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The formulation of polyhedral boranes for the boron neutron capture therapy of cancer

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The early promise of boron neutron capture therapy as a method for the treatment of cancer has been inhibited by the inherent toxicity associated with therapeutically useful doses of ^{10}B-containing pharmacophores, the need for target-tissue specificity and the challenges imposed by biological barriers. Although developments in the synthetic chemistry of polyhedral boranes have addressed issues of toxicity to a considerable extent, the optimisation of the transport and the delivery of boronated agents to the site of action – the subject of this review – is a challenge that is addressed by the development of innovative formulation strategies.

Shortly after the discovery of the neutron by Chadwick [1] and following the observation of Fermi *et al.* [2] that some nuclides (such as ^{10}B and ^{6}Li) are capable of absorbing thermal neutrons, Locher [3] laid the foundations for the development of neutron capture therapy (NCT) by proposing the use of 'strong neutron absorbers into the regions where it is desired to liberate ionisation energy'. For use in NCT the nuclide must offer a high 'neutron-capture cross section' (σ_{th}); a quantity that provides a measure of the probability of capturing a neutron. ^{157}Gd, ^{113}Cd, ^{3}He and ^{10}B all exhibit large σ_{th} values [4].

Boron neutron capture therapy

Of the nuclides that are potentially useful in NCT, ^{10}B not only offers a good compromise between toxicity and stability but also a chemistry that is highly versatile and well established [5].

Boron neutron capture therapy (BNCT) is a two-step chemo-radiotherapeutic technique (Fig. 1) that involves the selective delivery of ^{10}B-rich agents to tumours and their subsequent irradiation with low-energy neutrons, which induces a nuclear fission reaction that causes the selective destruction of the targeted cells.

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Energetic alpha particles, produced by the interaction of ^{10}B with neutrons, have a high linear energy transfer (number of ionisations per unit distance), low oxygen enhancement ratio (a measure of the proportion of radiation doses that are needed to affect the same rate of cell survival under hypoxic or oxic conditions) and high relative biological effectiveness (the relative amount of damage that a fixed amount of ionising radiation of a given type can inflict on biological tissues). These particles are lethal, but – because of their size, energy and short path lengths (4.5–10 μm) – the effect is confined within the host cell [6]. Inevitably, capture reactions also involve ^{1}H and ^{14}N, but the σ_{th} for these nuclei are too small to be of concern [4].

Applications and strategies

Although there has been interest in the application of BNCT for the treatment of malignant melanomas, head and neck cancers and hepatomas [7], most studies have focused on the treatment of brain tumours, largely represented by glioblastoma multiforme (GBM) [8]. GBM is difficult to treat surgically and is associated with metastases to other organ sites. In contrast to irradiation with γ-photons and X-rays, BNCT offers the promise of non-repairable sublethal damage (SLD) and potential lethal damage (PLD) [9], which rationalises the suggested use for the treatment of tumours,

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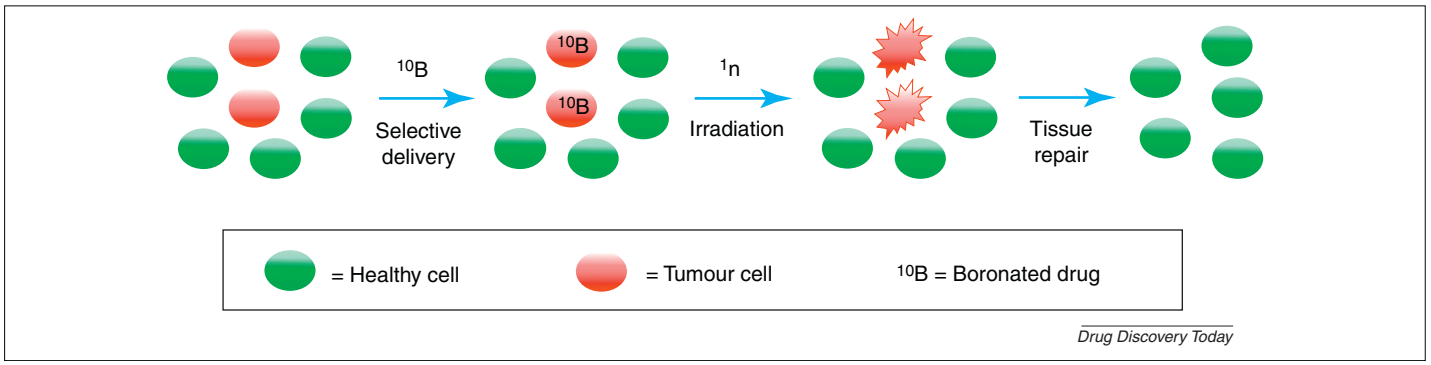

FIGURE 1

BNCT steps. The selective delivery of ^{10}B-containing drugs to tumour cells is followed by irradiation with slow neutrons (^{1}n), leading to tissue repair.

such as GBM, that are characterised by powerful DNA repair mechanisms [10].

Molecular design requirements

For potential applications in BNCT, a therapeutic agent [11,12] must: possess low toxicity (especially in the case of systemically administered agents); exhibit good tumour-cell selectivity; persist intracellularly at constant concentrations during the course of neutron radiation; be deliverable at $>10^9$ ^{10}B atoms per cell; be characterised by tumour:normal tissue and tumour:blood ratios higher than 3; and have the capacity to reach the target site through the blood stream by penetrating biological barriers, such as the blood–brain barrier (BBB). Early molecular-design approaches were guided by the observation that the BBB is more permeable in the diseased state than it is in the healthy state, but therapeutic strategies that emerged from these approaches did not prove successful, mainly because isolated clusters of tumour cells, protected by the normal BBB, retain the potential to become the foci for tumour recurrence. The multitude of performance demands that ^{10}B-containing drugs need to satisfy before they can be used in clinical trials are reflected by the number of ^{10}B compounds that have reached this stage [13,14]: 4-dihydroxyborylphenylalanine (BPA) [15] and sodium mercaptoundecahydrododecaborate (BSH) [16] (Fig. 2).

Primary among these demands is the delivery of therapeutic quantities of ^{10}B at the site of action. It has been calculated that, if a neutron fluence of 10^{12} neutrons/cm^2 is to be employed, the boronated agent needs to be maintained at ~30 μg ^{10}B/g of tumour throughout the period of irradiation [17]. A commonly employed strategy for achieving such concentrations involves the design of therapeutic agents that incorporate polyhedral borane moieties [18]. Among these, the carboranes [19] (neutral lipophilic icosahedral dicarba-*closo*-dodecaboranes, $C_2B_{10}H_{12}$; Fig. 3) are of particular interest, not only because of their high ^{10}B content, good catabolic stability and low toxicity [20] but also because they are amenable to chemical functionalisation [21,22].

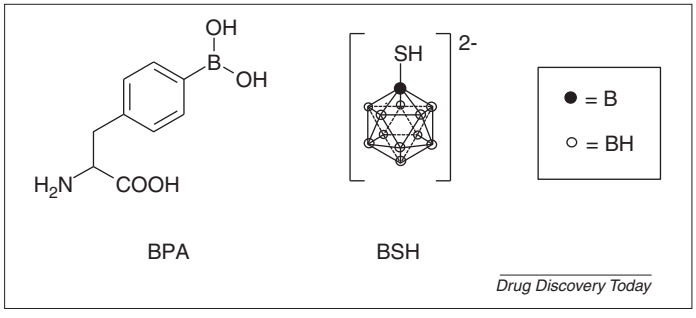

FIGURE 2

Structures of 4-dihydroxyborylphenylalanine (BPA) and mercaptoundecahydrododecaborate (BSH) anion.

Dosage form design

The major limitation in the early clinical application of BNCT has been the lack of availability of low-toxicity ^{10}B-containing compounds that can be selectively transported to the target tissue at the concentration level necessary to meet the therapeutic objective. Molecular-level structural modifications attempted to date have not been sufficient to address all these challenges and, as a consequence, much effort has been directed toward the development of complementary formulation strategies. Among these, emulsions, liposomes, dendrimers and, more recently, carbon nanotubes have received considerable attention.

Emulsions

Suzuki *et al.* [23] compared the pharmacokinetics of BSH, as a potential agent for the treatment of hepatomas, following intra-arterial administration in a biodegradable starch microsphere (DSM) formulation with those of an emulsion of BSH in lipiodol. The lipiodol-based delivery system exhibited high selectivity, effecting a tumour:liver boron concentration ratio >4 (a peak concentration ratio of ~14 was observed at 6 h from administration). In a separate effort [24], the same group exploited the propensity of

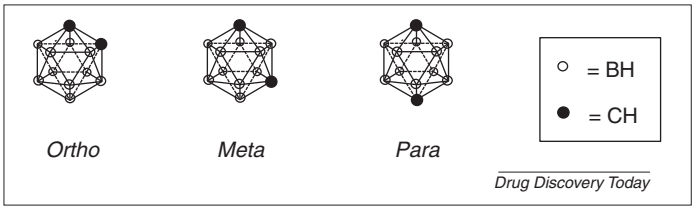

FIGURE 3

Structures of ortho-, meta- and para-carboranes.

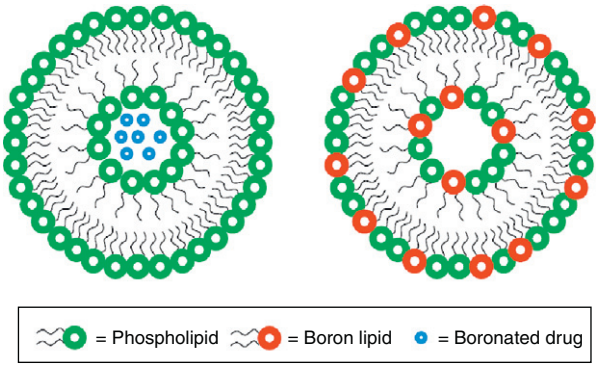

FIGURE 4

Schematic representation of liposomes showing encapsulation (left) and incorporation (right) of boron.

iodised poppy-seed oil (IPSO) mix emulsions to deposit selectively in hepatocellular carcinoma (HCC) cells as a more efficient means of delivering ^{10}B to the liver. The employment of BSH-entrapped water-in-oil-in-water (WOW) emulsions is reported [25,26] to effect HCC ^{10}B concentration ratios that are even higher than those associated with IPSO mix emulsions. A comparative study of the relative merits of emulsions as compared with other systems for the delivery of boron has been presented by Yanagie *et al.* [27].

Liposomes

Liposomes have the capacity to accommodate relatively large quantities of boron [28]. Two main strategies have evolved for the loading of liposomal systems with polyhedral boranes: encapsulation and incorporation (Fig. 4).

Encapsulation refers to the containment of boronated drugs within the internal cavity of liposomes, whereas incorporation is the integration of the polyhedral boranes within the bilayer structure. Incorporation allows the chemical binding of boranes to constituents of the bilayer (lipids or cholesterol) as their *nido*- or *closo*-congeners. The adopted formulation strategy is dictated by the complementarity of the respective physicochemical characteristics of the lipids and the active ingredient.

Encapsulation

Yanagie *et al.* [29] were the first to employ liposomes for the delivery of encapsulated boronated compounds *in vitro*. Selective delivery of the therapeutic agent was achieved by the encapsulation of BSH into immuno-liposomes that had been conjugated to monoclonal antibodies (mAbs) specific to carcinoembryonic antigen (CEA). *In vitro* experiments showed that thermal-neutron irradiation at 5×10^{12} neutrons/cm^2 inhibited tumour-cell growth, whereas *in vivo* experiments at 2×10^{12} neutrons/cm^2 demonstrated the capacity to suppress tumour growth. The significance of this work becomes apparent when the unsuccessful attempts to deliver boron by direct conjugation to antibodies are considered [30,31].

Hawthorne [32] encapsulated a variety of hydrolytically stable polyhedral borane anions into unilamellar liposomes and the formulation was tested via parenteral administration to tumour-bearing mice. Although the polyhedral anions did not exhibit any selectivity toward tumour cells, the employed liposome formulations were shown to be capable of effecting the selective delivery of borane anions to tumours (peak boron concentration = 40 μg ^{10}B/g tumour tissue; tumour:blood boron ratio = 5). Consistent with the performance demands of a therapeutically valuable colloidal delivery system, the encapsulation of $Na_3[B_{20}H_{17}NH_3]$ in liposomes of 5% PEG-2000-distearoyl phosphatidylethanolamine was seen to extend circulation time, as witnessed by the continuous accumulation of ^{10}B in the tumour over 48 hours (to a maximum of 47 μg ^{10}B/g tumour) [33].

Other strategies toward the selective delivery of boron to tumour cells have included the use of cationic liposomes, the conjugation of liposomes to a ligand targeting the folate receptor (FR), transferring (TF) receptor or epidermal growth factor receptor (EGFR) [32–42].

Rationalised in terms of potentially favourable electrostatic interactions with the negatively charged outer leaflet of mammalian plasma membranes, Ristori *et al.* [34] co-formulated ^{10}B-compounds with cationic liposomes. In experiments involving the use of DHD/K12/TRb rat colon carcinoma or B16-F10 murine melanoma cells to respectively induce liver or lung metastases, these cationic liposomes effected a greater than 30-fold increase in the cellular concentrations of ^{10}B relative to that achieved by BPA alone.

Pan *et al.* [35]demonstrated that FR-targeted liposomes afford an almost tenfold increase in the accumulation of ^{10}B in cancerous tissue. *In vitro* experiments involving human KB squamous epithelial cancer cells, which present an overexpression of FR, demonstrated an order-of-magnitude increase in cellular boron uptake as compared with the control (1584 μg/10^9 cells *vs.* 154 μg/10^9 cells). However, the encapsulation efficiency of such a system is low (6–15%). The concept of FR-targeting liposomes has also been exploited by Maruyama *et al.* [36] in the formulation of Na_2BSH with FR-PEG liposomes designed to reduce uptake by the reticuloendothelial system (RES). This approach offered an improvement in residence time and the consequent amplification in accumulation of ^{10}B in tumour cells [36]: at 72 hours post injection (dose = 35 mg ^{10}B/kg), the formulation effected a concentration of 30 μg ^{10}B/g tumour and a tumour:-plasma ratio of 6.

Doi *et al.* [37] illustrated the superior selectivity of TF-PEG liposomal carriers of BSH as compared with PEG-liposomes or BSH alone.

The use of EGFR-targeted liposomes has been explored by, among others, Kullberg *et al.* [38], who reported good ^{10}B uptake by glioma cells (6 μg/g of cells) in an *in vitro* experiment involving a water-soluble boronated acridine encapsulated in EGF-PEG liposomes. More recently, Tomizawa *et al.* [39] demonstrated the specific delivery of BSH to glioma cells by means of immunoliposomes. Specificity was achieved by the EGFRvIII functionalisation of liposomes; EGFRvIII is overexpressed in GBM (up to 57%), but undetectable in normal brain [38].

Incorporation

Hawthorne and colleagues [40,41] were the first to exploit the intercalation of *nido*-carborane into the bilayer of liposomes following functionalisation with a single hydrocarbon chain (CL; Fig. 5). *In vivo* studies of distearoylphosphatidylcholine (DSPC) and cholesterol-intercalated CL showed a maximal tumour

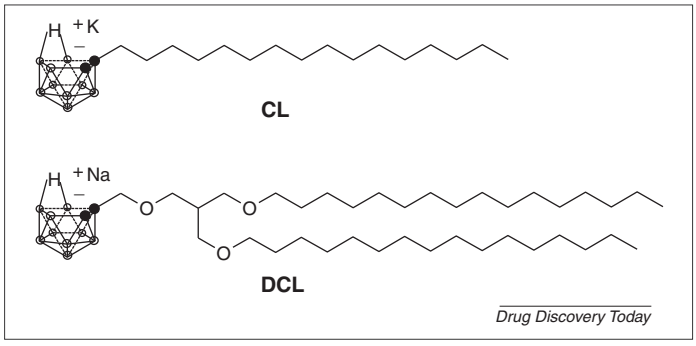

FIGURE 5

Single-(CL) [41] and double-tailed *nido*-carborane lipids (DCL) [42].

concentration of 35 μg ^{10}B/g tumour and a tumour:blood ratio of 8. The concept has been extended by Nakamura *et al.* [42] to double-tailed *nido*-carborane lipid (DCL; Fig. 5).

DCL is reported to form vesicles that exhibit good integrity in bovine blood samples [42]: injections at 7.2 mg ^{10}B/kg body weight to tumour-bearing mice effected a concentration of 22 μg ^{10}B/g tumour and extended survival rates following BNCT [43]. PEGylated congeners of these vesicles afforded increased circulation times and enhanced tumour-site accumulations, which was enhanced further following conjugation with TF. BNCT experiments involving the administration of TF-PEG-DCL liposomes to male BALB/c mice (37 minutes irradiation) have shown longer average survival rates (31 days, with one mouse surviving for 52 days after BNCT *vs.* 21 days) [28].

By drawing analogies with the chirality of phospholipids (such as DSPC), Nakamura *et al.* [44] and Lee *et al.* [45] designed BSH-functionalised lipids (Fig. 6) of relatively low toxicity.

The combination of DSPC and PEG with the lipid BSH-L1, or with BSH-L2, followed by extrusion through a 100 nm pore-diameter filter yielded nanovesicles. *In vivo* BNCT regimes (20 mg ^{10}B/kg body weight; 30 min irradiation at 0.9–1.4 × 10^{12} neutrons/cm^2) involving tumour-bearing mice have shown that over a period of two weeks the tumour growth rate in mice treated with ^{10}B-liposomes was a fifth of the controls [28].

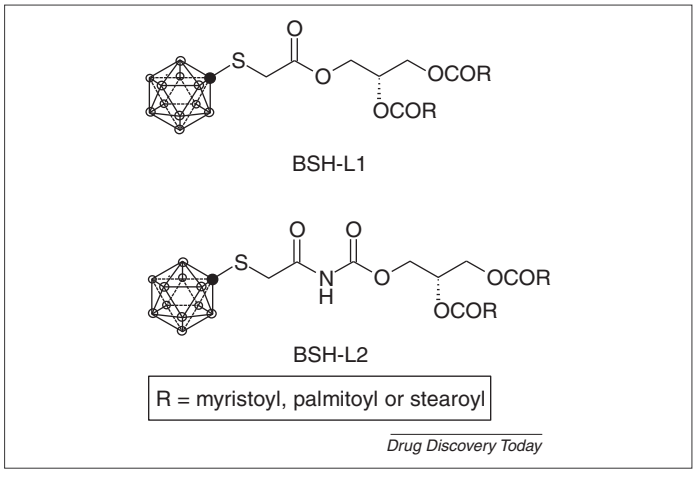

FIGURE 6

Structures of BSH-functionalised lipids.

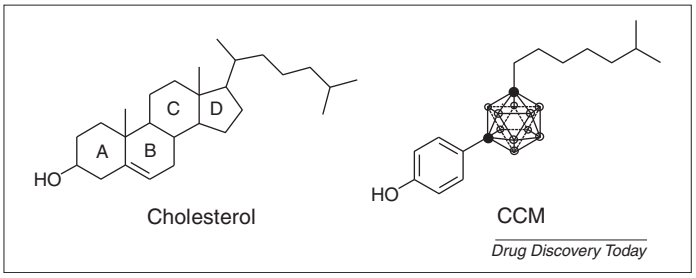

FIGURE 7

Structures of cholesterol and carboranyl cholesterol mimic (CCM).

Another strategy toward the incorporation of increased amounts of ^{10}B into liposomes involves the attachment of carboranes and BSH to cholesterol via alkyl chains [28,46].

Thirumamagal *et al.* [47] designed a molecule that was claimed to be capable of interacting with liposomes in a cholesterol-like manner (Fig. 7).

Jonnalagadda *et al.* [48] synthesised several α-carboranyl-α-acyloxy-amides, and also cholesterol and bishexadecyl-oxyglyceryl carboranes that, on preliminary evaluation, were shown to be non-cytotoxic, even at concentrations as high as 50 μM.

Dendrimers

Owing to their controllable architecture, monodispersivity and capacity to accommodate large numbers of boron atoms, dendritic BNCT agents, especially those involving conjugation with mAbs or EGFs, have received considerable attention.

Barth *et al.* [49] has shown that mAb-conjugated poly-amidoamine (PAMAM) 'starburst' dendrimers containing isocyanato polyhedral borane ($Na(CH_3)_3NB_{10}H_8NCO$) exhibit preferential accumulation in RES organs when tested *in vivo* against murine B16 melanoma. The concept of functionalisation with mAbs was also exploited by Wu *et al.* [50] who attached cetuximab to heavily boronated 'starburst' dendrimers. Because cetuximab is an EGF inhibitor it offers a further advantage in that it delays cell proliferation, which together with the five-generation dendrimer structure must be responsible for the high level of boron accumulation (92.3 μg ^{10}B/g of tumour) observed.

The linking of boronated starburst dendrimer to EGF was first attempted by Capala *et al.* [51] who synthesised molecular structures each containing more than 1000 ^{10}B atoms, but these dendrimers exhibited little affinity for EGFRs. Barth *et al.* [52] were the first to publish *in vivo* data indicating the significant therapeutic benefit associated with EGF-boronated dendrimers, either alone or in combination with BPA, as demonstrated by the increase in the life span of glioma-bearing rats [53]. Backer *et al.* [54] prepared boronated dendrimers of five-generation PAMAM that were conjugated with vascular EGF (VEGF) and possessed 1050–1100 ^{10}B atoms per dendrimer. *In vitro* studies using HEK293 cells, that had been engineered to express 2.5 × 10^6 VEGFR-2 per cell, showed negligible cytotoxicity and provided evidence for the uptake of dendrimers via a VEGF receptor-mediated mechanism. Although EGF targeting vehicles cannot act as stand-alone boron delivery agents (because of the heterogeneity of receptor expression in brain tumours) they can prove of value in therapies involving a combination of drugs [55].

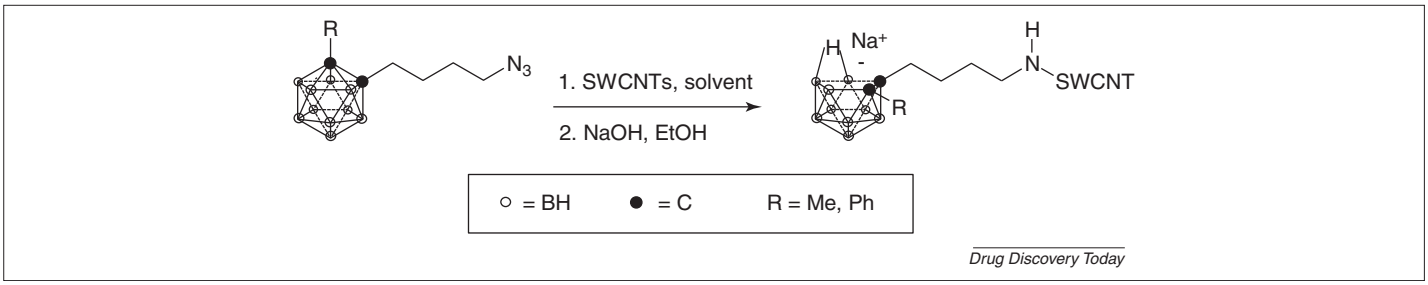

FIGURE 8

Functionalisation of SWCNTs with carboranyl clusters.

Graphite-based systems

Despite significant unknowns regarding tolerance and toxicity [56] and the absence of solubility or dispersivity [57–59], carbon nanotubes have received considerable attention because of their dual capability to penetrate biological barriers and to become internalised into the nucleus [12,56,60].

The relatively high surface area, and hence loading capacity, and the chemical and thermal stabilities of single-walled carbon nanotubes (SWCNTs; diameter 0.4–2.0 nm) [56,58] qualify them as promising BBB-crossing vehicles.

Yinghuai *et al.* [61] functionalised SWCNT with *closo*-carboranes by immobilising them onto the outer walls of the nanotubes. Subsequent degradation of the *closo*-carborane to its *nido*-form (Fig. 8), rendered the structures ionic, and hence water soluble. An *in vivo* investigation of the distribution of boron in tumour-bearing mice showed a long retention time (two days), considerable specificity toward tumour cells (concentrations as high as 28 μg ^{10}B/g tumour cells) and maximal tumour:blood ratios of 6 [61].

Additional to Yinghuai's approach for effecting solubility, two main strategies have evolved toward the prevention of the bundling of SWCNTs in liquid media: surface chemical functionalisation and the physisorption of tensioactive agents [62]. Surface chemical modification demands the disruption of the π cloud through re-hybridisation of some of the carbon atoms forming the SWCNTs, which however impacts upon the stability of these materials. The physisorption of tensioactive agents – which involves the use of surfactants or polymers to stabilise SWCNTs in aqueous media – is regarded as the method of choice for achieving dispersion, as is exemplified by the work of Yannopoulos *et al.* [62] who immobilised *o*-carborane on stable aqueous dispersions of *lyso*-phosphatidylcholine-functionalised SWCNTs.

Despite the lower toxicity of multi-walled carbon nanotubes (MWCNTs; diameter of 1.4–100 nm) [56,58] relative to SWCNTs, such materials have received little attention. The need for further investigations can be rationalised in terms of the findings of Montiro-Riviere *et al.* [63] who, having studied the capability of MWCNTs to enter cells, reported their highly preferential localisation into the cytoplasm and also the direct proportionality of the relationships between sample concentration or exposure time and concentration in the cytoplasm.

Analogous to the SWCNTs, carbon nanoparticles have recently been investigated as possible delivery vessels of ^{10}B. Hwang *et al.* [64], who examined the FR-targeting of carbon nanoparticles containing ^{10}B to HeLa cancer cells, reported that upon thermal neutron irradiation these particles induced acute cell death to the extent of 52% and also suppressed the proliferation capacity of HeLa cells that had survived.

Nanoparticles

In addition to their potential for use as tools for the controlled release of actives, nanoparticles [65] are highly stable in biological fluids, including blood. Studies [66,67] involving the use of radiolabelled drugs have revealed that appropriately designed nanoparticles are capable of overcoming the BBB and of depositing their therapeutic content in the brain. Reports claim that such structures afford up to a 10-fold increase in concentration of drug in the brain, a lessened burst effect, slow clearance and improved half-life [66,67]. Mandal *et al.* [68] tested gold nanoparticles that had been multi-functionalised with BPA, folic acid and fluorescein isothiocyanate against three cancer cell lines that are known to overexpress FR, and observed tumour:normal cell uptake ratios of 5 in the perinuclear region of cancer cells. Most promisingly, a method for the low-temperature, solution synthesis of surface-functionalised boron nanoparticles is now available [69]. In this method, the reduction of BBr_3 with sodium naphthalenide followed by the reaction of the resulting bromide-capped intermediate with octanol yields organo-capped boron nanoparticles.

Concluding remarks

Research activities for BNCT encompass not only the improvement of neutron beam characteristics and the design, synthesis and evaluation of more-selective tumour-targeting agents but also the optimisation of the transport and delivery of boronated pharmacophores. The relatively high costs associated with the construction of the neutron beam appear to have been the main factor inhibiting the evolution of a technique that offers the promise of significant therapeutic benefits, but the very considerable improvements in the efficiency and specificity of the delivery of boronated agents could provide the driving force that will bring the technique into the main stream of cancer treatments. Just as the advent of polyhedral-borane chemistry has addressed the demands of molecular design, developments in formulation strategies are appearing to address the challenges presented by the demands of the site-specific delivery of therapeutically useful quantities of these agents, but this still remains to be tested in the clinic.

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