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TF(+)-PEG-CL liposomes are prepared by the coupling of TF to the PEG-CO_2H moieties of PEG-CL liposomes (Ishida *et al.*, 2001). To 1 ml of PEG-CL liposomes (5 μmol lipids) in Mes buffer (10 m*M* Mes/150 m*M* NaCl, pH 5.5), 21 μmol of EDC and 28 μmol of S-NHS are added, and the mixture is incubated for 15 min at room temperature. The mixture is loaded into a Sephadex G25 column equilibrated with Mes buffer and liposome fractions are collected. The desired amount of TF, and if necessary, a trace amount of ^{125}I-thyraminyl inulin, is then added to the liposome solution, and the mixture is incubated for 3 h at room temperature with gentle stirring. Purification is accomplished by ultracentrifuging at 200,000$\times g$ for 20 min at 4 °C, and the pellets obtained are resuspended in PBS. TF-PEG-CL liposomes obtained are converted into the diferric form by treatment with $FeCl_3$–nitriloacetic acid solution. After the reaction, the suspension is purified by ultracentrifugation and resuspended in PBS.

5.2. Biodistribution of TF-PEG-CL and PEG-CL liposomes

Biodistribution studies are performed using male BALB/c mice (6 weeks old, 16–18 g, Nihon SLC). Tumor-bearing mice are prepared by inoculating subcutaneous (s.c.) injection of a suspension (5×10^6 cells) of colon 26 cells directly into their back. The mice are kept on regular mouse diet and water, and maintained under a standard light/dark cycle in an ambient atmosphere. These experiments are performed when the tumor is 7–9 mm in diameter. ^{125}I-Thylaminyl inuline solution is encapsulated in the liposomes, and 100–400 μl of liposomes is injected into the mice (three per group) via the tail vein at a selected dose of ^{10}B. At selected time intervals after administration, the mice are lightly anesthetized, bled via the retro-orbital sinus, sacrificed by cervical dislocation and dissected. Their organs are excised and their ^{125}I content is estimated by a liquid scintillation counter.

The time-dependent distributions of TF(−)-PEG-CL and TF(+)-PEG-CL liposomes in various tissues are shown in Fig. 10.6. The rapid clearance of TF(−)-PEG-CL and TF(+)-PEG-CL liposomes was observed in the blood, lung, and kidney after 3 h of injection. In general, PEGylated liposomes possess a longer circulation time compared to nonstealth liposomes (Mumtaz *et al.*, 1991; Vaage *et al.*, 1992). We examined the effect of CL on the circulation time of PEGylated liposomes and found that CL influenced a stealth property of the PEG-CL liposome. The enhanced accumulation of TF(+)-PEG-CL liposomes in comparison with TF(−)-PEG-CL liposome accumulation was observed in the spleen within 72 h. TF(+)-PEG-CL liposomes accumulated in the liver and tumor gradually, whereas TF(−)-PEG-CL liposomes were released from those organs 72 h after injection, although percentage doses of these liposomes were similar 24 h after injection. Surprisingly, more than 1.5% of the total dose injected

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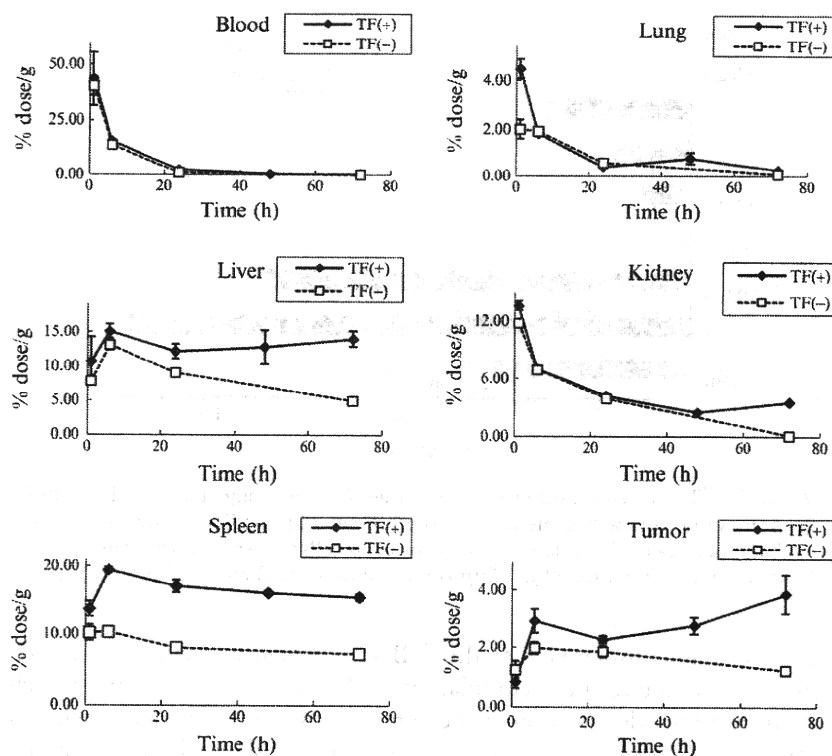

Figure 10.6 Time course of biodistribution of TF(−)-PEG-CL liposome (TF(−)) and the TF(+)-PEG-CL liposome (TF(+)). Liposomes encapsulating ^{125}I-tyraminyl inulin (500 g lipid/200 μl) were injected into male BALB/c mice (7 weeks old, weighing 20–25 g) via the tail vein. The distribution of liposomes was measured by determining the radioactivity of each tissue. The % dose/g in each tissue is plotted on the vertical axis, and the time (h) after administration is plotted on the horizontal axis.

accumulated in tumor tissues. This enhanced accumulation of TF(+)-PEG-CL liposomes may reflect marked receptor-mediated endocytosis after binding to tumor cells (Iinuma *et al.*, 2002).

^{10}B-enriched TF(+)-PEG-CL liposomes are injected into tumor-bearing mice, in which colon 26 cells were transplanted into their left thigh, via the tail vein at a dose of 7.2 mg ^{10}B/kg (200 μl of a liposome solution). Seventy-two hours after administration, ^{10}B concentration in each organ is measured by prompt γ-ray spectroscopy. The results are shown in Fig. 10.7. No boron accumulation was observed in the muscle, heart, and brain; however, the boron concentrations in the lung, kidney, and blood were approximately 10 ppm. Since no accumulation of TF(+)-PEG-CL liposomes labeled with ^{125}I-tyraminyl inulin was observed, as shown in

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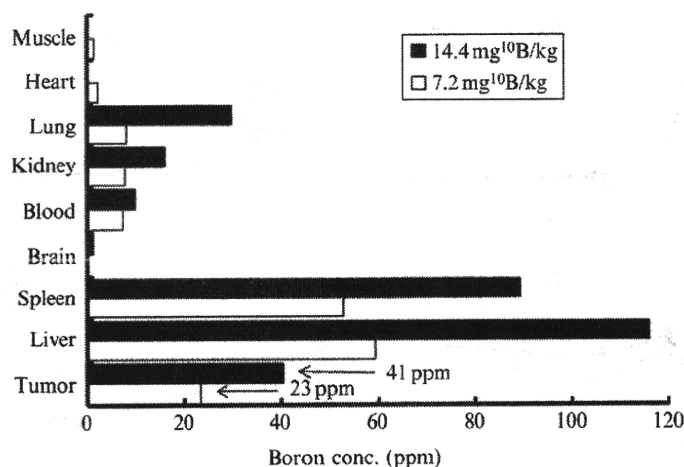

Figure 10.7 ^{10}B concentration in various tissues 72 h after injection of TF(+)-PEG-CL liposomes into tumor-bearing mice. ^{10}B-enriched TF(+)-PEG-CL liposomes were injected into tumor-bearing mice, in which colon 26 cells were transplanted into the left thigh, via the tail vein with 200 μl of liposome solutions (7.2 and 14.4 mg^{10}B/kg).

Fig. 10.6, it is considered that the ^{10}B concentrations detected in such organs may be due to the accumulation of the *nido*-carborane lipid, which was caused by the degradation of the parent liposomes. Enhanced accumulation of ^{10}B was observed in the spleen and liver, and this does not conflict with the result of the biodistribution of TF(+)-PEG-CL liposomes as shown in Fig. 10.6. A high level of ^{10}B concentration (22 ppm) in the tumor was observed in tumor tissues 72 h after the administration of TF(+)-PEG-CL liposomes. Furthermore, almost twice ^{10}B concentrations in each organ were observed in the mice injected with double dose of TF(+)-PEG-CL liposomes (14 mg ^{10}B/kg body weight).

5.3. Survival of tumor-bearing mice after BNCT

Besides the determination of ^{10}B concentration in various organs, the mice are anesthetized with sodium pentobarbital solution 72 h after the administration of TF(+)-PEG-CL liposomes and placed in an acrylic mouse holder, where their whole bodies, except their tumor-implanted leg, are shielded with acrylic resin. Neutron irradiation is carried out for 37 min at a rate of 2×10^{12} neutrons/cm^2 in the KUR atomic reactor. The antitumor effect of BNCT is evaluated on the basis of the survival of the mice, as shown in Fig. 10.8. The untreated mice did not survive after 32 days of neutron irradiation, and their average survival rate was 21 days. Long survival rates were observed in the mice treated with TF(+)-PEG-CL liposomes; one of

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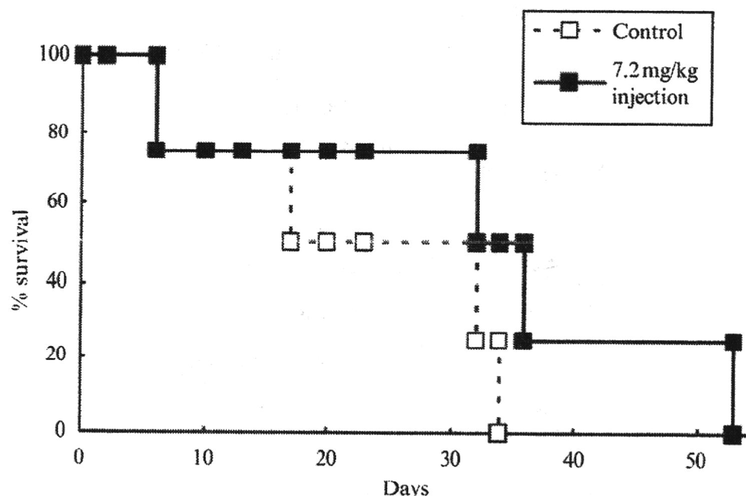

Figure 10.8 Survival curve of tumor-bearing mice after neutron irradiation for 37 min in KUR atomic reactor. The mice were injected with 7.2 mg ^{10}B/kg of the TF(+)-PEG-CL liposome and incubated for 72 h before irradiation. Control indicates survival rates of tumor-bearing mice after neutron irradiation without administration of TF(+)-PEGCL liposomes.

them even survived for 52 days after neutron irradiation. The average survival rate of the treated mice was 31 days.

However, the acute toxicity has been observed in the mice with a double dose injection (14 mg ^{10}B/kg body weight) within 1 day. Similar toxicity has been observed in the liposome prepared from the *nido*-carborane lipid **3** (Li *et al.*, 2006). Therefore, we modified the boron lipids based on biomimetic composition of phosphatidylcholines combined with the *closo*-type boron anion cluster to meet a sufficiently low toxic requirement. We introduced BSH, as an alternative hydrophilic function, to the boron lipids and examined the BNCT effects of *closo*-dodecaborate lipid liposomes on tumor-bearing mice (Lee *et al.*, 2007; Nakamura *et al.*, 2007).

6. *CLOSO*-DODECABORATE LIPID LIPOSOMES

BSH is known as a water-soluble divalent "*closo*-type" anion cluster and significantly lowered toxicity, and thus has been utilized for clinical treatment of BNCT. We have succeeded in the synthesis of the *closo*-dodecaborate lipids (**8** and **9**) (Lee *et al.*, 2007), which have a $B_{12}H_{11}S$-moiety as a hydrophilic function with a chirality similar to that of natural phospholipids, such as DSPC, in their lipophilic tails (Fig. 10.9).

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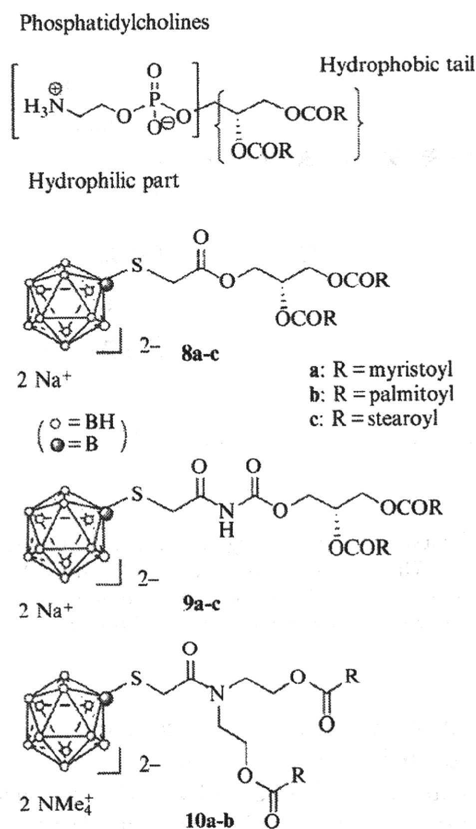

Figure 10.9 Design of *closo*-dodecaborate lipids based on biomimetic composition of phosphatidylcolines.

Recently, the symmetric *closo*-dodecaborate lipids **10** were reported by Gabel and coworkers (Justus *et al.*, 2007). The detailed protocols for synthesis of the the *closo*-dodecaborate lipids (**8** and **9**) and *in vivo* BNCT effects of the *closo*-dodecaborate lipid-liposomes are described.

6.1. Synthesis of *closo*-dodecaborate lipids

Synthesis of the hydrophobic tail functions of **8** and **9** is shown in Schemes 10.2 and 10.3. The chiral alcohol **11** is protected with benzylbromide using NaH and the resulting dioxolane **12** is converted into the diol **13** using aqueous AcOH in 83% yield. The ester formation of the diol **13** with various carboxylic acids is promoted by dicyclohexylcarbodiimide in the presence of catalytic amounts of *N*,*N*-dimethylaminopyridine in CH_2Cl_2 to

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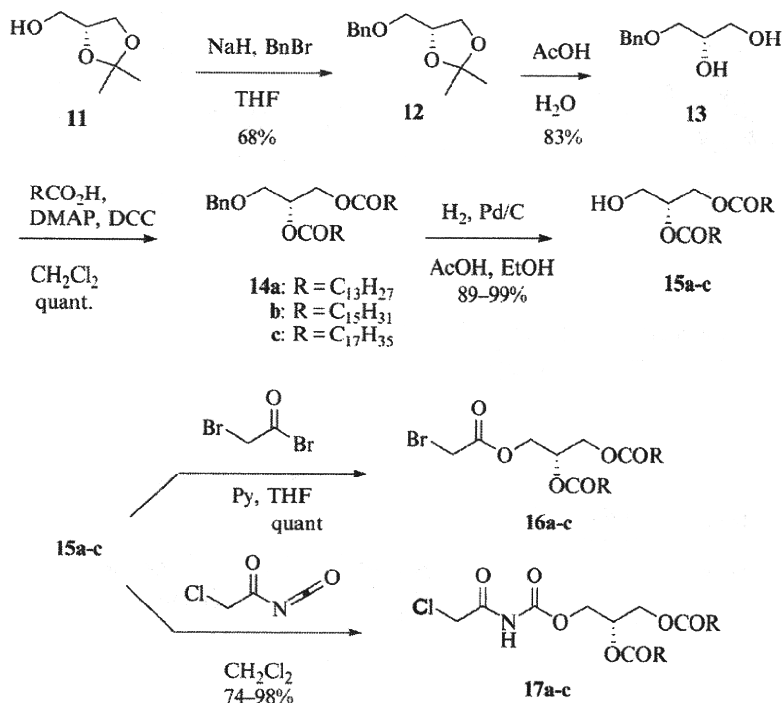

Scheme 10.2 Synthesis of the hydrophobic tail functions.

afford the precursors **14a–c**, quantitatively. Deprotection of the benzyl group of **14a–c** by hydrogenation gives the corresponding alcohols **15a–c** in 89–99% yields. The ester formation of **15a–c** with bromoacetyl bromide in pyridine gave **16a–c**, quantitatively, and the carbamate formation with chloroacetyl isocyanate in CH_2Cl_2 gave **17a–c** in 74–98% yields.

Introduction of BSH into the hydrophobic tail functions **16** and **17** is examined using the "protected BSH (**20**)," which is prepared according to Gabel's protocol, as shown in Scheme 10.3. Briefly, Na_2BSH is treated with 2 equiv. of 2-iodopropionitrile in acetonitrile and the resulting dicyanoethylated BSH **19** is precipitated as tetramethyl ammonium salts. Dealkylation of **19** proceeds in the presence of 1 equiv. of tetramethylammonium hydroxide in acetone to afford the protected BSH **20**. The *S*-alkylation of **20** with **16a–c** proceeds in acetonitrile at 70 °C for 12–24 h, giving the corresponding *S*-dialkylated products (**21**), which are immediately treated with tetramethylammonium hydroxide (1 equiv.) in acetone followed by ion exchange with Dowex X-100 to give **8a–c** as sodium salts. In a similar manner, **9a–c** are obtained from **17a–c**.

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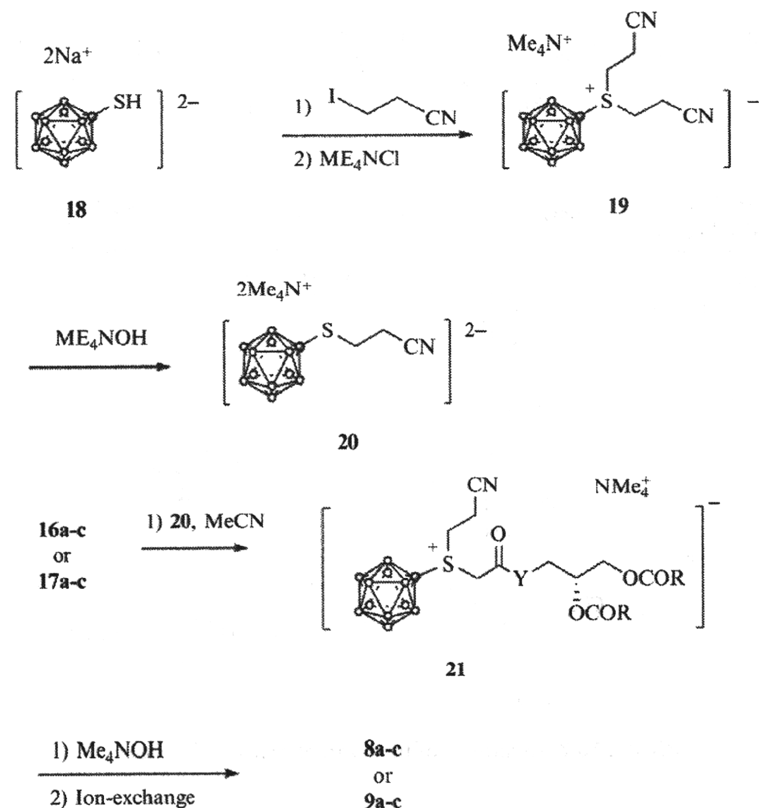

Scheme 10.3 Synthesis of *closo*-dodecaborate lipids **8** and **9**.

6.2. Synthesis of *closo*-dodecaborate cholesterols

Carborane-conjugated cholesterols (Feakes *et al.*, 1999) and carborane-containing cholesterol mimic (Thirumamagal *et al.*, 2006) have been developed as an alternative content of liposomal membranes. Our laboratory has focused on the structure ofBSH, as an alternative water-soluble boron cluster and succeeded in the synthesis of dodecaborate-conjugated cholesterols **23a–b** (Scheme 10.4) for liposomal boron delivery systems. Cholesterol is treated with bromoacetyl bromide in the presence of pyridine to give the corresponding ester **22a** in 83% yield. The chloroacetylcarbamate **22b** is also synthesized from chlesterol treating with chloroacetyl isocyanate in the presence of trimethylamine. The protected BSH **20** with **22a–b** proceeds in acetonitrile and the resulting sulfoniums are treated with 1 equiv. of

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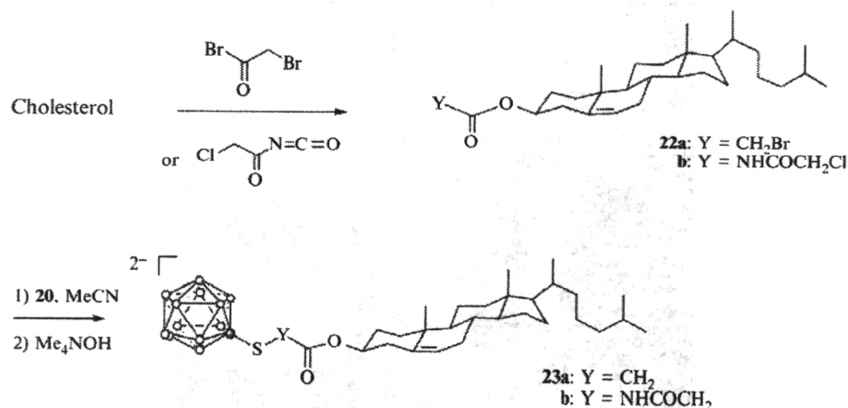

Scheme 10.4 Synthesis of *closo*-dodecaborate cholesterols 23.

tetramethylammonium hydroxide in acetone to give the corresponding thioesters **23a–b** in 73 and 47% yields, respectively, in two steps.

6.3. Preparation of *closo*-dodecaborate lipid-liposomes

Bare boron liposomes and PEG boron liposomes are prepared from DSPC, boron lipids, Chol (X:1 − X:1, molar ratio, $0 < X < 1$) and DSPC, boron lipids, Chol, DSPE-PEG (X:1 − X:1:0.11, molar ratio, $0 < X < 1$), respectively. These boron liposomes are prepared according to the REV method. Total lipids of 200 mg are dissolved in 6 ml of chloroform/diisopropyl ether mixture (1:1, v/v) and 3 ml of distilled water is added to the mixture to form a w/o emulsion. The emulsion is sonicated for 3 min, and then, the organic solvents are removed under the reduced pressure in a rotary evaporator at 60 °C for 30 min to obtain a suspension of liposomes. The liposomes obtained are subjected to extrusion 10 times through a polycarbonate membrane of 100 nm pore size, using an extruder device thermostated at 60 °C. Purification is accomplished by ultracentrifugation at 200,000×g for 60 min at 4 °C, and the pellets obtained are resuspended in 0.9% NaCl solution or PBS buffer. The size distribution of the boron liposomes are measured by an electrophoretic light scattering spectrophotometer. The composition of boron lipids and DSPC in liposome is calculated from simultaneous measurement of boron and phosphine concentrations by ICP-AES. Transmission electron micrograph (TEM) is carried out by using negative attaining method with uranyl acetate. Figure 10.10 shows the TEM image of 25% DSBL liposomes after sizing with 100 nm pore-diameter polycarbonate membranes.

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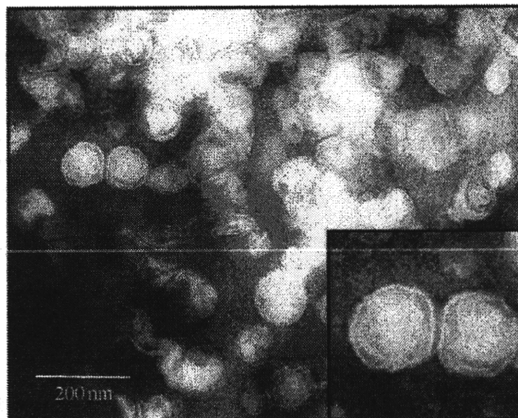

Figure 10.10 Electron micrographs of the boron liposomes composed of 25% DSBL after extrusion using 100 nm filter.

6.4. Fluorescent-labeled *closo*-dodecaborate lipid-liposomes

PKH67-labeled boron liposomes are prepared according to the conventional cell membrane labeling method. Briefly pellets of boron liposomes are dissolved in 250 μl of Diluent C, and then the boron liposomes solution are dropped into 1 μl of PKH67 dye stock solution. The mixture is maintained at 20 °C for 5 min, and then free PKH67 is removed by ultracentrifugation at 200,000×g for 60 min at 4 °C, and the obtained PKH67-labeled boron liposomes are resuspended in PBS.

Human epithelial carcinoma cell line HeLa cells are maintained at 37 °C under 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. For the subsequent experiments, the cells are seeded at a density of 5×10^4 cells in a φ-35 mm diameter dish (Greiner) and incubated at 37 °C for 20 h. The cells are incubated for further 3 h in the presence of PKH67-labeled boron liposomes in medium. After incubation, the cells are fixed with 4% paraformaldehyde in PBS for 10 min, and then treated with 0.1% Triton X-100 in PBS for 10 min. The cells are mounted on a slide after incubation for 1 h in PBS containing Hoechst 33342 nuclear stain at room temperature and analyzed by fluorescent microscope (IX71, OLYMPUS, Japan). Intracellular localization of the DSBL liposomes is visualized in the cytoplasm of the cells as shown in Fig. 10.11. Suspension of PKH67-labeled DSBL liposomes in PBS is detected by fluorescent microscopy (Fig. 10.11A). After HeLa cells are incubated with this liposome suspension for 3 h, the liposomes are internalized into the cell cytoplasm, but not the cell nucleus (Fig. 10.11(B–D)), without any ligands conjugated on the surface of the liposomes.

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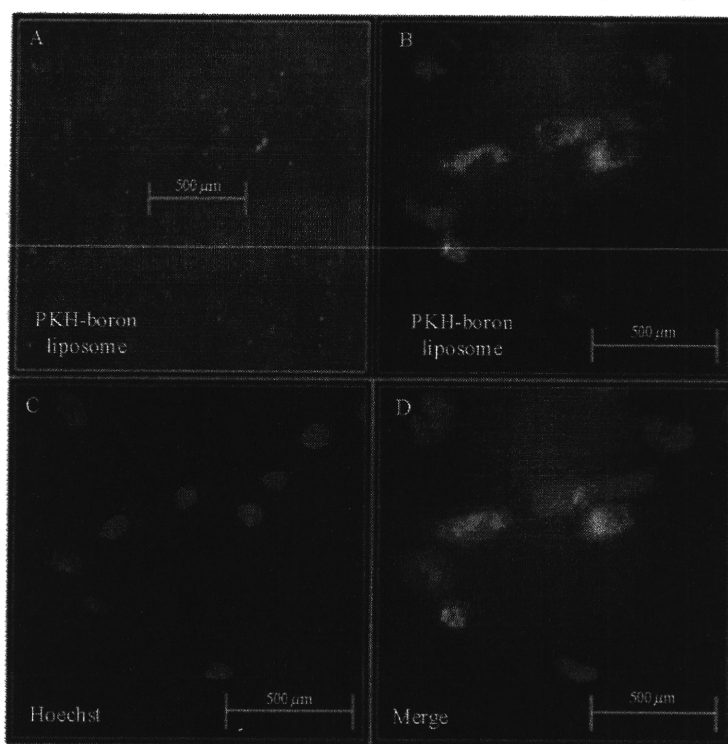

Figure 10.11 PKH67-labeled boron liposomes and intracellular location of PKH-labeled boron liposomes. (A) PKH67-labeled 25% DSBL liposomes in PBS were visualized in fluorescent microscope. (B) Intracellular (HeLa) location of PKH67-labeled 25% DSBL liposomes were visualized in fluorescent microscope. (C) Hoechst-labeled nucleuses in HeLa were visualized in fluorescent microscope. (D) The merge image of PKH67-labeled 25% DSBL liposomes (B) and Hoechst-labeled nucleus (C) is shown in (D).

6.5. Acute toxicity and accumulation of liposomes prepared from *closo*-dodecaborate lipids and cholesterols in healthy mice

Acute toxicity and distribution of boron liposomes in mice are examined. The results are summarized in Table 10.1. Although the acute toxicity was observed in mice injected with the 50% DSBL and BCC liposomes at a dose of 30 mg ^{10}B/kg body weight, the lethal toxicity was not observed at lower boron concentrations. The boron concentration in various organs including liver and spleen is measured 3 weeks after injection of various boron liposomes. Boron accumulation was not detected in liver and spleen of the healthy mice injected with boron liposomes composed of double-tailed

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Table 10.1 Acute toxicity and accumulation of boron liposomes in mice

Boron lipids		Content (%)	Injection dose (mg B/kg)	Liver (ppm B)	Spleen (ppm B)	Kidney (ppm B)	Toxicity in 72 h (%)
DSBL	**8c**	25	15	0.71 ± 0.05	1.01 ± 0.07	0.18 ± 0.01	0
		50	30	0.59 ± 0.18	0.96 ± 0.29	0.20 ± 0.00	33
			20	0.22 ± 0.00	0.17 ± 0.00	0.07 ± 0.00	0
DPBL	**8b**	25	15	0.77 ± 0.00	1.28 ± 0.00	0.19 ± 0.00	0
			10	0.27 ± 0.06	0.34 ± 0.10	0.24 ± 0.03	0
		50	20	0.39 ± 0.10	0.36 ± 0.08	0.56 ± 0.11	0
			15	0.26 ± 0.00	0.61 ± 0.00	0.50 ± 0.00	0
DSCBL	**9c**	25	15	0.79 ± 0.01	1.59 ± 1.00	0.19 ± 0.00	0
			10	0.44 ± 0.08	0.88 ± 0.57	0.41 ± 0.08	0
		50	20	0.78 ± 0.04	1.38 ± 0.32	0.26 ± 0.03	0
DPCBL	**9b**	25	10	1.03 ± 0.03	1.59 ± 0.26	0.24 ± 0.01	0
			5	0.83 ± 0.27	0.64 ± 0.21	0.30 ± 0.02	0
		50	20	0.68 ± 0.21	0.82 ± 0.36	0.24 ± 0.02	0
			15	0.73 ± 0.21	0.96 ± 0.38	0.19 ± 0.03	0
			10	0.61 ± 0.12	0.99 ± 0.03	0.18 ± 0.03	0
BC	**23a**	25	10	34.3 ± 2.65	34.3 ± 1.21	1.94 ± 0.20	0
			5	22.4 ± 0.10	19.5 ± 0.34	1.36 ± 0.11	0
		50	10	42.9 ± 0.00	37.3 ± 0.00	1.10 ± 0.00	0
			5	21.2 ± 1.66	21.3 ± 2.25	0.74 ± 0.07	0
BCC	**23b**	25	15	60.8 ± 8.84	38.0 ± 2.95	4.66 ± 0.22	0
			10	39.1 ± 5.14	15.9 ± 0.33	1.76 ± 0.26	0
		50	30	78.5 ± 12.5	60.7 ± 3.62	7.11 ± 0.00	33
			20	79.1 ± 0.00	54.9 ± 0.00	3.98 ± 0.00	0

Data are expressed as mean ± S.E.M.

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boron lipids, **8a–c** and **9a–c**. However, high boron concentrations in those organs were observed in the mice injected with the liposomes composed of the boron cholesterols **23a–b**.

6.6. Biodistribution of *closo*-dodecaborate lipid-liposomes in mice

Tumor-bearing mice (female, 5–6 weeks old, 16–20 g) are prepared by inoculating subcutaneously a suspension (2.5 × 10^6 cell/mouse) of colon 26 cells directly into the right thigh. The mice are kept on a regular mouse diet and water, and maintained under a standard light/dark cycle at an ambient atmosphere. Biodistribution experiments are performed when the tumor size is in the range from 7 to 9 mm in diameter. The tumor-bearing mice are injected via the tail vein with 200 μl of Na_2BSH (6000 ppm B) in 0.9% NaCl solution or 200 μl solutions of 25% DSBL (**8c**) PEG-liposomes. The mice are lightly anesthetized and blood samples are collected from the retro-orbital sinus 24 h after injection. The mice are then sacrificed by cervical dislocation and dissected. The various organs, including liver spleen, kidney, heart, brain, lung, muscle, and tumor, are excised, washed with 0.9% NaCl solution, and weighed. The excised organs are digested with 2 ml of conc. HNO_3 (ultratrace analysis grade; Wako, Tokyo, Japan) at 90 °C for 1–3 h, and then the digested samples are diluted with distillated water. After filtration with hydrophobic filter (13JP050AN, ADVANTEC), the boron concentration is measured by ICP-AES. Figure 10.12 shows the boron concentrations in various organs of tumor-bearing mice 24 h after injection of the boron liposomes composed of DSBL (25% and 50%) at doses of 20 mg B/kg, and 1 h after injection of

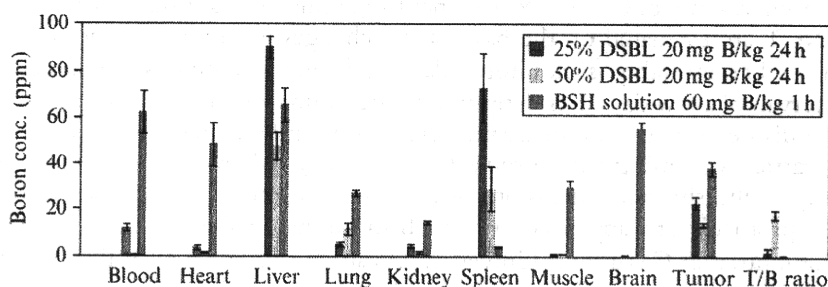

Figure 10.12 Biodistributions of mice (Balb/c, female, 6 weeks old, 14–20 g) bearing colon 26 solid tumors 24 h after i.v. injection of 25% and 50% DSBL PEG-liposomes and BSH solution. ■, 25% DSBL PEG-liposomes (20 mg B/kg); ▒, 50% DSBL PEG-liposomes (20 mg B/kg); ■, BSH solution (60 mg B/kg). Data are expressed as mean ± S.E.M. (n = 4–5).

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Na_2BSH solution at a dose of 60 mg B/kg. The boron concentrations in tumor were 22.7 and 13.4 ppm in the mice injected with the 25% and 50% DSBL liposomes (20 mg B/kg), respectively. The 25% DSBL liposomes showed higher boron concentrations in blood compared to the 50% DSBL liposomes. The tumor/blood ratio of the 50% DSBL liposomes increased to 7.22, which was higher than those of 25% DSBL liposomes (1.94) and BSH solution (0.48). These boron liposomes also accumulated in the liver and spleen. Na_2BSH accumulated in various tissues including the liver, spleen, heart, muscle, blood, and tumor, nonselectively and a higher boron concentration was also obtained in the tumor in comparison with DSBL liposomes. However, the injected boron dose of BSH was much higher than that of DSBL liposomes; therefore the percent injected boron dose per tumor (%ID) accumulation of BSH and DSBL liposomes is calculated. The %ID value of 25% DSBL liposomes was 5.68, where as those of 50% DSBL liposomes and Na_2BSH were 3.36 and 3.15, respectively. These results indicate that the 25% DSBL liposomes accumulate in tumors more selectively than BSH and 50% DSBL liposomes.

6.7. Tumor growth in mice administer DSBL-liposomes after neutron irradiation

DSBL-25% liposomes, which are prepared from the ^{10}B-enriched DSBL (**8c**), DSPC, Chol, and DSPE-PEG (0.25:0.75:1:0.11, molar ratio), are injected into colon 26 tumor-bearing mice (female, 6–7 weeks old, 16–20 g) via the tail at a dose of 20 mg ^{10}B/kg (2000 ppm of ^{10}B concentration; 200 μl of boron liposome solution). The mice are anesthetized with isoflurane (Forane, Abbott, Japan) and placed in an acrylic mouse holder 24 h after *i.v.* injection. The mice are irradiated in the JRR4 for 30 min at a rate of $0.9–1.4 \times 10^{12}$ neutrons/cm^2. The antitumor effects of BNCT are evaluated on the basis of the changes in tumor volume of the mice. The mortality is monitored daily and tumor volume is measured at intervals of a few days. For determining the tumor volume, two perpendicular diameters of the tumor are measured with a slide caliper, and calculation is carried out using the formula $0.5\ (A \times B^2)$, where A and B are the longest and shortest dimensions of the tumor in millimeters, respectively. All protocols are approved by the Institutional Animal Care and Use Committee in Gakushuin University. As shown in Fig. 10.13, the tumor growth rate in mice treated with the boron liposomes was significantly inhibited after thermal neutron irradiation. Suppression of tumor growth was observed during 2 weeks after neutron irradiation, although rapid tumor growth was observed in the mice without injection of the boron liposomes after neutron irradiation.

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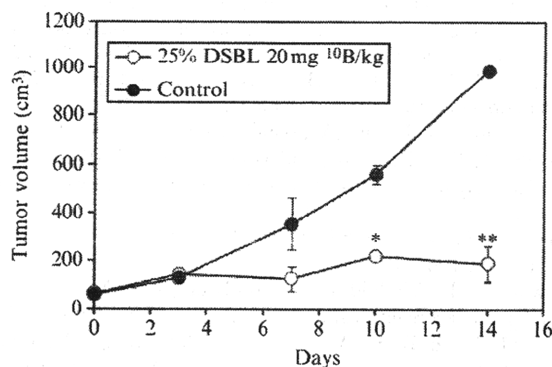

Figure 10.13 Tumor growth of mice (Balb/c, female, 6 weeks old, 14–20 g) bearing colon 26 solid tumor after thermal neutron irradiation for 30 min. The mice were injected with 25% DSBL PEG-liposomes (20 mg B/kg) incubated for 24 h before irradiation. Control indicated tumor growth of mice after neutron irradiation without administration of 25% DSBL PEG-liposomes. Data are expressed as mean ± S.E. ($n = 4$).

7. Concluding Remarks

BNCT is a binary system of thermal neutrons and neutron absorbers for the treatment of cancer. The clinical treatment with BNCT has been limited to the location and number of patients, because thermal neutrons are available only from atomic reactors. Recent development of accelerator technologies displays a possibility of accelerator-based BNCT in the near future, and in fact several accelerators are now under development for this purpose. The second component of this binary system involves the boron delivery system. Sufficient boron accumulation in the tumor tissues is the most important requirement for efficient BNCT. Liposomal boron delivery technologies described in this chapter may become one of the effective tools for boron delivery to tumor tissues. Accompanied with the establishment of hospital-based accelerators and development of new boron delivery technologies, BNCT will become the major modality for the next generation of cell-selective radiation therapy of cancers.

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8 Liposomal Boron Delivery System for Neutron Capture Therapy of Cancer

Hiroyuki Nakamura

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8.1 INTRODUCTION

The high accumulation and selective delivery of ^{10}B into the tumor tissue are the most important requirements to achieve efficient boron neutron capture therapy for cancer (BNCT), because the cell-killing effect of BNCT depends on the nuclear reaction of two essentially nontoxic species, boron-10 (^{10}B) and thermal neutrons, whose destructive effect is well documented in boron-loaded tissues (Barth, 2003; Barth, 2009; Hawthorne, 1993; Soloway et al., 1998). Two boron compounds, sodium mercaptoundecahydrododecaborate ($Na_2^{10}B_{12}H_{11}SH$; ^{10}BSH) (Soloway et al., 1967) and L-*p*-boronophenylalanine (L-^{10}BPA) (Snyder et al., 1958), have been clinically utilized for the treatment of patients with malignant brain tumors (Nakagawa et al., 1997) and malignant melanoma (Mishima **Q1** et al., 1989). According to the theoretical estimations as well as clinical data, three important parameters should be considered in the development of boron carriers for fatally damaging tumor cells with BNCT: (1) boron concentrations in the tumor should be in the range of 20–35 μg ^{10}B/g; (2) the tumor/normal tissue ratio should be greater than 3; and (3) the toxicity should be sufficiently low (Barth, R. F. et al., 2005). Recently, BNCT has been applied to various cancers, including head and neck cancer (Aihara et al., 2006; Kato et al., 2004), lung cancer, hepatoma (Suzuki et al., 2007), chest wall cancer, and mesothelioma (Ono et al., unpublished). Therefore, the development of new boron carriers is one of the most important issues that should be resolved to extend the application of BNCT to various cancers.

In the last decade, boron carrier development has taken two directions: small boron molecules and boron-conjugated biological vehicles. Unlike approaches using pharmaceuticals, boron carriers