that the *N*-3-substituted carboranyl thymidines were good substrates for TK1 but not for TK2. In fact, a small library of *N*-3-substituted carboranyl thymidine analogues, either with or without additional hydroxyl groups attached to the carborane cage, has been synthesized, and further evaluated as enzyme substrates using recombinant TK1 and TK2 (Wang *et al*., 1999) in phosphoryl transfer assays.

Several *N*-3 substituted carboranyl thymidine analogues have been synthesized and, together with some non-boronated nucleosides, have been evaluated in phosphoryl transfer assays with recombinant human TK1 and TK2 (Tjarks *et al*., 2001). In some cases increased uptake in tumour cell nuclei compared with the surrounding cytoplasm was detected *in vitro* (Tjarks *et al*., 2001).

A small library of 3-(carboranylalkyl)thymidines consisting of two series of thymidine derivatives containing *o*-carboranylalkyl groups at the *N*-3 position has been prepared (Al-Madhoun *et al*., 2002) in order to evaluate the influence of factors such as water solubility and tether length between the carborane cage and the nucleoside scaffold on the TK1 substrate properties.

Hydrocarbon spacers of various lengths (2-7 methylene groups) between the carborane and the nucleoside moiety were used to put the bulky boron cluster away from the nucleoside decreasing possible steric interference of the carborane cage with the binding of the nucleoside to the active site of TK1.

The advantage of the carboranyl thymidine derivatives is their high boron content, which could be important in obtaining the necessary ^{10}B concentrations for lethal tumour cell damage. However, the carborane moiety is bulky and highly lipophilic (Soloway *et al*., 1998; Tjarks, 2000) and such properties may prevent the interaction of carboranyl thymidines with TK1. Moreover, their relatively low water solubility may prevent an accurate assessment in TK1 phosphoryl transfer assays. One approach to improve the low water solubility of carboranyl thymidines has been the conversion of the *closo*-carborane moiety to the negatively charged *nido* form (Soloway *et al*., 1998; Tjarks, 2000). However, with a negative charge, such thymidine analogues are unlikely to pass cell membranes by passive diffusion, which may render them ineffective (Tjarks, 2000; Tjarks *et al.*, 2000). However, attaching additional hydroxyl groups to the

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carborane cage may improve upon the water solubility of *closo*-carboranyl thymidines and increase their affinity for TK1 phosphorylation while still ensuring the necessary hydrophilicity/ hydrophobicity balance for passive diffusion through various lipophilic barriers. It is possible that carboranyl thymidine analogues are substrates for the cellular nucleoside transporter.

In one series, an additional dihydroxypropyl substituent was introduced at the second carbon atom of the carborane cage. In the series of *N*-3-substituted carboranyl thymidines without additional dihydroxypropyl substituent, three steps were required in overall yields as high as 75%, while in the series of *N*-3-substituted carboranyl thymidines with additional dihydroxypropyl substituent, 9-10 steps were necessary with significantly lower overall yield.

Some compounds were synthesized (Dervan and Santilli, 1980; Wang and Chu, 1984; Tellier *et al*., 1993; Nemoto *et al.*, 1994; Hariharan *et al*., 1995; Rong *et al.*, 1997; Feakes *et al*., 1999; Lunato *et al*., 1999; Tjarks *et al*., 2000, 2001).

All target compounds were good substrates of human cytosolic thymidine kinase 1 while they were, if at all, poor substrates of the mitochondrial thymidine kinase 2 (Al-Madhoun *et al*., 2002).

Al-Madhoun *et al.* (2002) have carried out TK1 phosphoryl transfer assays with the library of some *N*-3-substituted carboranyl thymidines at lower substrate concentrations of 5 and 10 μM in the presence of 1% solubilizing dimethyl sulfoxide (DMSO), and a more defined pattern emerged with respect to the tether concept. There was only a minor difference in phosphorylation rates between *N*-3-substituted carboranyl thymidines with additional dihydroxypropyl substituents with thymidine kinase 1 (range: 13-49% relative to thymidine) and those lacking this group (range: 11-57% relative to thymidine).

Tether lengths of two and five methylene groups in both series gave the highest enzyme activities in that study. In both series, the compounds with a spacer of five methylene groups showed significantly better TK1 activities than their immediate neighbors with spacers of four and six methylene groups (Al-Madhoun *et al*., 2002).

The *N*-3 position of thymidine has been previously identified as the optimal substitution site for bulky carboranylalkyl groups to preserve the TK1 activity of carboranyl thymidine analogues (Lunato *et al*., 1999). It was also established that hydrophilicity has an impact on the phosphorylation rates of *N*-3-substituted carboranyl thymidines (Tjarks *et al.*, 2000, 2001). Al-Madhoun *et al.* (2002) have synthesized and evaluated an extended library of *N*-3-substituted carboranyl thymidines to further study the influence of factors such as water solubility and tether length between the carborane cage and the nucleoside scaffold on the TK1 substrate characteristics. The results indicated that spacers of two and five methylene groups between carborane and thymidine were optimal for the binding of *N*-3-substituted carboranyl thymidines to the active site of TK1. This information is of crucial importance in the design and synthesis of carboranyl thymidine analogues that may eventually be used in clinical BNCT. However, the physicochemical properties of *N*-3-substituted carboranyl should be improved for optimal binding to TK1.

The carboranyl cluster is a new and versatile modifying entity for nucleotides and nucleic acids (Lesnikowski, 2003). Three types of carboranyl (-$C_2B_{10}H_{11}$) group-containing DNA-oligonucleotides have been described: (1) CBMP- oligonucleotides, consisting of the carborane cage within an internucleotide linkage, (2) CDU-oligonucleotides, containing the carborane cage attached to a nucleobase, and (3) 2'-CBM-oligonucleotides, with the carborane cage linked to a sugar residue at the 2' position (Lesnikowski, 2003).

The method of synthesis and the physicochemical and biochemical features of these novel modifications have been recently discussed, together with structure-property relationships (Lesnikowski, 2003). The carboranyl cluster-containing oligonucleotides form a crossover between (carba)borane chemical and molecular biology. They are potentially useful as antisense agents for antisense oligonucleotide therapy (AOT) and boron carriers for BNCT (Lesnikowski, 2003). The chemical of carborane-modified nucleic acids has implications beyond BNCT and AOT. Owing to the unique properties of carborane clusters they have potential for further development as molecular probes for molecular medical diagnostics and bio-inorganic material for emerging technologies (Lesnikowski, 2003).

Boron-containing nucleosides have been evaluated both *in vitro* and *in vivo* as potential delivery agents for BNCT of brain tumours. The rationale for their synthesis was based on the fact that proliferating neoplastic cells have increased requirements for nucleic acid precursors, and, therefore, they should preferentially localize in the tumour. A series of 3-carboranlyalkyl thymidine analogues has been recently synthesized and a subset, designated N4, N5, and N7, and the corresponding 3-dihydroxypropyl derivatives, designated N4-2OH, N5-2OH, and N7-2OH, have been selected for evaluation (Barth *et al.*, 2004). Using these compounds as substrates for recombinant human thymidine kinase-1 and the

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mitochondrial isoenzyme thymidine kinase-2, the highest phosphorylation levels relative to thymidine were seen with N5 and the corresponding dihydroxypropyl analogue N5-2OH. In contrast, N4, N4-OH, N7, and N7-OH had substantially lower phosphorylation levels. To compare compounds with high and low thymidine kinase-1 substrate activity, N5 and N7 and the corresponding dihydroxypropyl derivatives were selected for evaluation of their cellular toxicity, uptake and retention by the F98 rat glioma, human MRA melanoma, and murine L929 cell lines, all of which are thymidine kinase-1 (+), and a mutant L929 cell line that is thymidine kinase-1 (-). N5-2OH was the least toxic (IC_{50}, 43-70 μM), and N7 and N7-2OH were the most toxic (IC_{50}, 18-49 μM) (Barth *et al.*, 2004). The highest boron uptake was seen with N7-2OH by the MRA 27 melanoma and L929 wild-type (wt) cell lines. The highest retention was seen with L929 (wt) cells, and this ranged from 29% for N5-2OH to 46% for N7. Based on the *in vitro* toxicity and uptake data, N5-2OH was selected for *in vivo* biodistribution studies either in rats bearing intracerebral implants of the F98 glioma or in mice bearing either *s.c.* or intracerebral implants of L929 (wt) tumours. At 2.5 h after convection-enhanced delivery, the boron values for the F98 glioma and normal brain were 16.2 ± 2.3 and 2.2 μg/g, respectively, and the tumour to brain ratio was 8.5 (Barth *et al.*, 2004). Boron values at 4 h after convection-enhanced delivery of N5-2OH to mice bearing intracerebral implants of L929 (wt) or L929 thymidine kinase-1(-) tumours were 39.8 ± 10.8 and 12.4 ± 1.6 μg/g, respectively, and the corresponding normal brain values were 4.4 and 1.6 μg/g, thereby indicating that there was selective retention by the thymidine kinase-1(+) tumours.

Based on these favourable *in vitro* and *in vivo* data, these authors have indicated that BNCT studies will be started using N5-2OH in combination with two non-cell cycle dependent boron delivery agents, BPA and BSH (Barth *et al.*, 2004).

CARBOHYDRATES

Studies on the structure of the complex of the BNCT drug, *L*-BPA, with fructose and related carbohydrates have been carried out, especially chemical and ^{13}C NMR evidence for the *beta-D*-fructofuranose 2,3,6-(*p*-phenylalanyl-*ortho*-boronate) structure have been reported (Shull *et al.*, 2000).

The complex of *L-p*-BPA with fructose has been used in clinical trials of BNCT to treat both melanoma and GBM. The structure of this complex in water buffered at physiological pH was not established. In the ^{1}H NMR spectra of the complex of *L-p*-BPA with various carbohydrates, the upfield chemical shifts of the aromatic protons of *L-p*-BPA confirmed that the boron atom was negative charged and tetrahedral (Shull *et al.*, 2000). In the ^{13}C NMR spectrum of the complex of *L-p*-BPA with U-^{13}C labeled fructose, the chemical shifts and coupling constants were consistent with fructose adopting the *beta-D*-fructofuranose form.

Furthermore, the coupling constants along with the binding constants measured for *L-p*-BPA with a series of monosaccharides and disaccharides suggested that the *beta-D*-fructofuranose 2,3,6-(*p*-phenylalanyl-*ortho*-boronate) structure strongly predominated, with free *L-p*-BPA and fructose the only other species detected (Shull *et al.*, 2000).

Recently, a new approach to the preparation of conjugates of *ortho*-carboranes with the carbohydrate ligands of lectins has been described (Orlova *et al.*, 2003). It is based on the use of prespacer strategy, which is highly flexible and efficient and allows a preparation of libraries of neoglycoconjugates from only one saccharide glycoside with a functional group in the terminal position of a rather simple prespacer aglycon (Orlova *et al.*, 2003). The application of this approach to the synthesis of the conjugates of 1,2-dicarba-*closo*-dodecaborane (*ortho*-carborane) with the disaccharide lactose, which is the ligand of lectins that are expressed on the surface of melanoma cells, has been discussed (Orlova *et al.*, 2003).

BNCT RESEARCH CARRIED OUT IN ARGENTINA

The main research groups of boron neutron capture therapy (BNCT) in Argentina are in Bariloche Atomic Center (*Centro Atómico Bariloche*), National Atomic Energy Commission (*Comisión de Energía Atómica,* CNEA), and University of Buenos Aires (*Facultad de Ciencias Exactas y Naturales, Facultad de Odontología*, *Facultad de Farmacia y Bioquímica*). Development of new potential BNCT-agents, experimental models, clinical trials, Phase I/ Phase II clinical trials are being carried out in these laboratories. Some recent reports are included in this section.

The development of new BNCT potential agents are being carried out in our laboratories, in particular nitrogen-containing heterocycles and biogenic amines (Sintas *et al.*, 2000a,b).

The hamster cheek pouch model of oral cancer for BNCTstudies has been proposed and validated (Kreimann *et al*., 2001a,b). The animals were used for biodistribution and pharmacokinetic studies of BPA.

The first evidence of the usefulness of BNCT for the treatment of oral cancer in an experimental model was reported by the Department of Radiobiology, CNEA (Kreimann *et al*., 2001a). The response of

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hamster cheek pouch tumours, precancerous tissue, and normal oral tissue to BPA-mediated BNCT using the thermalized epithermal beam of the RA-6 Reactor at the Bariloche Atomic Center has been reported (Kreimann *et al*., 2001a). BNCT has led to complete remission by 15 days post-treatment in 78% of tumours and partial remission in an additional 13% of tumours with virtually no damage to normal tissue.

Potentially therapeutic absolute boron concentrations, and tumour/normal tissue and tumour/blood ratios have been achieved in the hamster oral cancer model using BPA as the delivery agent (Kreimann *et al*., 2001b). Blood, tumour, precancerous pouch tissue surrounding tumour, normal pouch tissue, tongue, skin, cheek mucosa, palate mucosa, liver, and spleen, were sampled at 0-12 h after administration of 300 mg BPA/kg. The data revealed selective uptake of BPA by tumour tissue and, to a lesser degree, by precancerous tissue. Mean tumour boron concentration was 36.9 ± 17.5 ppm at 3.5 h and the mean boron ratios were 2.4/1 for tumour/normal pouch tissue and 3.2/1 for tumour/blood. Higher doses of BPA (600 and 1200 mg BPA/kg) increased tumour uptake (Kreimann *et al*., 2001b).

The biodistribution and pharmacokinetics of a lipophilic, carborane-containing tetraphenylporphyrin (CuTCPH) in the hamster oral cancer model was also reported (Kreimann *et al*., 2003). This non-toxic compound CuTCPH was developed in the Department of Radiobiology of CNEA. For potentially effective BNCT, tumour boron concentrations from a new agent should be greater than 30 ppm and tumour/blood and tumour/normal tissue boron concentration ratios should be greater than 5/1 without causing significant toxicity. Kreimann *et al*. (2003) administered CuTCPH intraperitoneally (i.p.) as a single dose of 32 microg/g body weight (bw) (10 microg B/g bw) or as four doses of 32 microg/g bw over 2 days. Blood and tissues were sampled at 3, 6, 12, 24, 48, and 72 h in the single-dose protocol and at 1-4 days after the last injection in the multidose protocol. The tissues sampled were tumour, precancerous tissue surrounding tumour, normal pouch, skin, tongue, cheek and palate mucosa, liver, spleen, parotid gland and brain. The maximum mean B ratios for the single-dose protocol were tumour/normal pouch: 9.2/1 (12h) and tumour/blood: 18.1/1 (72 h). The B value peaked to 20.7 ± 18.5 ppm in tumour at 24 h. The multidose protocol maximum mean ratios were tumour/normal pouch: 11.9/1 (3 days) and tumour/blood: 235/1 (4 days). Absolute boron concentration in tumour reached a maximum value of 116 ppm and a mean value of 71.5 ± 48.3 ppm at 3 days (Kreimann *et al*., 2003). The fact that absolute and relative B values markedly exceeded the BNCT therapeutic threshold with no apparent toxicity may confer on this compound a therapeutic advantage. CuTCPH-mediated BNCT has shown to be potentially useful for the treatment of oral cancer in an experimental model.

The biodistribution of a non-toxic boron compound, GB-10 ($Na_2{}^{10}B_{10}H_{10}$) has been also reported in the hamster cheek pouch model in order to assess its potential for BNCT or BNCT enhanced Fast Neutron Therapy (BNCT-FNT) (Heber *et al*., 2004). The uptake and retention of GB-10 in tumour and precancerous tissue and in potentially dose-limiting, clinically relevant normal tissues have been evaluated (Heber *et al*., 2004).

Mean tumour boron concentration delivered by GB-10 (50 mgB/kg) peaked to 77.7 ± 28.0 ppm at 20 min post-administration and remained at therapeutically useful values of 31.9 ± 21.4 ppm at 3 h. The clearance rate for normal tissues was faster than for tumour tissue. The consistently low brain and spinal cord values would preclude normal tissue toxicity. The uptake of GB-10 by precancerous tissue has shown to be of potential use in the treatment of field cancerized areas. GB-10 was deposited homogeneously in different tumour areas, an asset when treating heterogenous tumours. The data suggested that the joint administration of BPA and GB-10 might improve the therapeutic efficacy of BNCT. According to these findings GB-10 has been shown as a potential boron carrier for BNCT of head and neck tumours and for BNCT-FNT (Heber *et al*., 2004).

The selective uptake of BPA by undifferentiated human thyroid cancer (UTC) ARO cells both *in vitro* and *in vivo* has shown the possibility of applying BNCT for the treatment of UTC (Dagrosa *et al*., 2002). In *in vitro* studies, the uptake of *p*-^{10}BPA by the UTC cell line ARO, primary cultures of normal bovine thyroid cells (BT), and human follicular adenoma (FA) thyroid was studied. No difference in BPA uptake was observed between proliferating and quiescent ARO cells. The uptake by quiescent ARO, BT, and FA showed that the ARO/BT and ARO/FA ratios were 4 and 5, respectively ($p < 0.001$). In *in vivo* studies, ARO cells were transplanted into the scapular region of NIH nude mice, and after 2 weeks BPA (350 or 600 mg/kg bw) was injected i.p.. With 350 mg, tumour uptake was highest after 60 min and the tumour/normal thyroid and tumour/blood ratios were 3 and 5, respectively. When 600 mg/kg bw BPA were administered, after 90 min the tumour/blood, tumour/normal thyroid, and tumour/distal skin ratios for ^{10}B concentrations per gram of tissue were

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approximately 3, showing a selective uptake by the tumour (Dagrosa *et al*., 2002).

Moreover, a 50% histologic cure of the UTC mice was observed when the complete BNCT was applied (Dagrosa *et al*., 2003). The experimental design consisted of four groups of mice: (1) no treatment; (2) neutron beam alone; (3) 350 mg/kg bw BPA *plus* irradiation; (4) 600 mg/kg bw BPA *plus* irradiation. Follow-up was performed by measurement of tumour volume, histologic analysis, and assessment of DNA damage using the comet assay. The tumour continued to grow in Groups 1 and 2. In Group 3, a slow-down of tumour growth was observed in all mice, and a complete stop was observed in 100% of mice of Group 4. Complete disappearance of the tumour was observed in 50% of the mice that had an initial tumour volume of less than 50 mm^3 (Groups 3 and 4). DNA damage showed a progressive increase from Group 1 through 4. These data have shown, for the first time, that UTC is amenable to treatment by BNCT (Dagrosa *et al*., 2003).

More recently these authors have analysed the biodistribution of BOPP (tetrakis-carborane carboxylate ester of 2,4-*bis*-(*alpha*,*beta*-dihydroxyethyl)-*deutero*-porphyrin IX) and have shown that when BOPP was injected 5 days before BPA, and the animals were sacrificed 60 min after the i.p. injection of BPA, a significant increase in boron uptake by the tumour was found (38-45 ppm with both compounds *vs*. 20 ppm with BPA alone). Five days post the i.p BOPP injection and 1 h after BPA the ratios were: tumour/blood 3.75; tumour/distal skin 2. Other important ratios were tumour/thyroid 6.65 and tumour/lung 3.8. Thus, studies were performed in mice transplanted with ARO cells and injected with BOPP and BPA (Viaggi *et al*., 2004). Only in mice treated with the neutron beam and injected with the boronated compounds a 100% control of tumour growth was observed. Two groups of mice received different total absorbed doses: 3.00 and 6.01 Gy, but no further improvement in the outcome was found compared to the previous results using BPA alone (4.3 Gy) (Viaggi *et al*., 2004).

Since applications of BNCT to transplanted mice showed a 100% control of growth and a 50% histological cure of tumours with an initial volume of 50 mm^3 or less, a further study with four dogs with diagnosis of spontaneous UTC dogs has been performed (Dagrosa *et al*., 2004). A BPA-fructose solution was infused during 60 min and dogs were submitted to thyroidectomy. Samples of blood and from different areas of the tumours (and in one dog from normal thyroid) were obtained and the boron was determined by ICP-OES. Selective BPA uptake by the tumour was found in all animals, the tumour/blood ratios ranged between 2.02 and 3.76, while the tumour/normal thyroid ratio was 6.78. Individual samples had tumour/blood ratios between 8.36 and 0.33. These ratios were related to the two histological patterns observed: homogeneous and heterogeneous tumours. These results have confirmed the selective uptake of BPA by spontaneous UTC in dogs (Dagrosa *et al*., 2004).

Further studies have been recently performed in order to optimize this treatment with a boronated porphyrin, both alone and in combination with BPA (Dagrosa *et al*., 2005). *In vitro* studies with cells in culture showed that BOPP is localized intracellularly, with a highest concentration in the 11500*g* (mitochondrial-enriched pellet) fraction. When BOPP was administered alone to NIH nude mice transplanted with UTC human cells, no significant tumour uptake or selectivity in our *in vivo* model was observed. In contrast, when BOPP was injected 5-7 days before BPA and the animals were sacrificed 60 min after administration of BPA, a significant increase in boron uptake by the tumour was found (38-45 ppm with both compounds *vs* 20 ppm with BPA alone). On day 5 the tissue boron selectivity ratios were tumour/blood approximately 3.8 and tumour/distal skin approximately 1.8. Other important ratios were tumour/thyroid approximately 6.6 and tumour/lung approximately 2.9. These results have shown the possibility of improving the efficacy of BNCT for the UTC treatment (Dagrosa *et al*., 2005).

A clinical trial of the ^{10}B-enriched *p*-BPA-fructose complex (^{10}BPA-F) infusion procedure in potential BNCT patients, including four melanoma of extremities and two high-grade gliomas (glioblastoma and ganglioglioma) has been performed (Liberman *et al*., 2004). Tumour/blood and skin/blood ratios for ^{10}B concentrations in tumour, blood and skin were determined. The tumour/blood ratio for the glioblastoma was in the 1.8-3.4 range. The ganglioglioma did not show any significant boron uptake. For the nodular metastasic melanoma tumour/blood values were between 1.5 and 2.6 (average 2.1 ± 0.4), corresponding to the lower limit of the mean values reported for different melanoma categories. This result might suggest a lower boron uptake for nodular metastasic melanomas. Skin/blood was 1.5 ± 0.4. An open two-compartment pharmacokinetic model has been applied to predict the boron concentration during the course and at the end of a BNCT irradiation (Liberman *et al*., 2004).

A voxel model in BNCT treatment planning has been also studied (González *et al*., 2005). A new and improved material assignment algorithm implemented in NCTPlan treatment planning system for BNCT has been reported. Based on previous

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works, the performances of the 1 cm based voxel methods used in the MacNCTPlan and NCTPlan treatment planning systems were compared by standard simulation tests. In addition, the NCTPlan voxel model was benchmarked against in-phantom physical dosimetry of the RA-6 reactor of Argentina.

This investigation has shown the 1 cm resolution to be accurate enough for all reported tests, even in the extreme cases such as a parallelepiped phantom irradiated through one of its sharp edges. The skin was considered one of the organs at risk in all BNCT treatments and, in the particular case of cutaneous melanoma of extremities, limits the delivered dose to the patient (González *et al*., 2005).

A theoretical analysis has been carried out to assess the distortion caused by homogenization and material percentage rounding processes. Then, a new strategy for the treatment of surface voxels was proposed and tested using two different irradiation problems (González *et al*., 2005).

Research carried out in the Department of Oral Pathology (Faculty of Dentistry, University of Buenos Aires) has shown the *in vivo* response to heavy particle irradiation in rat tail epidermis using silver-stained nucleolar organizer regions (AgNOR) as the end-point (Itoiz *et al*., 2002). The energy degradation of the beam across the circular section of the tail allowed the study of the damage elicited by two different LET regions of a helium beam, i.e. non-Bragg peak (NBP) and Bragg peak (BP), at different sites on the same sample. The tails were locally irradiated with a helium ion beam at different fluences. The AgNO-regions provided quantitative evidence of differential damage in neighboring tissue areas exposed to different LET regions of a helium-ion beam (Itoiz *et al*., 2002).

A Phase I/II protocol for treating cutaneuos melanomas with BNCT was designed in Argentina by CNEA and the oncologic medical center Instituto Roffo. The first of a cohort of thirty planned patients was treated on October 9, 2003 (González *et al*., 2004a).

The protocol-based procedure was reported (González *et al*., 2004a). This report accounted for the first clinical case, treatment regime and planning, patient irradiation, retrospective dosimetric analysis and clinical outcome. Considering the low acute skin toxicity and the complete response in 21 of the 25 subcutaneous melanoma nodules treated, a second irradiation was performed in a different location of the extremity of the same patient. The corresponding clinical outcome is still under evaluation (González *et al*., 2004a).

A new approach to determine the tumour/blood ^{10}B concentration ratio in BNCT has been recently carried out in CNEA (González *et al*., 2004b). The performance of this statistical method was shown in a clinical case of cutaneous multiple nodular melanomas. The calculations involved a detailed dosimetry analysis, the determination of tumour control probabilities for the different nodules, the maximum likelihood estimation itself, and a parametric bootstrap to obtain confidence intervals for the tumour/blood ratio. The obtained ratio was 3.05 ± 0.46 with a 95%-confidence interval. Moreover, a single patient with multiple nodules proved enough to get statistically relevant results. The proposed method did not involve surgery and could be performed after a BNCT treatment without being invasive for the patient (González *et al*., 2004b).

The performance of two NCT treatment planning systems, *e.g.,* NCTPlan developed by CNEA and the Harvard-MIT group, and SERA developed by the INEEL/Montana State University group has been evaluated (Casal *et al*., 2004). This study was performed in some simple geometries with the therapeutical hyperthermal beam of the RA-6 facility at Bariloche, Argentina. The first geometry was a rectangular phantom and calculations and measurements were made along the central beam axis and along a parellel axis, 4 cm apart from the central beam axis. Measurements and calculations were also performed in a cylindrical phantom, to explore the behaviour of the treatment planning systems in a geometry simulating an extremity, in accordance with the CNEA clinical protocol. Comments on differences in source definitions and cross sections libraries have been also included (Casal *et al*., 2004). According to this report, both codes have given acceptable results on the central beam axis and on a lateral axis, showing good agreement with experimental results.

The progress of the modeling and experimental characterization of the RA-6 reactor neutron beam in Bariloche Atomic Center has been reported (Blaumann *et al*., 2004). Both beam set up procedure and performance have been evaluated for BNCT clinical trials of skin melanomas, being the first theoretical analysis of such beam performance.

Surface source modeling and assessment, beam dosimetry, treatment planning system calibration, and treatment planning optimization have been taken into account (Blaumann *et al*., 2004). Several methods and criteria were established in order to provide guidance for future clinical studies conducted in this facility. Following a realistic model, the theoretical analysis was based on a clinical case of malignant melanoma in extremities. Owing to the complex geometry of the tumour, this particular clinical case represented one of the most difficult lesions to be

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treated. Results showed that only 4% of the tumour volume was underdosed in cases of mean blood ^{10}B concentration values, even in the most unfavourable analysis (Blaumann *et al*., 2004).

CONCLUSIONS

Boron neutron capture therapy (BNCT) is an experimental cancer treatment modality based on the targeting of ^{10}B-enriched compounds to the tumour, the capture of low-energy neutrons by ^{10}B, which results in the emission of extremely cytotoxic $^{7}Li^{+}$ nuclei and *alpha*-particles [$^{10}B(n,alpha)^{7}Li$].

BNCT is an adjuvant therapy that has the potential to control local tumour growth. A selective delivery of sufficient amounts of boron to individual tumour cells, compared to surrounding normal tissues, is the key for successful BNCT.

A variety of boronated chemical structures have been synthesized and are under development, however, there is a need for improvements in adequate techniques to estimate tumour boron content before treatment, and especially in clinical BNCT implementation.

Polyhedral borate anions and carboranes have shown to be prominent substructures of the boron delivery agents. Current development of the following BNCT agents have been shown: corticosteroid-carborane esters, oligomeric phosphate diesters, ADP derivatives, *o*-carboranes carrying 1,3,5-triazine units, amines and polyamines, platinum (II)-amine complexes, porphyrins, phenanthridinium derivatives, benzimidazoles, amino acids, peptides, the antibacterial protein avidin, boron derivatives of harmane, nucleosides, and carbohydrates. Tumour-directed antibodies or their immunoreactive fragments are attractive candidates for the selective delivery of ^{10}B, provided that *ca*. 1000 ^{10}B atoms can be attached to each immunoreactive protein without significantly altering its biological properties. Targeting liposomes to tumour endothelial cells for BNCT proved to be efficient.

Most boron-derivatives chemical compounds which have been developed require to be clinically studied. Further structure modifications would be then carried out in order to improve BNCT efficacy.

BNCT has been shown to extend significantly the lifespan of patients with brain tumours, and in particular, glioblastoma multiforme, and a number of BNCT reagents are currently in Phase I and II clinical trials. Results from BNCT trials of melanoma are encouraging and complete or partial tumour control has been observed in several cases. However, there is a lack of sufficient clinical studies and clinical trials leading to prove the therapeutic efficacy of the synthetic boronated compounds for use in BNCT.

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