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Model studies directed toward the boron neutron-capture therapy of cancer: Boron delivery to murine tumors with liposomes

(vesicles)

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ABSTRACT **The successful treatment of cancer by boron neutron-capture therapy (BNCT) requires the selective concentration of boron-10 within malignant tumors. The potential of liposomes to deliver boron-rich compounds to tumors has been assessed by the examination of the biodistribution of boron delivered by liposomes in tumor-bearing mice. Small unilamellar vesicles with mean diameters of 70 nm or less, composed of a pure synthetic phospholipid (distearoyl phosphatidylcholine) and cholesterol, have been found to stably encapsulate high concentrations of water-soluble ionic boron compounds. The hydrolytically stable borane anions $B_{10}H_{10}^{2-}$, $B_{12}H_{11}SH^{2-}$, $B_{20}H_{17}OH^{4-}$, $B_{20}H_{19}^{3-}$, and the normal form and photoisomer of $B_{20}H_{18}^{2-}$ were encapsulated in liposomes as their soluble sodium salts. The tissue concentration of boron in tumor-bearing mice was measured at several time points over 48 h after i.v. injection of emulsions of liposomes containing the borane anions. Although the boron compounds used do not exhibit an affinity for tumors and are normally rapidly cleared from the body, liposomes were observed to selectively deliver the borane anions to tumors. The highest tumor concentrations achieved reached the therapeutic range (>15 μg of boron per g of tumor) while maintaining high tumor-boron/blood-boron ratios (>3). The most favorable results were obtained with the two isomers of $B_{20}H_{18}^{2-}$. These boron compounds have the capability to react with intracellular components after they have been deposited within tumor cells by the liposome, thereby preventing the borane ion from being released into blood.**

Boron neutron-capture therapy (BNCT), first proposed by Locher in 1936 (1), is based upon the propensity of the ^{10}B nucleus to undergo the $^{10}_{5}B + {}^{1}_{0}n \rightarrow {}^{7}_{3}Li + {}^{4}_{2}He$ reaction with thermal neutrons. This process releases 2.28 MeV of kinetic energy, which is distributed between the α-particle and the $^{7}Li^{+}$ ion. The effective distance of travel of these two ions in tissue is limited to approximately one cell diameter. During their passage through the interior of a cell, the energetic fission products cause ionization-tracking and cellular damage with associated cytotoxicity. Since the neutron capture cross-section of the ^{10}B nucleus is 10^3 to 10^4 greater than that of all elements of physiological importance, the selective concentration of ^{10}B atoms within cancer cells, followed by irradiation with thermal neutrons, should result in the destruction of the tumor cells even in the presence of neighboring normal cells.

The development of effective targeting strategies for the selective transport of boron to cancer cells has been the single most urgent problem in the area of BNCT. Successful therapy requires the site-specific delivery of relatively large amounts (15–20 μg of B per g of tissue) of boron to tumors (2). Strategies employed have included the use of boron compounds with some natural affinity for tumors, such as 4-(dihydroxyboryl)phenylalanine (BPA) (3) or the mercaptoundecahydro-*closo*-dodecaborate dianion ($B_{12}H_{11}SH^{2-}$; BSH) (4); the attachment of boron-containing species to other molecules such as porphyrins (5); and the conjugation of boron-rich compounds with tumor-specific monoclonal antibodies and their fragments (6). In any targeting strategy, the primary difficulties to be overcome are the delivery of therapeutic quantities of boron to tumor, lack of tumor specificity, and the mitigation of such specificity after incorporating useful amounts of boron in the targeting agent.

Liposomes present a novel approach for the solution of many of these problems. Although liposomes in general do not concentrate specifically in tumors, it has been demonstrated that certain physically robust liposomes of an appropriate size will accumulate in tumors in high concentration relative to normal tissues, including blood. As described below, such liposomes have been exploited by us as specific carriers of boron-containing compounds to cancerous cells for the purpose of BNCT.

There have been numerous attempts, generally unsuccessful, to target tumors with lipid vesicles *in vivo* (7). Many previous studies have been thwarted by the use of lipids of arbitrary chemical composition and/or inappropriate vesicle size. Impurities in phospholipid components can adversely affect the stability of the liposome in serum (8), and the surface presented by the lipid vesicle affects its interaction with cellular membranes and serum components. Such interactions will determine the specificity of cellular uptake. In addition, the size of the vesicle is related to its rate of clearance from the blood by the reticuloendothelial system as well as its ability to diffuse into tissues (9).

While the precise mechanism for selective uptake of liposomes by tumor cells remains somewhat obscure, transport out of circulation is probably aided by the increased and immature vasculature of a rapidly growing tumor mass in a manner that is independent of tumor type. Once presented to the tumor through this leaky vasculature, the liposomes may be internalized by endocytosis via an indeterminate mechanism, such as a coated pit to coated vesicle transformation, and distributed among cellular organelles and cytoplasm (10). In this manner, liposomes may provide a direct pathway for the selective introduction of a desired species to the interior of a tumor cell.

The liposomal delivery of drugs, when successful, has several attractive consequences. Sequestering the effector species in vesicles can provide it with an extended circulation lifetime (8, 11, 12), thereby increasing its opportunity to be taken up by tissue. The liposome also offers protection for the

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Abbreviations: BNCT, boron neutron-capture therapy; *n*-$B_{20}H_{18}^{2-}$ and *i*-$B_{20}H_{18}^{2-}$, normal form and photoisomer of $B_{20}H_{18}^{2-}$.

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occluded species from attack by normal physiological agents *in vivo* and reduces its potential toxic effects (7, 12, 13). Since the selective delivery and cell-entry mechanisms are provided by the liposome, the delivered species need not have a natural affinity for the targeted tumor cells, thus making this method amenable to a wide variety of effector molecules.

Previous research, utilizing pure synthetic phospholipids, has produced useful unilamellar vesicles that have been shown to deliver their contents preferentially to tumor cells in animals (11, 14) and humans (15, 16) such that the tumor levels of effector molecules are 5–10 times that of normal tissue, including blood.

The constituents of the lipid membranes of the synthetic liposomes described in this study are ubiquitous to mammalian physiology. Consequently, no adverse reactions were observed in recently conducted clinical trials of similar liposomes with comparable compositions (15, 16) and in the preclinical animal toxicity tests that preceded them. Since the tumor-targeting characteristics of liposomes are essentially independent of their encapsulated contents, we have examined the potential of liposomes for the selective delivery of therapeutic quantities of ^{10}B to tumors (>15 ppm).

MATERIALS AND METHODS

Materials. The polyhedral borane anion derivatives used were prepared by published methods (17–21). Disodium mercaptoundecahydro-*closo*-dodecaborate ($Na_2B_{12}H_{11}SH$) was a gift from the Callery Chemical (Pittsburgh). Distearoyl phosphatidylcholine was from Avanti Polar Lipids, and cholesterol was supplied by Calbiochem or Sigma.

Vesicle Preparation. Liposome emulsions were prepared by probe sonication of a dried film composed of equimolar amounts of the phospholipid and cholesterol with the hydrating solution (typically 5 ml, 250–300 mM in the boron-containing salt) at 65°C for 15–30 min. The vesicles were separated from the remaining free borane salt by eluting through a column of Sephadex G-25 (medium) with isotonic phosphate-buffered saline or lactose. Liposomal preparations were diluted with the appropriate buffer to a lipid concentration of 23–24 mg/ml and sterilized by filtration through a 0.22-μm Millipore membrane. The integrity of the encapsulated borane salt was confirmed by ^{11}B NMR at 162 MHz. The volume-weighted mean vesicle diameter of the liposomes was determined by dynamic light scattering.

Murine Studies. All murine biodistribution studies utilized female BALB/c mice (16–20 g), with EMT6 tumors implanted in the right flank 7–10 days prior to the experiment. Tumor mass at the time of sacrifice was 125–350 mg. Injections of liposome emulsions (200 μl) were made in the tail vein. Prior to sacrifice, each mouse was anesthetized with Halothane and bled into heparinized syringes via cardiac puncture. The blood was then placed into tared cryogenic tubes. While under anesthesia, mice were euthanized via cervical dislocation. The tumor, liver, and spleen were dissected and also placed in tared cryogenic tubes. Blood and tissues were stored frozen until analyzed. Animal experiments were done in accord with guidelines of the Animal Welfare Act and in compliance with protocols approved by the Vestar, Inc. Institutional Animal Care and Use Committee. Boron analyses of tissues and of liposome emulsions were performed by inductively coupled plasma–atomic emission spectroscopy (ICP-AES) (22) at the Idaho National Engineering Laboratory except in the case of $Na_2B_{10}H_{10}$, which was determined colorimetrically *in vitro* and by scintillation counting of tritiated $Na_2B_{10}H_{10}$ in animal tissues (*vide infra*). Each data point represents the average of five mice. For clarity, error bars are not shown in the graphical data; standard deviations were typically 5–15% of the average values.

RESULTS

Physical Characterization. The encapsulation efficiencies of the liposome preparations were approximately 2–3%. This low efficiency is typical for the sonication procedure employed. The borane salt not encapsulated in the vesicles was routinely recovered by elution from the gel column and recycled. The mean diameters of the liposomes containing the borane species averaged slightly more than 50 nm, with the smallest mean diameter of 43 nm for $Na_2B_{12}H_{11}SH$ and the largest of 70 nm for $Na_2B_{10}H_{10}$.

Because of the relatively high concentrations of the borane anion solutions encapsulated, the stability of several liposomal preparations in buffered solutions was examined. For example, in the case of the $B_{10}H_{10}^{2-}$ liposomes, after 3.5 months storage at room temperature, only about 6% of the original contents had escaped from the liposomes. This is a clear indication of the stability of the bilayer employed, since the entrapped solution (250 mM $Na_2B_{10}H_{10}$) has an osmolarity 2.5 times that of the external buffer. Attempts to determine the stability of the liposomes in serum at 37°C have been experimentally complicated by the nonspecific binding of the borane anions to serum proteins, the subject of another study. Indirect evidence that the liposomes are stable in serum comes from the observation that high blood levels of boron in mice persist for many hours, whereas breakdown of the vesicles would lead to rapid clearance of boron.

Biodistribution Studies in Mice. The biodistribution of vesicles loaded with the tritiated disodium salt of $B_{10}H_{10}^{2-}$ is presented in Fig. 1*A* (110 μg of B injected dose, ≈6 mg/kg of body weight). The tumor initially accumulates boron, but the dose that is delivered is quickly expelled from the tumor (46% loss between 6 and 24 h), and the ultimate concentration of

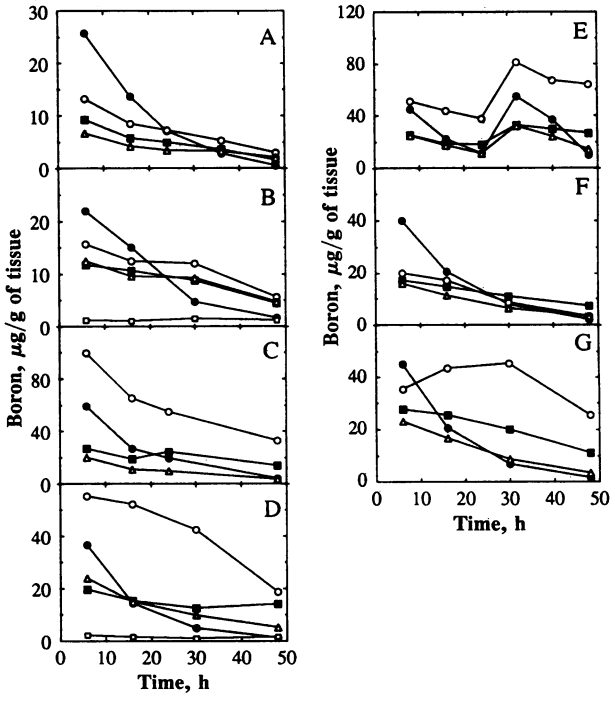

FIG. 1. Murine tissue boron concentrations from delivery of borane salts by liposomes. ●, Blood; ■, tumor; ○, liver; △, spleen; □, muscle. Borane salts with the injected doses were $Na_2B_{10}H_{10}$, 110 μg of boron (≈6 mg/kg of body weight) (*A*); $Na_2B_{12}H_{11}SH$, 126 μg of boron (≈7 mg/kg of body weight) (*B*); $Na_2(n\text{-}B_{20}H_{18})$, 273 μg of boron (≈15 mg/kg of body weight) (*C*); $Na_2(i\text{-}B_{20}H_{18})$, 206 μg of boron (≈11 mg/kg of body weight) (*D*); $Na_2(i\text{-}B_{20}H_{18})$, 280 μg of boron each injection (≈15 mg/kg of body weight) (*E*); $K_4B_{20}H_{17}OH$, 200 μg of boron (≈11 mg/kg of body weight) (*F*); and $Na_3B_{20}H_{19}$, 134 μg of boron (≈8 mg/kg of body weight) (*G*).

boron remains below the therapeutic level. The blood boron concentration does not drop below that of the tumor until late in the 48-h experiment when the tumor boron concentration is too low to be useful. In fact, all tissues rapidly lose their delivered dose as time progresses.

Liposomes loaded with the disodium salt of $B_{12}H_{11}SH^{2-}$ exhibited the biodistribution displayed in Fig. 1*B* (126 μg of B injected dose, ≈7 mg/kg of body weight). Here the blood is cleared at approximately the same rate as that observed in the case of $B_{10}H_{10}^{2-}$, but because the boron species escapes from the tumor cells at a lower rate (25% loss between 6 and 30 h), the overall results are more favorable. Blood-boron concentrations have dropped below those of the tumor 24 h after injection, and at 30 h the tumor/blood ratio is nearly 2 with a tumor boron concentration of 8.8 μg/g. As tissue levels continue to fall, the tumor/blood ratio reaches 2.6 at 48 h. These results do not represent therapeutically useful values.

The biodistribution of the liposomes containing the disodium salt of the normal form of $B_{20}H_{18}^{2-}$, designated $Na_2(n\text{-}B_{20}H_{18})$, is displayed in Fig. 1*C* (273 μg of B injected dose, ≈15 mg/kg of body weight). As expected, the use of a 20-boron-atom species has significantly raised the boron dose delivered to tissue. This is most noticeable in the liver values, which at 6 h represent about 35% of the injected dose per g. However, while the blood and liver clear rapidly, the tumor level remains fairly constant, decreasing only 11% between 6 and 24 h. At this point the tumor/blood ratio is 1.2 with 24.1 μg of B per g in tumor. The tumor/blood ratio increases to 3.3 at 48 h, while the tumor level dropped to 13.6 μg/g.

An isomer of $n\text{-}B_{20}H_{18}^{2-}$, designated $i\text{-}B_{20}H_{18}^{2-}$, results from the photochemical rearrangement of the normal isomer. Fig. 1*D* presents the even more promising results obtained with the liposomes containing the disodium salt of the photoisomer (206 μg of B injected dose, ≈11 mg/kg). With this species, the tumor-boron concentration persists quite well; the tumor-boron concentration at 48 h (13.9 μg/g) is still 71% of the value at 6 h. This long retention allows sufficient time for the blood boron concentration to decrease to very low levels, resulting in a tumor/blood ratio of 12. This represents a potentially useful accumulation of boron in the tumor with an extremely low blood boron concentration (1.2 ppm).

Free $Na_2(i\text{-}B_{20}H_{18})$ in buffer solution was injected, and the results are presented in Table 1. The injected dose was 200 μg of boron, approximately the same dose used in the liposome experiment described above. The borane anion showed some affinity for liver, although it was not as high as that of the encapsulated material. The remaining tissues examined contained only traces of boron 6 hr after injection.

The liposomes containing $Na_2(i\text{-}B_{20}H_{18})$ described above were investigated in a double injection experiment in which liposomes were injected at 0 and 24 h. Each of these injections contained 280 μg of boron (*ca.* 15 mg of boron per kg of body weight, and mice given both injections received 560 μg of boron or 30 mg/kg). The data obtained during the first 24 h (Fig. 1*E*) resembles that observed in the previous experiment with $Na_2(i\text{-}B_{20}H_{18})$. The second injection then results in an increase in the boron concentrations of all tissues, followed by the clearance of the tissues at their usual rates. This results in a tumor boron concentration of 26.6 μg/g at 48 h and a tumor to blood ratio of 2.6.

Table 1. Murine tissue boron concentrations after injection of free $Na_2(i\text{-}B_{20}H_{18})$ (200 μg of boron, ≈11 mg/kg of body weight) in buffered solution

Tissue	Boron concentration, μg/g of tissue ± SD	
	6 h	24 h
Blood	0.9 ± 0.1	1.9 ± 0.7
Liver	18.1 ± 0.8	8.9 ± 1.5
Spleen	0.9 ± 0.1	1.5 ± 0.5
Tumor	1.2 ± 0.3	1.9 ± 0.6

The biodistribution of the potassium salt of the ion $B_{20}H_{17}OH^{4-}$, the hydrolysis product of $i\text{-}B_{20}H_{18}^{2-}$, is shown in Fig. 1*F* (200 μg of B injected dose, ≈11 mg/kg). The blood quickly loses 80% of its boron content between 6 and 30 h, resulting in a 48-h tumor/blood ratio of 3.4. Although the liver and spleen rapidly clear, the tumor does not retain the boron. There is a 57% decrease in the tumor boron concentration over the allotted time period, with a final boron concentration of only 7.3 μg/g.

Liposomes loaded with the sodium salt of $B_{20}H_{19}^{3-}$, the reduction product of $B_{20}H_{18}^{2-}$, displayed the biodistribution exhibited in Fig. 1*G* (134 μg of B injected dose, ≈8 mg/kg). The liver accretes boron over a period of 30 h, resulting in a maximum concentration of 45.3 μg/g, which then decreases by 44% over the next 18-h time period. Blood and spleen are cleared during the 48-h time period, as observed in the other systems. Although the tumor boron concentration decreases steadily (60% loss between 6 and 48 h), the final concentration is still 11.2 μg/g because of the very high initial uptake of boron by tumor. This observation is especially interesting considering the relatively low injected dose. The final tumor/blood ratio of boron is 7.5.

DISCUSSION

A primary concern related to the delivery of boron for BNCT is the need to obtain sufficiently high boron concentrations in tumors to allow successful therapy. Small unilamellar vesicles are attractive delivery vehicles because of their ability to encapsulate relatively large amounts of water-soluble borane species in their aqueous core. Since the borane salts employed here exhibit high water solubility, the ultimate borane concentration that can be used is limited by liposome stability. It has been determined previously that liposomes constructed from chemically homogeneous, pure phospholipids exhibit enhanced stability *in vitro* and *in vivo* (8, 11). In the present study, the use of a pure synthetic phospholipid in conjunction with cholesterol has produced a sturdy bilayer membrane capable of encapsulating hyperosmotic solutions of these borane salts with up to 3 times the osmotic pressure of the external physiological buffer. This has allowed the preparation and examination of liposome emulsions containing nearly 1.5 mg of boron per ml.

The injected doses of boron used in this work (6–15 mg/kg of body weight) are much lower than those usually employed in BNCT studies that attempt to approach therapeutic concentrations of boron in tumors by using boron delivery methods and vehicles other than liposomes (23–28). The quantity of boron administered in these other studies is typically in the range of 30–50 mg of boron per kg of body weight. The tumor-targeting characteristics of the type of liposomes employed here allowed small injected doses of boron to achieve therapeutic concentrations in tumor.

The biodistribution of boron observed by using liposomal delivery is clearly due to the mode of transport, since free polyhedral borane anion salts such as $i\text{-}B_{20}H_{18}^{2-}$ and some of their simple derivatives have been shown to be cleared rapidly from circulation with little tissue uptake. In the case of free $i\text{-}B_{20}H_{18}^{2-}$, only negligible boron concentrations were observed in tissue; only liver exhibited an appreciable accretion of boron. Throughout the present study, liver and spleen tissues were routinely examined because these tissues, in contrast to other normal tissues, normally take up liposomes to a significant degree.

The *closo*-decahydrodecaborate dianion was selected for our initial investigations of liposomal delivery of boron

because it is conveniently prepared in high yield from decaborane (Eq. 1) (17, 29), and its sodium salt is relatively inert and known to be nontoxic (28). The $B_{10}H_{10}^{2-}$ ion also reacts quantitatively with phenyldiazonium cation (30) to produce an intensely colored azo dye (Eq. 2), used to determine decahydrodecaborate concentrations *in vitro*. For *in vivo* analyses, the anion was tritiated by an acid-catalyzed hydrogen-exchange process (31), which is rapid at pH < 1 (Eq. 3).

$$B_{10}H_{14} + 2Et_3N \rightarrow [Et_3NH]_2B_{10}H_{10} + H_2 \quad [1]$$

$$B_{10}H_{10}^{2-} + C_6H_5N_2^+ \rightarrow 1\text{-}B_{10}H_9NH{=}NC_6H_5^- \quad [2]$$

$$B_{10}H_{10}^{2-} + {}^3H_2O \rightleftarrows B_{10}H_9{}^3H^{2-} + H^3HO \quad [3]$$

Although the liposomes that contained $B_{10}H_{10}^{2-}$ did not provide therapeutically useful concentrations of boron in tumor, this initial biodistribution experiment did provide an excellent point of reference. A therapeutically useful technique requires significant amounts of boron to remain within the tumor in order to provide sufficient time for the clearance of boron from other tissues. Although the boron was not retained in the tumor for sufficient periods of time when using $Na_2B_{10}H_{10}$, this early experiment indicated that liposomes could be used to deliver polyhedral borane salts to tumors.

In an attempt to deliver and retain additional boron in the tumor we next examined disodium mercaptoundecahydro-*closo*-dodecaborate, $Na_2B_{12}H_{11}SH$, a compound previously demonstrated to have some unspecified affinity for tumors (24–27). Although the biodistribution data reported here are generally quite similar to that observed with $B_{10}H_{10}^{2-}$, the retention of $Na_2B_{12}H_{11}SH$ by tumors is supported to some degree by the data obtained. Thus, the reduction of the tumor-boron concentration over time observed with $B_{12}H_{11}SH^{2-}$ is approximately 20% less than the corresponding reduction observed with $B_{10}H_{10}^{2-}$.

In search of a species that would persist within the tumor cells for an extended period of time, we turned our attention to *n*-$B_{20}H_{18}^{2-}$, the oxidatively coupled dimer of $B_{10}H_{10}^{2-}$. This species is conveniently prepared (32) from $B_{10}H_{10}^{2-}$ by ferric ion oxidation. The *n*-$B_{20}H_{18}^{2-}$ anion was expected to improve the biodistribution in two ways: (*i*) the number of boron atoms per particle is twice that of $B_{10}H_{10}^{2-}$, allowing an increase in the amount of boron delivered at the same osmolarity of encapsulated solution with no change in required stability of the liposome bilayer; and (*ii*) the chemical reactivity of the *n*-$B_{20}H_{18}^{2-}$ ion may enhance the observed biodistribution.

The two B_{10} cages in *n*-$B_{20}H_{18}^{2-}$ are linked by a pair of three-center two-electron bonds between boron atoms (33). This region of the anion is electron deficient and reacts readily with nucleophiles (20), as illustrated in Fig. 2 for the reaction with hydroxide ion. The initial product of this reaction, $B_{20}H_{18}OH^{3-}$, is reversibly deprotonated (pK_a = 6.8) to form $B_{20}H_{17}OH^{4-}$. It was expected that a similar reaction by $B_{20}H_{18}^{2-}$ could occur with a protein residue (such as an ε-amino group originating at a lysine residue) once the *n*-$B_{20}H_{18}^{2-}$ anion had been deposited within a cell by liposome rupture. This hypothetical reaction would covalently bond the borane species within the cell, the preferred location for most effective BNCT (2, 34).

With liposomal *n*-$B_{20}H_{18}^{2-}$, both the injected dose and the initial boron concentration delivered to the tumor are approximately twice that of any boron compound observed previously, as would be anticipated by changing from a 10-boron to a 20-boron species. However, in the case of this boron agent, the dose delivered to the tumor is retained over a much longer period of time. This results in a final tumor-boron concentration of 13.6 μg/g after 48 h, which is substantially higher than the corresponding concentrations obtained with other boron-containing species. Most importantly, the observed tumor/blood ratio (3.3), combined with the high boron content, places these liposomes in a therapeutically useful range.

Another useful facet of the *n*-$B_{20}H_{18}^{2-}$ ion is its photochemical reactivity (19). This anion rearranges upon exposure to ultraviolet light to produce a photoisomer (*i*-$B_{20}H_{18}^{2-}$) as shown in Fig. 3. This photoisomerization process can be reversed by prolonged thermal soaking. In the photoisomer, the two B_{10} cages are linked by a pair of B—H—B bridges (35). This bonding arrangement, like that of *n*-$B_{20}H_{18}^{2-}$, is also reactive toward nucleophiles and produces additional isomers of $B_{20}H_{17}OH^{4-}$ in its reaction with hydroxide ion. In general, the photoisomer tends to be more reactive in these nucleophilic reactions than the normal isomer.

Even more promising results than those obtained with *n*-$B_{20}H_{18}^{2-}$ were obtained with *i*-$B_{20}H_{18}^{2-}$ entrapped in vesicles. When this species was delivered by liposomes the tumor-boron concentration demonstrated improved persistence with time and the liver-boron values were significantly lower. The tumor-boron concentration at 48 h (13.9 μg/g) was still 71% of the level observed at 6 h. This good tumor retention coupled with rapid blood clearance provides a tumor/blood ratio of 12. This represents a therapeutically useful accumulation of boron in the tumor combined with an extremely low blood-boron concentration (1.2 ppm).

Following the promising results obtained with Na_2(*i*-$B_{20}H_{18}$), a double injection experiment was performed. Since the boron retention in the tumor was adequate, this experiment would indicate whether another incremental liposome dose, delivered after the blood was essentially cleared, would continue to load the tumor to attain even higher boron concentrations. This supposition proved to be correct. The data obtained during the first 24 h resembled the previous single-injection experiment. The second injection increased the boron concentrations of all tissues, after which the tissues cleared boron at their usual rates. Because the tumor boron is retained, this results in a tumor-boron concentration of 26.6 μg/g at 48 h, the highest concentration observed in this set of experiments, while the tumor/blood ratio at this point is 2.6.

It was desirable to determine whether the improved tumor retention observed with the $B_{20}H_{18}^{2-}$ isomers was due to retention of the encapsulated compounds or their hydrolysis products, isomers of $B_{20}H_{17}OH^{4-}$ (19, 20), which could be

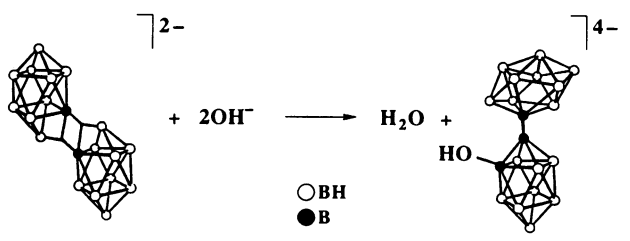

FIG. 2. Hydrolysis of *n*-$B_{20}H_{18}^{2-}$ by hydroxide ion.

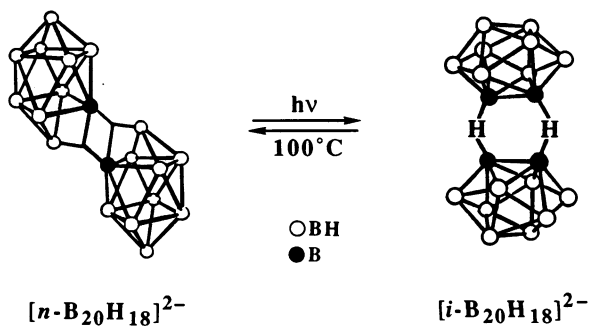

FIG. 3. Isomerization of $B_{20}H_{18}^{2-}$.

formed relatively rapidly at biological pH. In an attempt to answer this question, the hydrolysis product of the photoisomer was prepared and encapsulated in liposomes. While the initial delivered dose of the hydrolysis product is similar to that of the other 20-boron species, the biodistribution data indicate little tumor retention over the 48-h period. The biodistribution is obviously not analogous to that of $i\text{-}B_{20}H_{18}^{2-}$, indicating that the $i\text{-}B_{20}H_{18}^{2-}$ is being retained in the tumor as a result of its own characteristic chemical properties rather than as the hydrolysis product. This argument most likely applies to $n\text{-}B_{20}H_{18}^{2-}$ as well.

The reduction of $n\text{-}B_{20}H_{18}^{2-}$ by mild reducing agents (18) produces $B_{20}H_{18}^{4-}$, which in aqueous solution is in equilibrium with the protonated species $B_{20}H_{19}^{3-}$. Biodistribution experiments with the sodium salt of this anion demonstrated that although the tumor-boron level is steadily reduced with time, the concentration at 48 h is still 11.2 μg/g because of the very high initial uptake (27.7 μg at 6 h). The high boron uptake by the tumor, despite the relatively low injected dose (134 μg) is very promising. Over the first half of the experiment, the tumor-boron concentration (expressed as the percent of the injected dose per g of tissue) is twice that previously observed with other species examined in this study. Unfortunately, this boron-containing species is not efficiently retained in tumor. However, a good tumor/blood ratio of 3 is observed as early as 30 h after injection and reaches 7.5 at the end of the experiment (48 h).

The results obtained to this point suggest that unilamellar liposomes of appropriate size and composition provide a viable modality for the selective delivery of therapeutic concentrations of boron to tumors for BNCT.

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