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PRESENT STATUS OF BORON NEUTRON CAPTURE THERAPY

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The neutron capture reaction $^{10}B(^{1}n, ^{4}He)^{7}Li$ produces two energetic particles, $^{4}He^{2+}$ and $^{7}Li^{3+}$ that are strongly cell toxic. Due to the short range of these nuclear fragments (5–9 μm) mainly those cells that have bound or internalized a ^{10}B-containing substance are growth-inactivated. The most critical and difficult step in an efficient boron neutron capture therapy (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. The boron-containing substances presently considered for therapy are sulfhydryl boron hydride (BSH) and boron-phenylalanine, (BPA) for the treatment of gliomas and malignant melanomas respectively. Other 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 is 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 produces protons with a range of about 10–11 μm and this gives a radiation dose to all cells in the neutron activated area. Calculations show that the local concentration of ^{10}B near the critical radiation target, DNA, must be higher than 10 ppm (10 μg/g). Increased emphasis will be put on the development of combinations of treatments that fulfil the requirements for attacking the microscopic spread of the tumour.

Boron and neutrons constitute together a 'binary weapon' against cancer. The target cancer cells have to be loaded with ^{10}B and at a chosen time this boron load is activated by applying a radiation field of low energy (thermal or epithermal) neutrons. The nuclear reaction $^{10}B(^{1}n, ^{4}He)^{7}Li$ that is initiated by neutron capture occurs with high probability and the energetic $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions are strongly cell toxic. These nuclear fragments have ranges of 9 and 5 μm in tissue, respectively (Fig. 1). The cross-section for neutron capture varies strongly with neutron energy and is large for low energy 'thermal' neutrons and lowest for high energy neutrons. In 94% of the capture reactions prompt gamma radiation is also formed. This gamma radiation does not deliver any dosage of therapeutical value but can be used to give a relative measure of the boron content in the irradiated area at the time of neutron activation.

Natural boron contains about 80% ^{11}B and 20% ^{10}B. Since only ^{10}B has a high cross-section for capture of thermal neutrons it is advantageous to use ^{10}B-enriched compounds. Several boron compounds are now commercially available in the enriched form. In BNCT the ^{10}B containing tumour-seeking substance given to the patient should be taken up efficiently by the tumour. This is a critical aspect, otherwise BNCT will be reduced to low energy neutron radiotherapy with suboptimal doses and poor geometrical definition. When the tumour specific substance has been allowed to accumulate in the tumour and, in the ideal case, disappear from blood and normal tissues, the areas thought to contain tumour cells are

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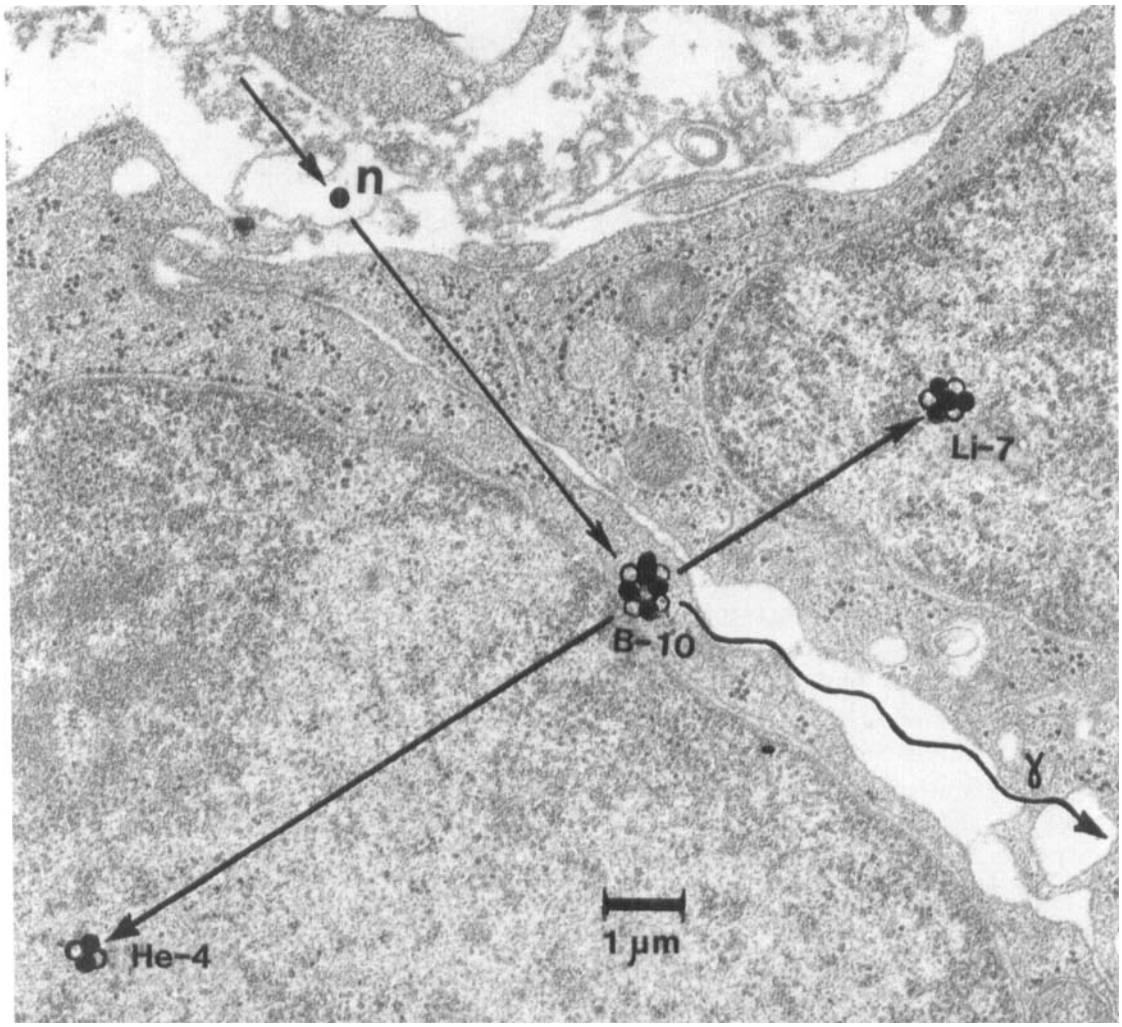

$$^{10}B + {}^{1}_{n} \rightarrow {}^{4}He + {}^{7}Li + \gamma(0.48\ MeV) + 2.31\ MeV \quad (94\%)$$
$$\rightarrow {}^{4}He + {}^{7}Li + 2.79\ MeV \quad (6\%)$$

Fig. 1. Capture of slow neutrons in ^{10}B produces the intensely ionizing particles $^{4}He^{2+}$ and $^{7}Li^{3+}$ with 9 and 5 μm range in tissue, respectively. Gamma radiation (γ) is formed in most of the capture processes.

treated with the neutrons. Due to the short range of the nuclear fragments mainly those cells that have bound or internalized the ^{10}B-containing substance are growth-inactivated. When the distribution of boron compounds within the tumour is heterogeneous, a cocktail of different boronated tumour seekers could be considered. Even when it is unlikely that all tumour cells can be treated efficiently, BNCT might be considered as a complement to other forms of therapy. The binary nature of BNCT is an advantage not only since the tumour-seeking substance can be activated at any chosen time but also since the neutron field can be delivered to selected areas so that exposure of critical healthy organs, which might contain large amounts of boron, can be avoided. The boron containing substance has to be non-toxic and the neutron field in itself should not give too much damage. Theoretically this gives good possibilities to save normal cells and tissues while the tumour cells are severely damaged. The selective action should work in spite of the fact that tumour cells are often infiltrated in normal tissue and thereby mixed with populations of normal cells.

History

BNCT was originally tested for treatment of malignant gliomas between 1953 and 1961 at Brookhaven National Laboratory and Massachusetts General Hospital (1–3). Poor results were obtained since the applied boron-containing substances had no or too little selective uptake in the tumours and by unfavourable physical characteristics of the neutron fields (4). Too high boron levels in the normal brain blood vessels and capillaries are believed to

have given severe damage to the vascular endothelium, with resulting injuries of healthy tissues. Another weak point in the early treatments was the fact that the biological action of neutrons was not as well known as today. It is possible that too high doses were given in some cases since the relative biological effectiveness (the RBE values) for the neutron fields applied might have been underestimated.

Better ^{10}B-containing substances have now been developed and promising approaches have recently been reported for the treatment of both malignant gliomas (5–9) and melanomas (10). The strategies for developing new boron-containing substances have been reviewed (11, 12) and were recently discussed at an international workshop held in Uppsala in May 1991. It was concluded that the chemistry and biology of BNCT is under intensive development and the efforts made at different laboratories around the world arouse hope for substances that are well characterized regarding their physiological behaviour, toxic action, and pharmacokinetics in tumours and normal tissues. Neutron capture in boron is today reconsidered as a possible therapeutical modality, with new principles for tumour cell targeting, progress in boron chemistry and improved neutron fields.

Targeting and BNCT

It is now possible to synthesize a large number of boron compounds (e.g. carboranes with ten ^{10}B atoms per molecule) and to use them alone or conjugated with tumour-seeking macromolecules, such as monoclonal antibodies or different types of polypeptides. Many types of tumours are candidates for BNCT, but selective tumour seekers are lacking for the large majority of tumour types. The characterization of tumour markers and tumour-associated antigens is a developing field (13–15) and the next 10 years will hopefully give increased valuable knowledge. In some cases there is already considerable knowledge about tumour-specific targets, such as the tumour-associated antigens, CEA, CA 19-9 and CA 50 in colon carcinomas (16, 17), melanin precursors in malignant melanomas (18), and amplified EGF receptors in some gliomas (19, 20) but the principles how to specifically deliver sufficient amounts of boron are not evident.

Colon carcinomas are often considered suitable for targeting since several interesting antigens have been characterized (16, 17) and this malignancy is one of the most studied types of tumours regarding antibody uptake. However, antibody-mediated BNCT for colon carcinomas is far from clinical applications. It is uncertain if enough boron can be delivered to all critical tumour cells in colon carcinomas through boronated monoclonal antibodies. The problem of antibody penetration into poorly vascularized tumour regions is now appreciated but has so far not been investigated in enough detail. Basic biological studies on transplanted colon carcinomas in experimental animals (21–24) and on cultured colon carcinoma spheroids (15, 25–27) have indicated large variations in the penetration and binding of monoclonal antibodies. The variations were both dependent on the actual cell-type and on the type of antibodies or antibody fragments applied. A problem in the case of colon carcinomas, and possibly also in other tumours, is the often seen antigenic heterogeneity (24, 28–30) which might give difficulties to target all critical tumour cells. Furthermore, there are difficulties to boronate antibodies without loosing their specificity (31).

In the case of gliomas the penetration of boron-containing substances through the blood brain barrier to areas with spread cells is a major problem (12, 32, 33). It is therefore not so easy to effectively utilize, for example, a possible tumour-specific EGF-receptor amplification for targeting of boron compounds. No clinical tests have so far been initiated where the EGF-receptor amplification is used as the target for BNCT of malignant gliomas. The clinical tests for treatment of malignant gliomas made so far concerns the use of a low molecular weight boron-containing thiol, BSH, whose binding structure is not known as will be described below.

Malignant melanomas seem well suited for clinical trials with BNCT since several tumour seeking boron compounds of interest recently have been described (10, 18, 34–37). The problem of the often reported heterogeneity of melanomas and melanoma metastases might be overcome if different substances can be mixed to 'cocktails' by which most critical cells might be reached. Whatever type of tumour is under consideration the targeting principle is presently the bottleneck in the chain leading to successful BNCT.

Boron concentrations

The required boron concentration in a tumour cell is given by the ratio between the dose due to neutron capture in ^{10}B and that of background capture in the normally existing elements nitrogen and hydrogen. Capture in nitrogen produces protons with a range of about 10–11 μm and this gives a 'background' radiation dose to all cells in the neutron activated area. The capture in hydrogen gives gamma radiation which locally not contributes very much to the dose but that may give significant effects when larger volumes of tissues are irradiated.

The cross sections for capture of thermal neutrons in nitrogen and hydrogen are not very large (1.75 and 0.33 versus 3 837 barns for ^{10}B) but both nitrogen and hydrogen are abundant in tissue and the resulting radiation doses are therefore not negligible. This means that rather high local concentrations of ^{10}B have to be present in the target cells to give a significant boron-dependent radiation dose. Some calculations, based on experiments, have been performed with regard to the necessary amounts of ^{10}B in the target

cells and effects of different spatial ^{10}B distributions (38–41). From these calculations it can be deduced that the local concentration of ^{10}B in the neigbourhood of the critical radiation target, DNA, must be more than 10 ppm (10 μg/g). If the targeting principle is working well this might not be difficult to achieve in the tumour cells to be inactivated. If, for example, a tumour-specific substance loaded with ^{10}B is internalized and degraded and the low molecular weight ^{10}B compound is intercalated in DNA, the local concentration in DNA would be much higher than the average concentration in the tissue. Certain tissues are infiltrated with few tumour cells and it is then difficult to judge if the targeting principle is working only by analysing the mean concentration of ^{10}B in the tumour infiltrated tissue. Analysis of the ^{10}B content in individual tumour cells is necessary for the judgement if the therapy is likely to be successful.

New administration principles might allow increased boron concentrations in tumour cells. It has recently been shown that dextran prevents the degradation of conjugates, based on the epidermal growth factor, EGF, after internalization in EGF-receptor amplified cultured glioma cells (42). Thus, the use of dextran-containing conjugates might allow long retention times of therapeutic agents at the cellular level. It could be questioned whether this would also give possibilities to readministrate the conjugate after new receptors have been formed and thereby load the tumour cells repeatedly with boron before the neutron irradiation. The intracellular concentration of boron might thereby be strongly increased. Such new approaches have to be considered to allow the targeting process to be optimized.

Boron-containing substances

The boron-containing substances presently considered for therapy can be divided into different groups and some examples are given in the schematic presentation in the Table. Several other substances, not mentioned in the Table have been proposed, such as porphyrins (43–46), LDL particles and liposomes (47–49), and other compounds (12, 50).

BSH Di-sodium undecahydro-mercapto-closo-dodecacarborate, BSH, (Fig. 2a) which is presently tried for treatment of malignant gliomas consists of twelve ^{10}B atoms and one SH group. It has not been possible, so far, to show good binding of BSH to isolated tumour cells but a significant uptake in malignant tumours (especially in gliomas) has been demonstrated for both BSH and the corresponding disulfide BSSB in experimental animals (51) and in surgical patient samples (6, 12).

It is possible that BSH accumulates locally in regions with poor vascularization. Such regions are often present in tumour tissues and BSH might accumulate due to the somewhat decreased pH, low pO_2 or proteolytic environment. It has, in a recent study, been shown that BSH binds in the central degenerative areas of cellular tumour spheroids (52) but not to the corresponding isolated viable tumour cells. Thus, it seems likely that BSH accumulation depends on some factor or local property related to the degenerative changes in tumours. Another possibility is that BSH through its lipophilic character is taken up in the myelin-rich brain tissue and that this mainly occurs in the regions near the primary tumour where the blood-brain barrier may be partly broken down. The levels of BSH in normal brain tissue are usually low. It has also been shown that the blood levels of BSH decrease rather fast and that, within one hour, a higher concentration of ^{10}B is obtained in the tumour mass than in the blood. Thus, the vascular endothelium will probably not be seriously damaged. Pharmacokinetic investigations of the properties of BSH have recently been reported at international meetings in Uppsala (Sweden) and in Petten (Netherlands) and will soon be published.

BPA Another substance, presently tested for therapy of malignant melanomas, is p-boronophenylalanine, BPA (Fig. 2b). Promising BNCT studies on experimental animals have been reported (10, 53) and the first clinical trial with BPA was performed by Mishima (10, 36, 54). The first patient suffered from an inoperable malignant melanoma lesion on the left occiput and the perilesional injections of BPA, followed by irradiation with thermal

Table

Examples of ^{10}B-containing substances used in clinical trials or with potential to be used in clinical trials with BNCT. The target structures and the types of tumours treated or to be treated are listed schematically

	Target structure	Tumour types treated	Tumour types to be treated
Presently tested in therapy			
BSH	Unkown	Malignant gliomas	
BPA	Unkown	Malignant melanomas	
Potential for future therapy			
Monoclonal antibodies	Tumour assoc. antigens		Antigen-rich tumours
Nucleosides	DNA		Fast growing tumours
Growth factors/hormones	Receptors		Receptor-amplified tumours
Thioureas	Melanin		Malignant melanomas

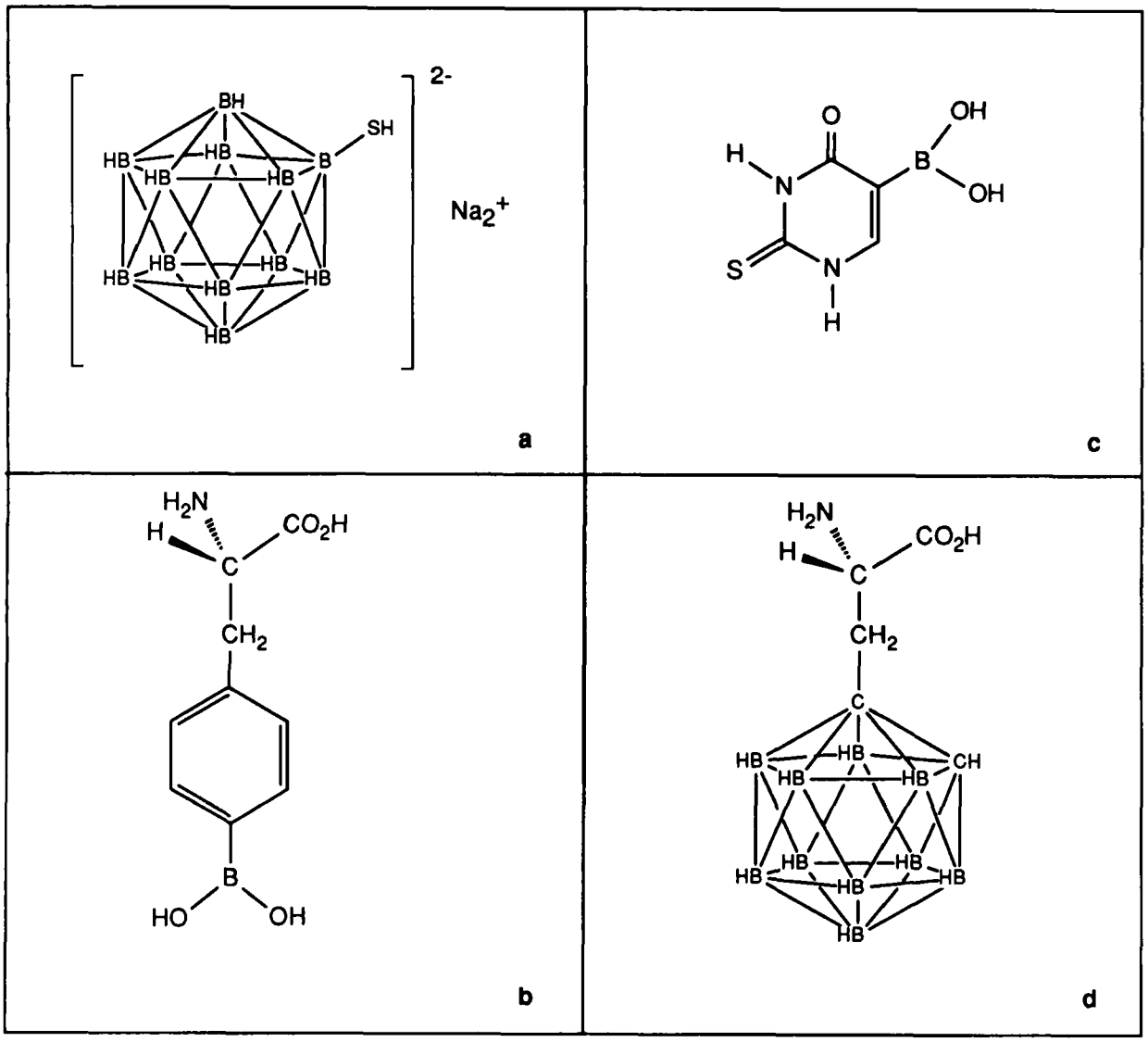

Fig. 2. Schematic structural formulas of some ^{10}B-containing substances used in clinical trials or with potential to be used in clinical trials with BNCT. a) di-sodium undecahydro-mercapto-closo-dodecacarborate, BSH. b) p-boronophenylalanine, BPA. c) 5-dihydroxyboryl-2-thiouracil. d) o-carboranylalanine.

neutrons, was successfully tested. There was no local reccurence of the treated tumour. Mishima and collaborators have later treated additional patients, and the results from these tests are to be published. The mechanism behind the retention of BPA in melanoma cells is so far not fully understood. Mishima (10) proposed that BPA selectively accumulates in melanoma tissue due to its structural similarity to tyrosine, which is the natural melanin precursor. According to Coderre (35), the BPA uptake in melanoma is dependent upon active amino acid transport rather than upon melanin synthesis. In distribution studies, on mice carrying Harding-Passey melanoma, Coderre and collaborators found that the L-isomer accumulated in actively dividing tumour cells. This was found by simultaneously comparing the ^{10}B and ^{3}H-thymidine distributions. The retention of boron in tumour tissue was transient, reaching a maximum approximately 6 h after injection. Incorporation into the melanin should give a much longer period of retention. Coderre et al. (35) also reported on results with a poorly pigmented B-16 murine melanoma indicating that BPA was still selectively accumulated in the tumour.

Thioureas. The use of thioureas as boron vehicles is another possible approach to the treatment of malignant melanoma (18, 37). Various thioureas, e.g. thiouracil, are known to be localized selectively in melanin during its synthesis (18). They are apparently bound to dopaquinone, an intermediate of the melanin synthetic pathway, and the adduct is gradually trapped in the melanin polymer during its formation. Various boronated thioureas, with specificity for growing melanin, have been synthesized and tested for

melanoma targeting in mice carrying murine melanoma. Examples are decarborane adducts with 5-(diethylamino) methyl-2-thiouracil and 1H-1,2,4-triazole-3-thiol (55), 5-dihydroxyboryl-2-thiouracil and its 6-propyl derivative (11, 56), a carboranyl-thiouracil adduct (57) and some other compounds (18). 5-dihydroxyboryl-2-thiouracil (Fig. 2c) was found to be selectively localized in B16 and Harding-Passey melanomas, transplanted to mice. The retention in the tumours was persistant in time, and 3 h after the injection, boron concentrations higher than 100 ppm were obtained, which is sufficient for possible application of BNCT (11). So far, none ot the boronated thioureas has been subject to clinical testing. A drawback of using boronated thioureas is the tendency of melanoma metastases to lose pigmentation. About 7% of the primary cutaneous melanomas are amelanotic, but as much as 31% of the distant metastases lack melanin (58). These conditions restrict the use of boronated thioureas for BNCT to melanotic melanoma, and if poorly pigmented cells are present in the tumours, the use of a 'cocktail' of boronated melanoma-seekers, e.g. BPA or boronated monoclonal antibodies added to the thiouracils will be necessary.

Monoclonal antibodies. Several attempts have been made to label monoclonal antibodies with boron (59–69). The main problem with antibodies has so far been loading them with large amounts of boron without losing the 'immunoreactivity', i.e. the binding capacity to the antigens. A possible approach is to attach the boron to the carbonhydrate domains on the long arms of the antibodies as has been made for chelators for the delivery of toxic radionuclides such as ^{90}Y and ^{212}Bi (70).

Nucleosides and oligonucleotides. Several types of nucleosides and oligonucleotides containing boron are presently synthesized and tested at the Department of Pharmacy, Columbus, Ohio State University (Albert Soloway, personal correspondance) and at some other laboratories (71). The hope is that the nucleosides are selectively incorporated in the DNA of the fast growing tumour cells which should increase the BNCT efficiency in tumour cell killing substantially. It might in the future be possible to synthesize boronated oligonucleotides with sequences that have homology with sequences in, for example, endogeneous mRNA and thereby they might bind and be retained in the cells. However, oligonucleotides can not easily penetrate into cells and the problem of membrane permeabilization is not solved so far. The possibilities to obtain selective tumour-binding through specific homology with nucleotide sequences which are unique for the tumour cells is a great challenge for the future. The target structure might be mRNA from an activated oncogene. Another possibility is to utilize 'normal' mRNA which, as a result of the tumour transformation, is overproduced through gene amplification or abnormal transcription as the target structure. Possible protooncogene and oncogene candidates are described in recent reviews (72–74). Such targeting principles seem very futuristic but considering the fast development in molecular biology it may be possible to test the principles in vitro within the near future.

Growth factors and hormones. Growth factor receptors might be targets for BNCT if they are overexpressed in the tumour cells. For example, it has been reported that the EGF receptors are overexpressed in 25–30% of highly malignant gliomas (19, 20, 75). The targeting substance can be monoclonal antibodies against the EGF-receptor labelled with ^{10}B or it can be EGF-based conjugates containing ^{10}B. Aminoalkylcarboranes (76) and carboranylaminoacids, such as o-carboranylalanine (77) (Fig. 2d), are considered for conjugations to EGF. The advantage of using EGF-based conjugates is that the binding to the receptor is very specific and the risk of unspecific binding is low. Furthermore, EGF-containing conjugates can be made rather small and this is an advantage both for the penetration into tumours and for the decreased risk of unwanted immunogenic effects. EGF-based conjugates have so far mainly been tested in vitro (42). It has been shown that conjugates between EGF and toxic substances selectively kill cells with large amounts of EGF receptors (78, 79). It has also been shown that EGF-guided targeting of ^{131}I give specific toxic effects to receptor-rich glioma cells (80–82). Other approaches might be to selectively target cancer cells, rich in hormone receptors, via specific binding of boron-containing hormones, e.g. to load estrogen or estramustin receptor-rich prostate cancer cells with boron through boron-containing estrogen or estramustin. One possible candidate for such a therapy might be prostatic cancers.

Neutron beams

The cross-section for capture of neutrons in ^{10}B is highest for low energy thermal or epithermal neutrons and such neutrons are therefore considered for BNCT. There might also be some possibilities to use fast neutrons in combination with BNCT (83). Thermal and epithermal neutrons are easily obtained from nuclear reactors. The clinical tests with BSH-based BNCT against gliomas in Brookhaven, Massachusetts, and more recently in Japan (5–9, 39, 84), showed that thermal neutrons do not have a good penetration across the skull and it is therefore thought that reactor-produced epithermal neutrons preferably should be used (39). The epithermal neutrons are thermalized within the brain and will then have the ideal energy for capturing by ^{10}B.

Neutrons can also be produced with conventional accelerators for non-relativistic acceleration of protons (85, 86). The present development is that of high fluxes of either high energy protons colliding with a heavy metal (87, 88) or low energy protons colliding with lithium (85, 86, 89). In both cases accelerators with extremely high beam currents have to be used (0, 1–1 mA) to induce enough

amounts of neutrons. The induced fast neutrons are then slowed down in moderators containing low atomic number elements and the induced gamma and roentgen radiation must be absorbed with high atomic number filters. An experimental set-up based on high energy protons for production of epithermal neutrons is presently tested at the Paul Scherrer Institute (PSI) in Switzerland in collaboration with scientists in Uppsala (87, 88). If this, and other efforts along the same lines, is successful it will be possible to use BNCT at many centres since the cost of high current proton accelerators is only a few times higher than that of conventional linear accelerators for electron and photon radiation therapy.

Yet another alternative to create neutrons is by use of neutron-emitting radionuclides such as ^{252}Cf (84, 90) which has been used in some centres for treatment of cervical carinoma. One possibility for BNCT could be to find a tumour-specific boron compound (e.g. boron loaded monoclonal antibodies with specific binding to the cervical cancer cells) and then to expose the tumour mass for neutrons through intracervical insertion of the ^{252}Cf source.

Thus, the creation of neutron fields of suitable energy and geometrical extension can be solved rather well today, and epithermal neutron beams which are 'thermalized' in the tissues can be produced. This has recently been shown both at the reactor at Brookhaven (BNL, Long Island, New York) and at the high flux reactor in Petten (the Netherlands). The reactor in Petten has an epithermal neutron beam designed for BNCT and will be used within an 'EC-concerted action' for treatment of malignant gliomas. The shape of the epithermal neutron fields, suitable for BNCT of malignant gliomas, was recently presented at an international meeting in Petten in September 1991.

Experimental therapy

Tests of the BNCT principle have been made for some boron containing substances in cell cultures, in animal models but also in patients (12, 91). Such tests have, when the targeting principle has been working, shown that the capture process in ^{10}B really gives an improved inactivation of tumour cell proliferation. The controls in such experiments are normally irradiated with neutrons without addition of ^{10}B-substances. Increased cell killing by a factor up to 5 has been reported in the animal and cell culture studies (12). It can be concluded that there will be therapeutical advantages of using BNCT as long as the targeting process is working and the ^{10}B mainly is associated with the tumour cells.

A question which has high priority for radiobiological research regards the tolerance dose for neutrons, and neutrons in combination with boron, of normal tissue (4, 12). This is of special importance to brain tissues in the planned treatments of malignant gliomas in Petten. Experiments are presently performed on dogs to analyse the tolerance dose with and without boron administration. It has previously been seen that normal brain tissue is rather sensitive and that fractionated radiation giving total doses higher than 30–35 Gy of conventional low-LET radiation might give late effects such as degeneration of the white matter.

Clinical trials with BSH in Petten

In Japan several patients with malignant gliomas (mainly grades III and IV) have been treated with BNCT (5–9). These treatments are based on the accumulation of BSH in the tumour tissue. The treatment of the most superficial tumours with BNCT has thereby given encouraging results. Highly malignant gliomas are among the tumours most difficult to treat and most patients die within one year after diagnosis. Nearly no patients survive for two years. Professor Hatanaka in Japan reports that some of these patients, who were diagnosed to have malignant gliomas grades III-IV, are considered cured as they have been free from recurrence for several years after treatment, in some cases even for ten years. This reported success has now led to the planned tests with BNCT for malignant gliomas in Petten.

The 'Petten-project' is administrated through a 'concerted action' by the EC-countries. Research groups in Sweden and Switzerland also take part as full members in this collaborative research project. The planning is to start patient treatments at the beginning of 1994. All participating countries are allowed to send glioma patients for treatment. The patient selection will be made by a team of physicians in each country and the coordination will be made by physicians in Petten. The costs of each treatment during the first years will, reduced by travelling costs, not much exceed those of conventional radiotherapy to brain tumours. It will be possible both to irradiate inoperable tumours and to treat patients after surgery. Measures to increase the tolerance dose of normal tissue to the neutron radiation, such as corticosteroid treatment, will of course be taken.

The 'Petten-project' should be considered a 'high risk' project. The underlying principles for BSH accumulation in tumour cells and in brain tumours are not clear (52). The cell biological basis for BSH-based BNCT of malignant gliomas is not well founded and if malignant gliomas were not such a severe disease therapy should be recommended not to start before more basic knowledge about BSH binding to glioma cells, cellular internalization kinetics and degradation patterns was better known. However, the encouraging reports by Professor Hatanaka in Japan (5–9) that malignant gliomas can really be cured makes it difficult to delay trials with BSH-based BNCT, especially when nearly all preparations already have been made in Petten. Many lives may be saved if the therapy works.

A treatment failure in the form of lacking growth retardation of the tumours or unacceptable side-effects on normal brain tissue may lead to critisism of this type of therapy and this may have negative consequences for planned future BNCT activities, based also on other substances than BSH. The whole research field might then be delayed or even stopped.

Single dose versus fractionation

It has been discussed whether the neutron doses should be fractionated or not when treating malignant gliomas in Petten. The clinical trials by Professor Hatanaka were made as single doses with the skull bone open to allow efficient passage of the thermal neutrons into the tumour area. An argument against fractionation is that the highest possible neutron dose should be applied in combination with the highest possible concentration of ^{10}B in the tumour and that these conditions will be difficult to achieve repeatedly.

An argument for use of at least two or more fractions is the fact that the tumour dose is to a large extent delivered by very high LET radiation from the helium and lithium ions while the surrounding normal brain tissue mainly will be influenced through neutron capture in nitrogen and hydrogen and to the prompt 0.48 MeV gammas which are formed in 94% of the neutron captures in ^{10}B. On the average, these components have a much lower LET than the helium and lithium ions. Effects of low LET can be repaired by cells while repair is not easily made after high LET radiation. This means that it should be an advantage for the normal tissue to use fractionated treatments since the normal tissue should then be allowed to recover between the irradiations while this could not be the case for the high LET irradiated tumour cells. A similar effect may be achieved through extended irradiation.

Considering the pharmacokinetics of BSH, as reported at the Petten meeting in September 1991, it is, when fractionated treatment is considered, necessary to give a second neutron irradiation within 24 h otherwise the ^{10}B concentration has decreased too much in the tumour. If for example, the interval is 48 h or longer, BSH has to be readministered to obtain sufficient concentration in the glioma tissue. It is not known how effective a 'retargeting' of BSH is after a previous radiation although there are indications from experiments on mice (92) that BSH is more easily taken up in the brain after a first treatment. Thus, it might be easier for BSH to penetrate and reach the tumour cells after the first radiation. This has to be analysed in more detail.

Combined treatments

A general approach for BNCT, whatever type of tumour is considered, could in the future be initially to try different combinations of surgery, external radiation, chemotherapy, hyperthermia or other suitable modalities and in some way combine them with BNCT. For example, metastases with limited spread or with rather well-known localization, possible to be included in the applied neutron field, could be targeted with boron-containing tumour seeking agents for BNCT. This is, according to the authors of this article, a reasonable approach since it might in many cases be difficult to erradicate all tumour cells in the primary tumour mass by BNCT only.

There are also some basic tumour biological aspects in favour of the use of BNCT in combination with other treatment modalities. Targeting boron-containing macromolecules might in most cases bind to tumour cells in well vascularized regions of the tumours. This means that mainly the tumour cells close to capillaries will be efficiently treated. Cells in poorly vascularized areas or cells that are positioned one or a few hundred microns from the nearest capillary are difficult to reach and this seems to be a common feature for all treatments based on tumour seeking macromolecules. The targeting process must in these cases be complemented with a treatment modality, e.g. external radiotherapy, that inactivates the remaining cells with proliferative capacity (93, 94).

Another possible complementary treatment modality, not yet tested, is based on the fact that cells in bad vascularized regions or far from the nearest capillaries probably suffer from a lower extracellular pH than do cells in other regions of the tumour (95). Hyperthermia and certain drugs interfering with intracellular pH control can be selectively toxic to such cells (95–97). From a theoretical point of view it should therefore be possible to combine a targeting modality, such as BNCT or radioimmunotherapy, RIT, with external radiation fields and with hyperthermia and/or administration of drugs that are mainly active at low extracellular pH.

Research groups in many countries try to clarify the advantages and the limitations of targeting therapy whether in the form of BNCT or RIT. We foresee that increased efforts will be made to develop treatment combinations that fulfil the requirements of attacking both the primary tumour and the spread of metastases. BNCT is one promising approach that should be considered in the efforts to develop such combined treatments.

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