and -independent mechanisms. Aromando et al. [32] reported that BNCT-induced control of hamster cheek pouch tumors would be an inhibitory effect on DNA synthesis and apoptosis does not have a significant role in tumor control. Masunaga et al. [31] examined the effect of BNCT on SAS xenografts in nude mice. After BNCT, the tumor cells were dissociated and the cell suspension was cultured for colony formation, the detection of apoptotic cells, and a micronucleus assay. The peak of apoptosis was observed at 6 h after BNCT at low levels, irrespective of the p53 status, suggesting that apoptosis occurred early on. We also observed an increase in the sub-G1 population and nuclear fragmentation early after BNCT in SAS/neo cells, and the level was maintained thereafter. In SAS/mp53 cells, however, the increase in apoptosis occurred subsequent to G2 arrest. Thus, p53 seems to be responsible for G1 arrest-associated apoptosis. In the present study, p53 led to a significant but limited increase of apoptosis. Differently, in colony formation and MTT assays, p53 has a much stronger impact on the survival fraction and proliferation of treated cells. This indicates that apoptosis is a form of cell death induced by BNCT. So far, different types of cell death have been documented. They include apoptosis, autophagy, mitotic catastrophe, necrosis and senescence [33]. Especially, participation of mitotic catastrophe, necrosis and senescence in BNCT-treated cancer cells should be clarified.

p21 binds to and inhibits the cyclin-dependent protein kinases that drive the cell cycle, and is responsible for G1 arrest [34-36]. In SAS/neo cells, we found that the expression and phosphorylation of p53 was markedly enhanced from 6 h after BNCT, and this level was maintained for 48 h. We also detected a transient increase in the expression of p21 which inhibited the transition from the G1 to S phase. In SAS/mp53 cells, however, p21 was not induced, and neither G1 arrest nor the induction of apoptosis was observed. This indicates that p21 is associated with cell cycle arrest at G1 down-stream of the p53 pathway.

After BNCT, cells that escaped G1 arrest accumulated at G2 to prevent mitotic entry after potentially lethal DNA damage. Cdc2 protein kinase activity is required for the G2-to-mitosis transition in all eukaryotic cells. Cdc25 activates the cdc2/cyclin B1 complex by dephosphorylating inhibitory threonine-14 and thyrosine-15 residues of cdc2 [37-39]. This step is indispensable to mitosis after IR. Wee1 protein kinase allows cdc2 inactivation by phosphorylation of cdc2 on tyrosine -15 [40,41]. Matsumura et al. [42] reported that carbon-ion irradiation was associated with the overexpression of Wee1 and phosphorylation of cdc2, followed by the prolongation of G2 arrest and subsequent induction of apoptosis. Consistent with their results, we found that BNCT induced the expression of Wee1 and cyclin B1 and increased the phosphorylation of cdc2 in both SAS/neo and SAS/mp53 cells around 12 h after BNCT. Therefore, it can be stated that Wee1, cdc2, and cyclin B1 are associated with G2 arrest in a p53-independent manner.

Carbon-ion beams reportedly induce apoptosis in oral SCC and lung cancer cells regardless of the p53 status at a high LET [17,18]. Why high LET BNCT leads to the p53-dependent suppression of cell survival and induction of cell cycle arrest at the G1 checkpoint is unclear. Probably, each tumor cell would be equally exposed to carbon-ion beams. In the case of BNCT, however, the path lengths of high LET α and Li particles are very short, so that the LET would decrease markedly, even within a cell, being dependent on the distance from the cytoplasmic boron to the nuclear DNA [7,8]. This may generate a variety of intracellular LET values, and yield appropriate energy to induce cell cycle arrest at G1, if the cells have functional p53. It may also be ascribed to the characteristics of the cell lines used. Indeed, the survival curve of SAS/mp53 cells is not exponential, but a shoulder curve. The form of the curve suggests that the LET was not very high. If the mutation may influence the intracellular accumulation of BPA, it may heavily influence the LET of the radiation and relative biological effect.

In conclusion, oral SCC cells with mutant-type p53 were more resistant to the cell-killing effect of BNCT than those with wild-type p53 under the present experimental conditions. A functional p53 is required for the induction of apoptosis related to G1 arrest. BNCT inhibits oral SCC cells via p53-dependent and -independent mechanisms. Recent clinical studies have shown that the delivery of wild-type p53 to cancer cells with p53 mutations significantly increases their radiation sensitivity [43,44]. Adenoviral-mediated gene therapy is a reliable method to introduce the wild-type p53 gene [45,46]. Such an approach may be applicable to oral SCCs with mutated p53 to promote the efficiency of BNCT.

Conflict of interests

The authors declare that they have no competing interests.

Authors' contributions

YF carried out the experiments in the study and drafted the manuscript. IK provided the compound and carried out the experiments. SI carried out the experiments. KO participated in the design of reactor irradiation. MS helped the measurement of boron concentration. YS helped reactor irradiation. KO provided cell lines and participated in the design of the study. TO provided cell lines and participated in the design of the study. YY conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

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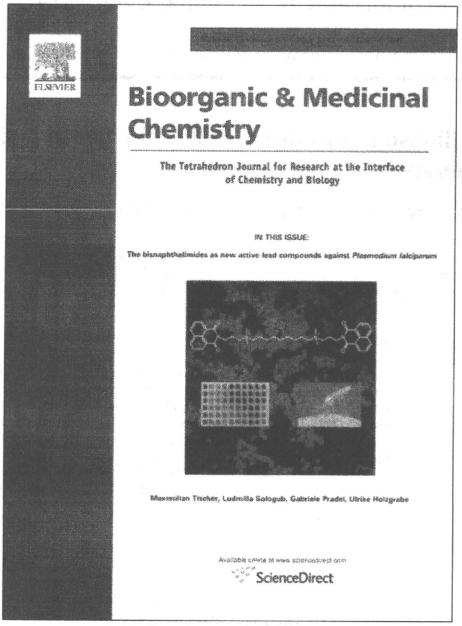
References

1. Genden EM, Ferlito A, Bradley PJ, Rinaldo A, Scully C: **Neck disease and distant metastases.** *Oral Oncol* 2003, **39**:207-212.
2. Palme CE, Gullane PJ, Gilbert RW: **Current treatment options in squamous cell carcinoma of the oral cavity.** *Surg Oncol Clin N Am* 2004, **13**:47-70.
3. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW: **Participation of p53 protein in the cellular response to DNA damage.** *Cancer Res* 1991, **51**:6304-6311.
4. Offer H, Zurer I, Banfalvi G, Reha'k M, Falcovitz A, Milyavsky M, Goldfinger N, Rotter V: **p53 modulates base excision repair activity in a cell cycle-specific manner after genotoxic stress.** *Cancer Res* 2001, **61**:88-96.
5. Yamazaki Y, Chiba I, Hirai A, Notani K, Kashiwazaki H, Tei K, Totsuka Y, Iizuka T, Kohgo T, Fukuda H: **Radioresistance in oral squamous cell carcinoma with p53 DNA contact mutation.** *Am J Clin Oncol* 2003, **26**:e124-129.
6. Niemantsverdriet M, Jongmans W, Backendorf C: **Radiation response and cell cycle regulation of p53 rescued malignant keratinocytes.** *xp Cell Res* 2005, **310**:E237-247.
7. Coderre JA, Morris GM: **The radiation biology of boron neutron capture therapy.** *Radiat Res* 1999, **151**:1-18.
8. Barth RF, Coderre JA, Vicente MG, Blue TE: **Boron neutron capture therapy of cancer: Current status and future prospects.** *Clin Cancer Res* 2005, **11**:3987-4002.
9. Fukuda H, Hiratsuka J, Kobayashi T, Sakurai Y, Yoshino K, Karashima H, Turu K, Araki K, Mishima Y, Ichihashi M: **Boron neutron capture therapy (BNCT) for malignant melanoma with special reference to absorbed doses to the normal skin and tumor.** *Australas Phys Eng Sci Med* 2003, **26**:97-103.
10. Kato I, Ono K, Sakurai Y, Ohmae M, Maruhashi A, Imahori Y, Kirihata M, Nakazawa M, Yura Y: **Effectiveness of BNCT for recurrent head and neck malignancies.** *Appl Radiat Isot* 2004, **2061**:1069-1073.
11. Kankaanranta L, Seppälä T, Koivunoro H, Saarilahti K, Atula T, Collan J, Salli E, Kortesniemi M, Uusi-Simola J, Mäkitie A, Seppänen M, Minn H, Kotiluoto P, Auterinen I, Savolainen S, Kouri M, Joensuu H: **Boron neutron capture therapy in the treatment of locally recurred head and neck cancer.** *Int J Radiat Oncol Biol Phys* 2007, **69**:475-482.
12. Miyatake S, Kawabata S, Yokoyama K, Kuroiwa T, Michiue H, Sakurai Y, Kumada H, Suzuki M, Maruhashi A, Kirihata M, Ono K: **Survival benefit of Boron neutron capture therapy for recurrent malignant gliomas.** *J Neurooncol* 2009, **91**:199-206.
13. Iwadate Y, Mizoe J, Osaka Y, Yamaura A, Tsujii H: **High linear energy transfer carbon radiation effectively kills cultured glioma cells with either mutant or wild-type p53.** *Int J Radiat Oncol Biol Phys* 2001, **50**:803-808.
14. Matsui Y, Asano T, Kenmochi T, Iwakawa M, Imai T, Ochiai T: **Effects of carbon-ion beams on human pancreatic cancer cell lines that differ in genetic status.** *Am J Clin Oncol* 2004, **27**:24-28.
15. Tsuboi K, Moritake T, Tsuchida Y, Tokuuye K, Matsumura A, Ando K: **Cell cycle checkpoint and apoptosis induction in glioblastoma cells and fibroblasts irradiated with carbon beam.** *J Radiat Res (Tokyo)* 2007, **48**:317-325.
16. Gong L, Jin X, Li Q, Liu J, An L: **Heavy ion beams induce survivin expression in human hepatoma SMMC-7721 cells more effectively than X-rays.** *Acta Biochim Biophys Sin (Shanghai)* 2007, **39**:575-582.
17. Takahashi A, Matsumoto H, Yuki K, Yasumoto J, Kajiwara A, Aoki M, Furusawa Y, Ohnishi K, Ohnishi T: **High-LET radiation enhanced apoptosis but not necrosis regardless of p53 status.** *Int J Radiat Oncol Biol Phys* 2004, **60**:591-597.
18. Yamakawa N, Takahashi A, Mori E, Imai Y, Furusawa Y, Ohnishi K, Kirita T, Ohnishi T: **High LET radiation enhances apoptosis in mutated p53 cancer cells through Caspase-9 activation.** *Cancer Sci* 2008, **99**:1455-1460.
19. Hainaut P, Soussi T, Shomer B, Hollstein M, Greenblatt M, Hovig E, Harris CC, Montesano R: **Database of p53 gene somatic mutations in human tumors and cell lines: updated compilation and future prospects.** *Nucleic Acids Res* 1997, **25**:151-157.
20. Balz V, Scheckenbach K, Götte K, Bockmühl U, Petersen I, Bier H: **Is the p53 inactivation frequency in squamous cell carcinomas of the head and neck underestimated? Analysis of p53 exons 2-11 and human papillomavirus 16/18 E6 transcripts in 123 unselected tumor specimens.** *Cancer Res* 2003, **63**:1188-1191.
21. Ota I, Ohnishi K, Takahashi A, Yane K, Kanata H, Miyahara H, Ohnishi T, Hosoi H: **Transfection with mutant p53 gene inhibits heat-induced apoptosis in a head and neck cell line of human squamous cell carcinoma.** *Int J Radiat Oncol Biol Phys* 2000, **47**:495-501.
22. Coderre JA, Button TM, Micca PL, Fisher CD, Nawrocky MM, Liu HB: **Neutron capture therapy of the 9L rat gliosarcoma using the p-boronophenylalanine-fructose complex.** *Int J Radiat Oncol Biol Phys* 1994, **30**:643-652.
23. Obayashi S, Kato I, Ono K, Masunaga S, Suzuki M, Nagata K, Sakurai Y, Yura Y: **Delivery of ^{10}boron to oral squamous cell carcinoma using boronophenylalanine and borocaptate sodium for boron neutron capture therapy.** *Oral Oncol* 2004, **40**:474-482.
24. Kamida A, Fujita Y, Kato I, Iwai S, Ono K, Suzuki M, Sakurai Y, Yura Y: **Effect of neutron capture therapy on the cell cycle of human squamous cell carcinoma cells.** *Int J Radiat Biol* 2008, **84**:191-199.
25. Mosmann T: **Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.** *J Immunol Methods* 1983, **65**:55-63.
26. McIlwrath AJ, Vasey PA, Ross GM, Brown R: **Cell cycle arrests and radiosensitivity of human tumor cell lines: dependence on wild-type p53 for radiosensitivity.** *Cancer Res* 1994, **54**:3718-3722.
27. Gallo O, Chiarelli I, Bianchi S, Calzolari A, Simonetti L, Porfirio B: **Loss of p53 gene mutation after irradiation is associated with increased aggressiveness in recurring head and neck cancer.** *Clin Cancer Res* 1996, **2**:1577-1582.
28. Cuddihy AR, Bristow RG: **The p53 protein family and radiation sensitivity: Yes or no?** *Cancer Metastasis Rev* 2004, **23**:237-257.
29. Lothaire P, de Azambuja E, Dequanter D, Lalami Y, Sotiriou C, Andry G, Castro G Jr, Awada A: **Molecular markers of head and neck squamous cell carcinoma: promising signs in need of prospective evaluation.** *Head Neck* 2006, **28**:256-269.
30. Harris SL, Levine AJ: **The p53 pathway: positive and negative feedback loops.** *Oncogene* 2005, **24**:2899-2908.
31. Masunaga S, Ono K, Takahashi A, Sakurai Y, Ohnishi K, Kobayashi T, Kinashi Y, Takagaki M, Ohnishi T: **Impact of the p53 status of the tumor cells on the effect of reactor neutron beam irradiation, with emphasis on the response of intratumor quiescent cells.** *Jpn J of Cancer Res* 2002, **93**:1366-1377.
32. Aromando RF, Heber EM, Trivillin VA, Nigg DW, Schwint AE, Itoiz ME: **Insight into the mechanisms underlying tumor response to boron neutron capture therapy in the hamster cheek pouch oral cancer model.** *J Oral Pathol Med* 2009, **38**:448-454.
33. Okada H, Mak TW: **Pathways of apoptotic and non-apoptotic death in tumour cells.** *Nat Rev Cancer* 2004, **4**:592-603.
34. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ: **The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases.** *Cell* 1993, **75**:805-816.
35. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D: **p21 is a universal inhibitor of cyclin kinases.** *Nature* 1993, **366**:p701-704.
36. el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B: **WAF1, a potential mediator of p53 tumor suppression.** *Cell* 1993, **75**:817-825.
37. Hoffmann I, Clarke PR, Marcote MJ, Karsenti E, Draetta G: **Phosphorylation and activation of human cdc25-C by cdc2--cyclin B and its involvement in the self-amplification of MPF at mitosis.** *EMBO J* 1993, **12**:53-63.
38. Solomon MJ, Harper JW, Shuttleworth J: **CAK, the p34cdc2 activating kinase, contains a protein identical or closely related to p40 MO15.** *EMBO J* 1993, **12**:3133-3142.
39. Leach SD, Scatena CD, Keefer CJ, Goodman HA, Song SY, Yang L, Pietenpol JA: **Negative regulation of Wee1 expression and Cdc2 phosphorylation during p53-mediated growth arrest and apoptosis.** *Cancer Res* 1998, **58**:3231-3236.
40. Russell P, Nurse P: **Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog.** *Cell* 1987, **49**:559-567.

41. Lundgren K, Walworth N, Booher R, Dembski M, Kirschner M, Beach D: **mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2.** *Cell* 1991, **64**:1111-1122.
42. Matsumura S, Matsumura T, Ozeki S, Fukushima S, Yamazaki H, Inoue T, Inoue T, Furusawa Y, Eguchi-Kasai K: **Comparative analysis of G2 arrest after irradiation with 75 keV carbon-ion beams and ^{137}Cs γ-rays in a human lymphoblastoid cell line.** *Cancer Detect Prev* 2003, **27**:222-228.
43. Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, Roth JA: **Adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation.** *Clin Cancer Res* 1996, **2**:1665-1671.
44. Li JH, Lax SA, Kim J, Klamut H, Liu FF: **The effects of combining ionizing radiation and adenoviral p53 therapy in nasopharyngeal carcinoma.** *Int J Radiat Oncol Biol Phy* 1999, **43**:607-616.
45. Zhang S, Li Y, Li L, Zhang Y, Gao N, Zhang Z, Zhao H: **Phase I study of repeated intraepithelial delivery of adenoviral p53 in patients with dysplastic oral leukoplakia.** *J Oral Maxillofac Surg* 2009, **67**:1074-1082.
46. Tian G, Liu J, Sui J: **A patient with huge hepatocellular carcinoma who had a complete clinical response to p53 gene combined with chemotherapy and transcatheter arterial chemoembolization.** *Anticancer Drugs* 2009, **20**:403-407.



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Dodecaborate lipid liposomes as new vehicles for boron delivery system of neutron capture therapy

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ABSTRACT

closo-Dodecaborate lipid liposomes were developed as new vehicles for boron delivery system (BDS) of neutron capture therapy. The current approach is unique because the liposome shell itself possesses cytocidal potential in combination with neutron irradiation. The liposomes composed of *closo*-dodecaborate lipids DSBL and DPBL displayed high cytotoxicity with thermal neutron irradiation. The *closo*-dodecaborate lipid liposomes were taken up into the cytoplasm by endocytosis without degradation of the liposomes. Boron concentration of 22.7 ppm in tumor was achieved by injection with DSBL-25% PEG liposomes at 20 mg B/kg. Promising BNCT effects were observed in the mice injected with DSBL-25% PEG liposomes: the tumor growth was significantly suppressed after thermal neutron irradiation (1.8×10^{12} neutrons/cm^2).

1. Introduction

The cytotoxic effect of boron neutron capture therapy (BNCT) is due to the nuclear reaction of two essentially nontoxic species, boron-10 and thermal neutrons (Eq. 1):[1]

$$^{10}B + {}^{1}n \rightarrow {}^{4}He(\alpha) + {}^{7}Li + 2.4\ MeV \quad (1)$$

The resulting α-particle and Li nuclei are high linear energy transfer (LET) particles that exert the cytotoxic effect. The fact that LET particles travel a short range (approximately 10 μm) limits radiation-induced damage to cells containing boron-10. Therefore, the high accumulation and selective delivery of boron-10 into tumor tissue are the most important requirements to achieve efficient BNCT of cancers.[2–5] The amount of boron-10 necessary to realize fatal tumor cell damage is 20–35 μg/g tumor tissue.[6] At the same time, boron concentration in surrounding normal tissues and blood should be kept low to minimize damage to those tissues. Although mercaptoundecahydrododecaborate (BSH; $Na_2B_{12}H_{11}SH$)[7,8] and L-*p*-boronophenylalanine (L-BPA)[9,10] have been utilized for BNCT, the development of new boron-10 carriers that deliver an adequate concentration of boron-10 atoms to a tumor is still an important task to achieve effective cancer therapy.[11–13]

Recently much attention has been focused on the liposomal boron delivery system (BDS). Liposomes are efficient drug delivery vehicles because they can transport their contents to the interior of various tumors in a manner that is essentially independent of their contents. Therefore, boron compounds-encapsulated liposomes are attractive vehicles to deliver adequate quantities of boron to the tumor cells for BNCT. Various boron compounds-encapsulated BDSs have been developed including passive targeting liposomes[14–16] and/or active targeting liposomes by conjugating tumor specific ligands, such as mAb,[17–19] folate,[20] epidermal growth factor,[21] and transferrin (TF).[22,23] However, in order to deliver therapeutic quantities of boron to the tumor cells, concentrated aqueous solutions of polyhedral borane salts must be encapsulated. This causes osmotic problem of the liposome production.

In contrast, the development of lipophilic boron compounds embedded within the liposome bilayer is an attractive means to increase the overall incorporation efficiency of boron-containing species, as well as to raise the gross boron content of the liposome in the formation. Selective boron delivery to tumors by lipophilic species incorporated in the membranes of unilamellar liposomes was first demonstrated by Hawthorne and co-workers.[24,25] We previously reported the first synthesis of *nido*-carborane lipid (**1**) having a double-tailed moiety conjugated with *nido*-carborane as a hydrophilic moiety and its vesicle formation from **1** (Fig. 1).[26] Furthermore, we investigated the possibility of actively targeting boron liposomes to solid tumor by conjugating TF to the surface of the liposomes. Boron concentration of 22 μg ^{10}B/g tumor was observed

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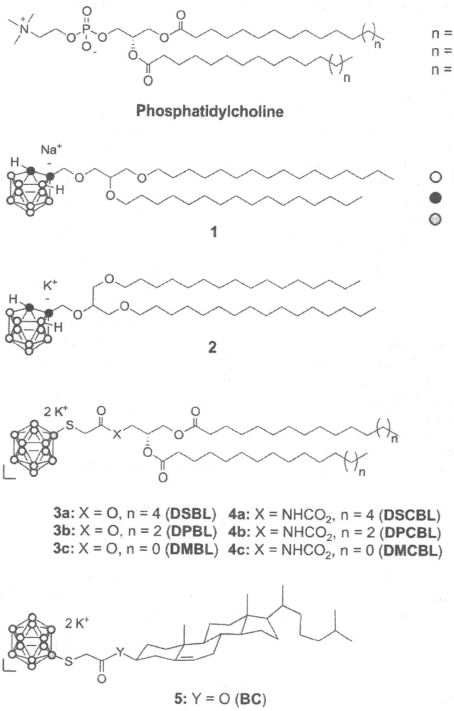

Figure 1. Structures of phosphatidylcholine, boron lipids (**1–4**).

in mice injected with the boron liposomes at 7.2 mg ^{10}B/kg body weight. However, the injection of a higher boron concentration (14 mg ^{10}B/kg body weight) resulted in acute toxicity to the mice.[27] Hawthorne and co-workers also recently reported similar acute toxicity in mice injected with *nido*-carborane lipid (**2**).[28] We surmised that this high toxicity may be caused by the *nido*-carborane structure, although the mechanism of *nido*-carborane cytotoxicity has not been studied in detail.

In order to overcome this drawback, we focused on BSH as an alternative hydrophilic boron cluster for boron lipids. BSH is a water-soluble divalent '*closo*-type' anion cluster that has significantly low toxicity. Because of this property, BSH has been utilized for BNCT. We were the first to synthesize *closo*-dodecaborate lipids (**3** and **4**) that possess the $B_{12}H_{11}S$ moiety as the hydrophilic function and have similar chirality to natural phospholipids, such as DSPC, in their lipophilic tails (Fig. 1).[29,30] We also focused on cholesterol, which is also one of the important contents for liposome formation, and synthesized *closo*-dodecaborate-conjugated cholesterols.[31] As we surmised, the liposomes prepared from the current *closo*-dodecaborate lipids and cholesterols did not show acute toxicity at 20 mg ^{10}B/kg body weight in healthy mice.[32] Recently, various boron compounds embedded within the liposome bilayer have been reported for BDS,[24,28,33–35] however their BNCT effects have not been reported yet. We found the significant BNCT effects of the mice treated with the current *closo*-dodecaborate lipid liposomes after neutron irradiation.[32] In this paper, we provide a full account of our BDS studies using *closo*-dodecaborate lipid liposomes.

2. Materials and methods

2.1. Chemicals

DSPC (MC-8080) and DSPE-PEG (SUNBRIGHT DSPE-020CN) were purchased from Nippon Oil and Fats (Japan). Cholesterol (Chol) was purchased from Kanto Chemical (Japan). $Na_2{}^{10}B_{12}H_{11}SH$ was kindly supplied by Stella Chemifa (Japan). DSBL (**3a**), DPBL (**3b**), DMBL (**3c**), DSCBL (**4a**), DPCBL (**4b**), DMCBL (**4c**), and BC (**5**) were synthesized as previously described[29–31] and transformed into sodium forms by an ion-exchange resin (Amberlite IR-120). PKH Linker Kit (MINI67-1KT) was purchased from Sigma (USA). All other chemicals were of the highest grade commercially available.

2.2. Preparation and composition of boronated liposomes

Boronated liposomes and PEG boronated liposomes were prepared from boron lipids, DSPC, Chol (X:1 – X:1, molar ratio, $0 < X < 1$) and boron lipids, DSPC, Chol, DSPE-PEG (X:1 – X:1:0.11, molar ratio, $0 < X < 1$), respectively. These boronated liposomes were prepared according to the reverse-phase evaporation (REV) method.[36] Total lipids of 200 mg were dissolved in 6 mL of chloroform/diisopropyl ether mixture (1:1, v/v) and 3 mL of distilled water was added to form a w/o emulsion. The emulsion was sonicated for 3 min and then, the organic solvents were removed under reduced pressure in a rotary evaporator at 60 °C for 30 min to obtain a suspension of liposomes. The liposomes obtained were subjected to extrusion 10 times through a polycarbonate membrane filter of 100 nm pore size (Whatman, 110605, FILTER, 0.1UM, 25MM, Gentaur Molecular Products, Belgium), using an extruder device (LIPEX™ Extruder, Northern Lipids, Canada) thermostated at 60 °C. Purification was accomplished by ultracentrifugation (himac cp 80 wx, Hitachi Koki, Japan) at 200,000g for 60 min at 4 °C, and the pellets obtained were resuspended in 0.9% NaCl solution or PBS. Particle size distribution of the boronated liposomes was measured with an electrophoretic light scattering spectrophotometer (Nano-ZS, Sysmex, Japan). The compositions of boron lipids and DSPC in liposomes were calculated from data obtained by the simultaneous measurement of boron and phosphorus concentrations by inductively coupled plasma atomic emission spectroscopy (ICP-AES, HORIBA, Japan).

2.3. Transmission electron microscopy analysis

An aliquot of the sample solution was applied to electron microscope carbon-coated grids covered with parlodion backing. The excess of the solution was blotted with filter paper. Grids were immediately negatively stained with 2.5% uranyl acetate for 10 min. The grids were examined in a Transmission Electron Microscope (JEM 2000FX) at an accelerating voltage of 200 kV.

2.4. Cell culture and neutron irradiation

The mouse colorectal carcinoma cell line, colon 26, was maintained at 37 °C under 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, HyClone, USA), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Invitrogen, USA). For subsequent experiments, the cells were seeded at a density of 5×10^3 cells/well in a 96-well plate (Greiner, Germany) and incubated at 37 °C for 20 h. Neutron irradiation was carried out in the Japan Research Reactor No. 4 (JRR4) of Japan Atomic Energy Agency.

Method A (no wash): The cells were incubated for 30 min in the presence of various concentrations of boron lipid-25% liposomes prepared from DSPC, ^{10}B-enriched boron lipids, and Chol (0.75:0.25:1, molar ratio) in medium. The cells were irradiated with thermal neutrons in the JRR4 for 30 min ($3.8–5.0 \times 10^{11}$ neutrons/cm^2). After irradiation, the cells were washed with PBS and incubated for 3 days in fresh medium. Cell viability was determined by the MTT assay.

Method B (wash): The cells were incubated for 30 min in the presence of various concentrations of boron lipid-25% liposomes (^{10}B-enriched) in medium. After the medium was exchanged with a fresh one, the cells were incubated for another 30 min and then irradiated with thermal neutrons in the JRR4 for 30 min ($3.8–5.0 \times 10^{11}$ neutrons/cm^2). After irradiation, the cells were washed with PBS and incubated for 3 days in fresh medium. Cell viability was determined by the MTT assay.

2.5. In vitro fluorescence imaging

PKH67-labeled boronated liposomes were prepared according to the conventional cell membrane labeling method (Sigma, PKH67 Green Fluorescent Cell Linker Kit). Briefly, pellets of boronated liposomes were dissolved in 250 μL of Diluent C, and then the boronated liposome solution was added dropwise into 1 μL of PKH67 dye stock solution. The mixture was maintained at 20 °C for 5 min and free PKH67 was removed by ultracentrifugation at 200,000g for 60 min at 4 °C. The obtained PKH67-labeled boronated liposomes were resuspended in PBS.

Colon 26 cells were 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 subsequent experiments, the cells were seeded at a density of 5×10^4 cells in a 35 mm diameter dish (Greiner) and incubated at 37 °C for 20 h. The cells were incubated at 37 °C or 4 °C in the presence of NaN_3 (1 mM)[37] for another 3 h in the presence of PKH67-labeled boronated liposomes in medium. To prevent pH changes under CO_2-free condition, Leibovitz's L-15 medium (Invitrogen, USA) were used. After incubation, the cells were fixed with 4% paraformaldehyde in PBS for 10 min, and coverslipped using Vectashield mounting medium (Vector Lavoratories, USA) for further analysis under a fluorescent confocal microscope (FV100D IX81, OLYMPUS, Japan).

2.6. Biodistribution of boronated liposomes in mice

Tumor-bearing mice (female, 5–6 weeks old, 16–20 g, Sankyo Labo Service, Japan) were prepared by injecting subcutaneously (s.c.) a suspension (2.5×10^6 cells/mouse) of colon 26 cells directly into the right thigh. The mice were kept on a regular chow diet and water and maintained under 12 h light/dark cycle in an ambient atmosphere. Biodistribution experiments were performed when the tumor diameter was 7–9 mm. The tumor-bearing mice were injected via the tail vein with 200 μL of BSH (6000 ppm B) in 0.9% NaCl solution or DSBL-25% and -50% PEG liposomes (2000 ppm B). At selected time intervals after administration, the mice were lightly anesthetized and blood samples were collected from the retro-orbital sinus. The mice were then sacrificed by cervical dislocation and dissected. Liver, spleen, kidney, heart, brain, lung, muscle, and tumor were excised, washed with 0.9% NaCl solution, and weighed. The excised tissues were digested with 2 mL of concd HNO_3 (ultratrace analysis grade, Wako, Japan) at 90 °C for 1–3 h, and then the digested samples were diluted with distilled water. After filtering through a hydrophobic filter (13JP050AN, ADVANTEC, Japan), boron concentration was measured by ICP-AES.

2.7. BNCT for tumor-bearing mice

Liposomes (DSBL-25%) were prepared from ^{10}B-enriched DSBL (**3a**), DSPC, Chol, and DSPE-PEG (0.25:0.75:1:0.11, molar ratio) and injected into colon 26 tumor bearing mice (female, 6–7 weeks old, 16–20 g) via the tail vein at a dose of 20 mg ^{10}B/kg (2000 ppm of ^{10}B concentration; 200 μL of boronated liposome solution). The mice were anesthetized with isoflurane (Forane, Abbott, Japan) and placed in an acrylic mouse holder 24 h after iv injection. The mice were irradiated in the JRR4 for 30 min at a rate of 1.8×10^{12} neutrons/cm^2. The BNCT effects were evaluated on the basis of the changes in tumor volume of the mice. Mortality was monitored daily and tumor volume was measured at intervals of a few days. To determine tumor volume, two perpendicular diameters of the tumor were measured with a slide caliper and calculation was 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 were approved by the Institutional Animal Care and Use Committee of Gakushuin University.

3. Results and discussion

3.1. Characterization of boronated liposomes

The boronated liposomes were prepared from DSBL (**3a**), DSPC, Chol (X:1 – X:1, molar ratio, X<1) and DSBL, DSPC, Chol, DSPE-PEG (X: 1 – X:1:0.11, molar ratio, X<1) by the REV method and particle sizes were measured with an electrophoretic light scattering spectrophotometer. As shown in Table 1, particle sizes (diameter) were distributed in the range of 95–105 nm. The boronated liposomes from each boron lipid (**3b–c** and **4a–c**) also displayed similar particle size distribution (data not shown). DSBL/DSPC lipid ratios in the liposomes were calculated from the concentrations of boron and phosphorus determined by ICP-AES. The mixing ratio of DSBL to DSPC (X value) in the preparation of the liposomes is plotted on the abscissa and the DSBL/DSPC ratio in the liposomes obtained is plotted on the ordinate, as shown in Figure 2. It was revealed that the DSBL/DSPC ratio in the liposomal membrane is proportional to the mixing ratio in the preparation. The formation of DSBL-25% liposomes was analyzed under a transmission electron microscope by the negative staining method after extrusion through a 100 nm filter. As shown in Figure 3, boronated liposomes were formed as unilamellar particles measuring 100 nm in diameter.

3.2. In vitro cytotoxicity of boronated liposomes after neutron irradiation

We next examined the effect of boronated liposomes (^{10}B-enriched) on colon 26 cells at various ^{10}B concentrations with thermal neutron irradiation. Cell viability with or without thermal neutron irradiation was measured by the MTT assay. In the case of method A (no wash), thermal neutron irradiation of cells was carried out in the presence of boronated liposomes prepared from DSBL (**3a**), DPBL (**3b**), DMBL (**3c**), DSCBL (**4a**), DPCBL (**4b**), and DMCBL (**4c**), BC (**5**) in the cell medium. BSH and $B(OH)_3$ were used as controls. As shown in Figure 4A, except DSCBL liposome, all boronated liposomes as well as BSH and $B(OH)_3$ controls showed no cytotoxicity when not irradiated with thermal neutrons. After thermal neutron irradiation, however, significant cell damage was observed in a boron-dose-dependent manner in all cases. Figure 4B shows BNCT effects on cells irradiated with thermal neutrons for 30 min after the cell medium was replaced with a boron-free one. Compared with liposomes composed of DMCBL and BC and boron compounds $B(OH)_3$ and BSH that showed no cytotoxicity, liposomes composed of DSBL, DPBL, DMBL, DSCBL, and DPCBL showed boron-dose-dependent cytotoxicity, and DSBL and DPBL were found to be potential boron lipids for liposomal boron vehicles. The results indicate that the boronated liposomes were taken up by the tumor cells and remained there for certain periods, whereas $B(OH)_3$ and BSH were readily washed out from the cells. In the current experiments, a slight difference in thermal neutron dose ranging from 3.8 to 5.0 × 10^{11} neutrons/cm^2 was observed on the 96-well plates. However, the difference did not affect cell viability remarkably.

Table 1
Particle size, polydispersity index, and zeta potential of DSBL liposomes

DSBL X value	Particle size[a] (nm)	Polydispersity index[a]	Zeta potential (mV)
0	94.8 ± 0.62	0.050 ± 0.003	−2.4
0.05	100.5 ± 0.17	0.033 ± 0.011	−42.8
0.1	95.6 ± 1.01	0.022 ± 0.011	−45.7
0.15	99.0 ± 0.20	0.029 ± 0.010	−42.5
0.25	102.0 ± 0.44	0.048 ± 0.005	−45.8
0.5	104.3 ± 0.61	0.034 ± 0.014	−46.7

[a] Data are expressed as means ± sem.

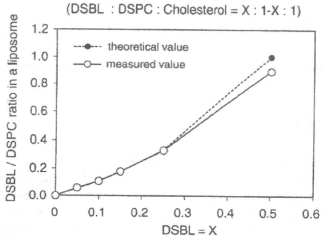

Figure 2. Composition of DSBL and DSPC in boronated liposome membranes. Boronated liposomes were prepared from DSBL, DSPC, and Chol (molar ratio is X:1 – X:1).

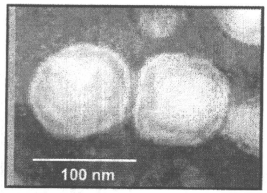

Figure 3. Transmission electron microscopy analysis of 25% DSBL liposomes.

3.3. Uptake of fluorescence-labeled boronated liposomes by endocytosis

Colon 26 cells were treated with DSBL-25% liposomes dyed green with PKH67. After 3 h incubation with the liposomes, the cells were washed with PBS and the PKH67-labeled DSBL liposomes were detected with a fluorescent confocal microscope. Figure 5A shows intracellular localization of PKH67-labeled DSBL liposomes at 37 °C. To confirm that the translocation of liposome is mediated by endocytosis, effects of endocytosis inhibitor sodium azide at low temperature.[37] As shown in Figure 5B, PKH67-labeled DSBL liposomes were localized on plasma membrane in the presence of sodium azide at 4 °C. These results indicate that DSBL liposomes were taken up into the cytoplasm by endocytosis without degradation of the liposomes.

3.4. Biodistribution of boronated liposomes in mice

Figure 6A and B show the time courses of boron concentrations in various tissues of tumor-bearing mice after injecting DSBL-25% PEG liposomes (20 mg B/kg) and BSH (60 mg B/kg). In the case of BSH, boron concentration in blood was 62.7 ppm 1 h after injection but dropped to 15.7 and 0.48 ppm 3 and 10 h after injection, respectively. Boron concentration in the other tissues decreased along with the disappearance of BSH in blood due to high renal clearance. Boron concentration of 37.8 ppm was observed in tumor with a tumor/blood ratio of ~0.6 at 1 h after injection. In contrast,

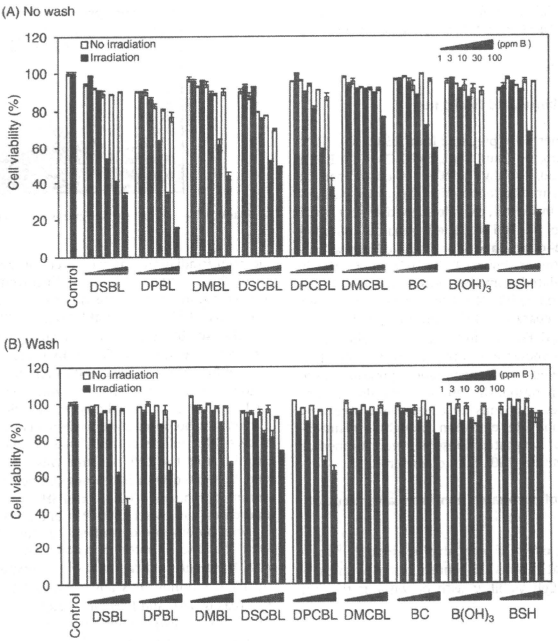

Figure 4. Effects of boronated liposomes on colon 26 cell viability after thermal neutron irradiation or no irradiation. All boronated liposomes were composed of 25% boron lipids or boron cholesterols. (A) Colon 26 cells were incubated in a 96-well microplate at 37 °C in 5% CO_2 in air for 30 min in the presence of boronated liposomes. The cells were irradiated with thermal neutrons for 30 min (no wash). Three days after irradiation, cell viability was determined by the MTT assay. (B) Colon 26 cells were incubated in a 96-well microplate at 37 °C in 5% CO_2 in air for 30 min in the presence of boronated liposomes. The cells were irradiated with thermal neutrons for 30 min after medium exchange (wash). Three days after irradiation, cell viability was determined by the MTT assay. Data are expressed as means ± sem ($n = 3$).

boron concentration of 22.7 ppm in tumor with a tumor/blood ratio of ~2 was observed 24 h after administration of DSBL-25% PEG liposomes, and boron concentration gradually decreased thereafter. High boron concentration was observed also in liver and spleen. The high boron concentration in spleen may be due to the instability of the DSBL-25% PEG liposomes present in blood. In general drug delivery systems, the high accumulation of drug-encapsulating or -attaching nanoparticles in other tissues, such as liver and spleen, sometimes induces side effects due to the cytotoxicity of the accumulated drugs. Current boron lipid liposomes displayed significantly low toxicity and were readily eliminated from the tissues within three weeks after injection. Therefore, it is considered that the high accumulation of boron in liver and spleen observed in Figure 6A would not have serious side effects unless thermal neutron irradiation is carried out on these tissues. In this regard, *BNCT is a double-targeting therapy that involves boron delivery to and neutron irradiation of cancers.*

3.5. BNCT effect of boronated liposomes on tumor-bearing mice

The cytotoxicity of DSBL-25% PEG liposomes was examined by irradiating colon 26 tumor bearing mice with thermal neutrons. When colon 26 cells transplanted into the left thighs of mice showed logarithmic growth, the mice were given saline as control and DSBL-25% PEG liposomes at a dose of 20 mg ^{10}B/kg. As the highest boron concentration in tumor (22.7 ppm at a dose of 20 mg B/kg) was observed 24 h after injecting DSBL-25% PEG liposomes, thermal neutron irradiation of the tumor-transplanted left thighs of mice was carried out 24 h after the injection while shielding bodies with the acrylic mouse holder. As shown in Figure 7, tumor volume in mice treated with DSBL-25% PEG liposomes was significantly inhibited after thermal neutron irradiation. The tumor volumes were ~20% of those of control mice two weeks after the neutron irradiation.

4. Conclusion

We newly prepared the *closo*-dodecaborate lipids containing liposomes as BDS vehicles for neutron capture therapy. The current approach is unique because *the liposome shell itself possesses cytocidal potential in combination with neutron irradiation.* The boronated liposomes composed of DSBL or DPBL, in particular, displayed high cytotoxicity with thermal neutron irradiation in colon 26 cells. The efficient in vitro BNCT effects were due to the uptake of the boro-

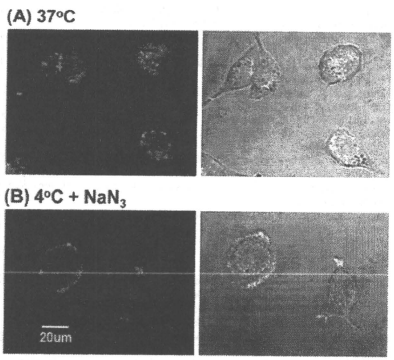

Figure 5. Intracellular localization of PKH-labeled boronated liposomes. (A) PKH67-labeled DSBL-25% liposomes were incubated at 37 °C for 3 h in colon 26 cells, and visualized under a fluorescent confocal microscope. (B) The cells were incubated at 4 °C for 3 h with PKH67-labeled DSBL-25% liposomes in the presence of NaN_3 (1 mM).

nated liposomes in the cytoplasm by endocytosis, which was observed in the fluorescence experiments using PHK67-labeled DSBL-25% liposomes. The previously developed boronated liposomes composed of *nido*-carborane lipid **1** showed acute toxicity within one day at a dose of 14 mg B/kg,[27] however the boronated liposomes described in this paper did not show acute toxicity toward healthy mice at a dose of 20 mg B/kg. Furthermore, the boronated liposomes composed of *closo*-dodecaborate lipids were readily eliminated from the body, whereas BSH-conjugated cholesterols BC and BBC were not eliminated and remained in tissues even after three weeks. Boron concentration of 22.7 ppm in tumor was achieved by injection with DSBL-25% PEG liposomes at 20 mg B/kg in tumor-bearing mice. As described, 20–35 ppm boron concentrations are required to realize fatal tumor cell damage, therefore the concentration observed by injection with DSBL-25% PEG liposomes in tumor-bearing mice is expected to result in the fatal tumor cell damage with BNCT. In fact, significant suppression of tumor growth was observed after thermal neutron irradiation. This is the first promising BNCT of tumor-bearing mice with the boron lipid liposomes, although various boron compounds embedded within the liposome bilayer have been reported. As the internal aqueous core of the examined boronated liposomes is still vacant, drugs, including boron compounds, can be encapsulated in it. In this regard, boron-10 and drugs may be simultaneously delivered

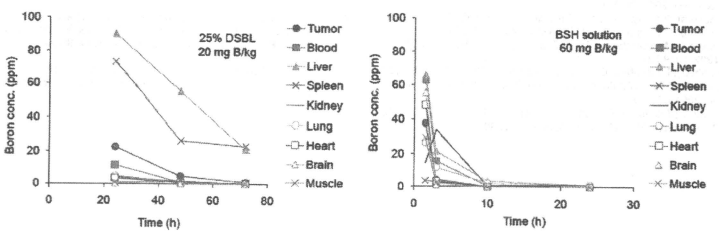

Figure 6. Time course of biodistribution of (A) DSBL-25% PEG liposomes (20 mg B/kg) and (B) BSH solution (60 mg B/kg). Each sample was injected into tumor-bearing mice (Balb/c, female, 6 weeks old, 14–20 g) via the tail vein.

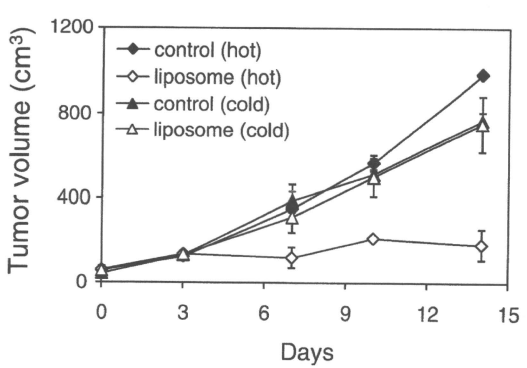

Figure 7. Tumor volume in mice (Balb/c, female, 6 weeks old, 14–20 g) bearing colon 26 solid tumor with thermal neutron irradiation (hot) for 30 min (1.8×10^{12} neutrons/cm^2) or without irradiation (cold). The irradiation was performed at 24 h after iv injection of DSBL-25% PEG liposomes (20 mg B/kg). Data are expressed as means ± sem ($n = 3$).

to a tumor, realizing combination therapy consisting of BNCT and chemotherapy. Investigation for further efficient BDS including active targeting to tumor by functionalization of the boronated liposomes are going on in our laboratory.

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References and notes

1. Locher, G. L. *Am. J. Roentgenol.* **1936**, *36*, 1.
2. Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F. G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
3. Barth, R. F.; Coderre, J. A.; Vicente, M. G.; Blue, T. E. *Clin. Cancer Res.* **2005**, *11*, 3987.
4. Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950.
5. Yinghuai, Z.; Cheng Yan, K.; Maguire, J. A.; Hosmane, N. S. *Curr. Chem. Biol.* **2007**, *1*, 141.
6. Barth, R. F.; Soloway, A. H.; Fairchild, R. G.; Brugger, R. M. *Cancer* **1992**, *70*, 2995.
7. Soloway, A. H.; Hatanaka, H.; Davis, M. A. *J. Med. Chem.* **1967**, *10*, 714.
8. Nakagawa, Y.; Hatanaka, H. *J. Neurooncol.* **1997**, *33*, 105.
9. Snyder, H. R.; Reedy, A. J.; Lennarz, W. J. *J. Am. Chem. Soc.* **1958**, *80*, 835.
10. Mishima, Y.; Ichihashi, M.; Hatta, S.; Honda, C.; Yamamura, K.; Nakagawa, T. *Pigment Cell Res.* **1989**, *2*, 226.
11. Kato, I.; Ono, K.; Sakurai, Y.; Ohmae, M.; Maruhashi, A.; Imahori, Y.; Kirihata, M.; Nakazawa, M.; Yura, Y. *Appl. Radiat. Isot.* **2004**, *61*, 1069.
12. Aihara, T.; Hiratsuka, J.; Morita, N.; Uno, M.; Sakurai, Y.; Maruhashi, A.; Ono, K.; Harada, T. *Head Neck* **2006**, *28*, 850.
13. Suzuki, M.; Sakurai, Y.; Hagiwara, S.; Masunaga, S.; Kinashi, Y.; Nagata, K.; Maruhashi, A.; Kudo, M.; Ono, K. *Jpn. J. Clin. Oncol.* **2007**, *37*, 376.
14. Shelly, K.; Feakes, D. A.; Hawthorne, M. F.; Schmidt, P. G.; Krisch, T. A.; Bauer, W. F. *PNAS* **1992**, *89*, 9039.
15. Feakes, D. A.; Shelly, K.; Knobler, C. B.; Hawthorne, M. F. *PNAS* **1994**, *91*, 3029.
16. Martini, S.; Ristori, S.; Pucci, A.; Bonechi, C.; Becciolini, A.; Martini, G.; Rossi, C. *Biophys. Chem.* **2004**, *111*, 27.
17. Yanagie, H.; Fujii, Y.; Takahashi, T.; Tomita, T.; Fukano, Y.; Hasumi, K.; Nariuchi, H.; Yasuda, T.; Seliguchi, M.; Uchida, H. *Human Cell* **1989**, *2*, 290.
18. Yanagie, H.; Tomita, T.; Kobayashi, H.; Fujii, Y.; Takahashi, T.; Hasumi, K.; Nariuchi, H.; Sekiguchi, M. *Br. J. Cancer* **1991**, *63*, 522.
19. Pan, X.; Wu, G.; Yang, W.; Barth, R. F.; Tjarks, W.; Lee, R. J. *Bioconjugate Chem.* **2007**, *18*, 101.
20. Pan, X. Q.; Wang, H.; Shukla, S.; Sekido, M.; Adams, D. M.; Tjarks, W.; Barth, R. F.; Lee, R. J. *Bioconjugate Chem.* **2002**, *13*, 435.
21. Kullberg, E. B.; Carlsson, J.; Edwards, K.; Capala, J.; Sjoberg, S.; Gedda, L. *Int. J. Oncol.* **2003**, *23*, 461.
22. Maruyama, K.; Ishida, O.; Kasaoka, S.; Takizawa, T.; Utoguchi, N.; Shinohara, A.; Chiba, M.; Kobayashi, H.; Eriguchi, M.; Yanagie, H. *J. Controlled Release* **2004**, *98*, 195.
23. Yanagie, H.; Ogura, K.; Takagi, K.; Maruyama, K.; Matsumoto, T.; Sakurai, Y.; Skvarc, J.; Illic, R.; Kuhne, G.; Hisa, T.; Yoshizaki, I.; Kono, K.; Furuya, Y.; Sugiyama, H.; Kobayashi, H.; Ono, K.; Nakagawa, K.; Eriguchi, M. *Appl. Radiat. Isot.* **2004**, *61*, 639.
24. Feakes, D. A.; Shelly, K.; Hawthorne, M. F. *PNAS* **1995**, *92*, 1367.
25. Watson-Clark, R. A.; Banquerigo, M. L.; Shelly, K.; Hawthorne, M. F.; Brahn, E. *PNAS* **1998**, *95*, 2531.
26. Nakamura, H.; Miyajima, Y.; Takei, T.; Kasaoka, S.; Maruyama, K. *Chem. Commun.* **2004**, 1910.
27. Miyajima, Y.; Nakamura, H.; Kuwata, Y.; Lee, J.-D.; Masunaga, S.; Ono, K.; Maruyama, K. *Bioconjugate Chem.* **2006**, *17*, 1314.
28. Li, T.; Hamdi, J.; Hawthorne, M. F. *Bioconjugate Chem.* **2006**, *17*, 15.
29. Lee, J.-D.; Ueno, M.; Miyajima, Y.; Nakamura, H. *Org. Lett.* **2007**, *9*, 323.
30. Nakamura, H.; Lee, J.-D.; Ueno, M.; Miyajima, Y.; Ban, H. *NanoBioTechnology* **2007**, *3*, 1551.
31. Nakamura, H.; Ueno, M.; Lee, J.-D.; Ban, H. S.; Justus, E.; Fan, P.; Gabel, D. *Tetrahedron Lett.* **2007**, *48*, 3151.
32. Nakamura, H.. In *Methods Enzymol*; Nejat, D., Ed.; Academic Press, 2009; Vol. 465, p 179.
33. Justus, E.; Awad, D.; Hohnholt, M.; Schaffran, T.; Edwards, K.; Karlsson, G.; Damian, L.; Gabel, D. *Bioconjugate Chem.* **2007**, *18*, 1287.
34. Shirakawa, M.; Yamamto, T.; Nakai, K.; Aburai, K.; Kawatobi, S.; Tsurubuchi, T.; Yamamoto, Y.; Yokoyama, Y.; Okuno, H.; Matsumura, A. *Appl. Radiat. Isot.* **2009**, *67*, S88.
35. Thirumamagal, B. T.; Zhao, X. B.; Bandyopadhyaya, A. K.; Narayanasamy, S.; Johnsamuel, J.; Tiwari, R.; Golightly, D. W.; Patel, V.; Jehning, B. T.; Backer, M. V.; Barth, R. F.; Lee, R. J.; Backer, J. M.; Tjarks, W. *Bioconjugate Chem.* **2006**, *17*, 1141.
36. Szoka, F. J.; Papahadjopoulos, D. *PNAS* **1978**, *75*, 4194.
37. Mudhakir, D.; Akita, H.; Tan, E.; Harashima, H. *J. Controlled Release* **2008**, *125*, 164.

—Review—

ホウ素の中性子捕捉反応を利用した低侵襲細胞選択的放射線療法

中村浩之

Minimally Invasive Cytoselective Radiation Therapy Using Boron Neutron Capture Reaction

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The cell-killing effect of boron neutron capture therapy (BNCT) is due to the nuclear reaction of two essentially nontoxic species, boron-10 (^{10}B) and thermal neutrons, whose destructive effect is well observed in boron-loaded tissues. High accumulation and selective delivery of boron into tumor tissue are the most important requirements to achieve efficient neutron capture therapy of cancers. This review focuses on liposomal boron delivery system (BDS) as a recent promising approach that meet these requirements for BNCT. BDS involves two strategies: (1) encapsulation of boron in the aqueous core of liposomes and (2) accumulation of boron in the liposomal bilayer. In this review, recent development of liposomal boron delivery system is summarized.

Key words——boron neutron capture therapy (BNCT); boron delivery system (BDS); liposome; boron cluster

1. はじめに

高齢化の進むわが国の死亡原因の第一位はがんであり，2人に1人ががんを発病し3人に1人ががんで死亡している．その年間死亡者数はおよそ30万人である．がん検診の普及，早期診断・早期治療，さらには初期治療としての手術・放射線療法・化学療法の複合治療によって，治癒率の改善が図られてきた．特に，近年サイバーナイフやガンマナイフ，重粒子線療法などの技術の進歩により放射線治療を受ける患者数は年々増加しており，最近では年間に18万人以上の患者が放射線治療を受けている．化学療法では分子標的治療薬が開発されいくつかのがんに対しては，副作用が抑えられるようになりつつあるが，しかし多くの場合，依然全身的な副作用との戦いが強いられている．放射線治療では，技術の進歩によりミリ単位で照射野を制御できるようにはなったが，浸潤しているがんを細胞レベルで照射することはできない．しかしながら，これらの化学療法と放射線療法の両方の原理をうまく利用し，細胞選択的に照射が可能であるホウ素中性子捕捉療法（BNCT: boron neutron capture therapy）が次世代放射線療法として注目されている．[1]

2. ホウ素中性子捕捉療法の原理

低エネルギーの熱中性子はエネルギーの高い高速中性子とは異なり，人体には無害である．しかしながら熱中性子とホウ素10との反応は，リチウムとヘリウム（α線）を生じ，これらのエネルギーは2.4 MeVとおよそ細胞1つを殺傷するのに十分であり，その飛程は細胞1つの直径（5–9 μm）である[Eq. (1)]．したがって，あらかじめホウ素分子をがん細胞にのみ選択的に取り込ませそこへ中性子照射を行えば，がん細胞のみを選択的に破壊することができる（Fig. 1）．これを利用するのがBNCTである．

$$^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He + 2.4\ MeV \qquad (1)$$

3. ホウ素中性子捕捉療法の歴史と現状

BNCTの概念は，1936年にLocherによって最初に提唱された．[2] その後，1951年から米国ブルックヘブン国立研究所（BNL）において悪性神経膠腫を対象とした最初の試験治療研究がFarr及びSweetらによって開始され，10年間で45例のBNCTが

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本総説は，日本薬学会第130年会シンポジウムS10で発表したものを中心に記述したものである．

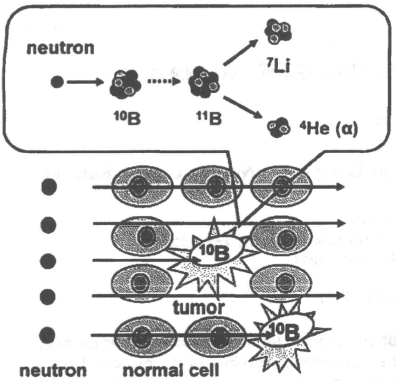

Fig. 1. Concept of BNCT

行われた．また，1953年からマサチューセッツ工科大学（MIT）でも治療が開始され，18例のBNCTが行われたが，ホウ素化合物の腫瘍選択性と中性子遮蔽の不十分さゆえに治療成績が悪く，1961年に治療が中断された．[3]

日本では，1968年，帝京大学の（故）畠中らは，Fig. 2に示すように非常に低毒性であるホウ素イオンクラスター（BSH: mercaptoundecahydrododecaborate）を用いて世界で初めて脳腫瘍のBNCTに成功した．[4] BSHは分子内に12個のホウ素原子を含む20面体の特異な化学構造を有しており，それ自身はがん細胞に対する選択性は低いが，高水溶性・低毒性である．健全な脳には血液脳関門現象（blood-brain barrier）があり，血液中の水溶性物質は正常な脳組織には取り込まれ難いが，脳腫瘍はこの血液脳関門が壊れているためBSHのような水溶性の化合物が脳組織内に取り込まれると考えられている．畠中らの成功以来，日本はこの分野をリードしてきており，現在まで脳腫瘍の治療実績は300症例を超えている．

一方，1987年神戸大学の三島らはアミノ酸誘導体であるBPA（*p*-boronophenylalanine）[5]を用いて悪性黒色腫のBNCTに成功した．[6] BPAは必須アミノ酸であるフェニルアラニンの類縁体として増殖の盛んながん細胞に選択的に取り込まれると考えられている．BPAは，中性領域下での溶解性が低いため，臨床ではD-フルクトースとの複合体として用いられている．現在まで悪性黒色腫の治療実績はおよそ30症例で5年生存率は60%を超えており，非常に治療効果が高い．また外科的手術と異なり機能温存できることからQOLの高さもBNCTの利点である．

1994年には，京都府立医大の今堀・上田らにより^{18}F-BPAを用いたPET (positron emission tomography) 診断法が開発された．この診断法によってあらかじめ腫瘍部位のホウ素蓄積量を見積もることができるようになっただけでなく，[7] ^{18}F-BPAが多くの悪性腫瘍に集積することも分かった．BPAは増殖しているがん細胞には選択的に取り込まれるものの，休止期腫瘍細胞への取り込みは低いことが弱点であったが，BSHとの併用により克服できるようになった．

2001年，大阪大の加藤・由良らは小野らと共同して世界に先駆けて頭頸部悪性腫瘍のBNCTに成功した．[8] 頭頸部悪性腫瘍は現在でも手術が中心であり，審美障害，嚥下・咀嚼障害などの機能障害が後遺することがある．彼らは，再発耳下腺がん患者に対し，BSHとBPAの併用BNCTを行い，9ヵ月後にはがんが完全に消失し，皮膚への放射線障害もほとんどみられなかった．この成功をきっかけにBNCTの適応拡大が進められ，頭頸部がん以外にも，肝臓がん，肺がん，胸壁腫瘍などの適応疾患の拡大とともにBNCTの症例数が増加している．[9-11]

その一方で，ホウ素薬剤である^{10}B濃縮したBSHとL体BPAは，海外からの輸入に頼っていたため，臨床に必要なホウ素薬剤の確保がしばしば困難であった．大阪府立大の切畑らはステラケミファ㈱と共同で，^{10}B濃縮したBSHとBPAの国産化に成功し，この2つのホウ素薬剤のGMPレベルでの供給体制が整った．BNCTに用いる熱中性子は現在のところ原子炉から得ているが，加速器から十

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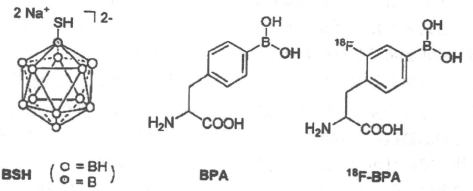

Fig. 2. Structures of BSH, BPA, and ^{18}F-BPA Used for Clinical Treatments

分な熱中性子が得られるようになれば，都市型病院への併設が可能となることから BNCT は放射線療法の 1 つとして一般に普及することが期待される．現在，BPA を用いた小型加速器 BNCT の臨床治験に向けた研究が，京都大学と住友重機械工業㈱・ステラファーマ㈱の産学共同研究で進められている．

4. 次世代型ホウ素デリバリーシステム

さて，効果的に BNCT を行うためには選択的かつ高濃度でがん細胞にホウ素 10 を送り込むことが必要である．実際の臨床では，BSH と L 体 BPA を併用して，腫瘍内ホウ素濃度が 25–100 ppm，腫瘍/血液のホウ素濃度比並びに腫瘍/正常組織のホウ素濃度比が 2–3 で行われている．はじめに述べたように，BNCT の望まれる条件（腫瘍内ホウ素濃度が 30 ppm 以上でなおかつ，腫瘍/血液並びに腫瘍/正常組織のホウ素濃度比が 5 以上）を達成するために，様々なホウ素キャリアーの開発研究が行われてきた．ホウ素キャリアーに望まれることは，(i) 500 mg/kg 程度の濃度で投与が可能なくらい毒性が低いこと，(ii) 十分に水溶性であること，(iii) 腫瘍細胞への蓄積が選択的であること，が挙げられる．これらの条件を満たすためには，従来の抗がん剤開発とは全く異なるアプローチが必要であり，リポソームを用いたホウ素デリバリーシステム（boron delivery system; BDS）が有望であると注目されている．

リポソームを用いる利点として，(i) 一度に大量のホウ素 10 をがん細胞に送り込むことが可能であること，(ii) ホウ素薬剤自体に薬理活性を持たせる必要がないこと，(iii) 受容体選択性を高めるためにリポソーム表面に対応するリガンドを導入することにより能動的な送達（アクティブターゲティング）が可能であること，が挙げられる．リポソームを用いたホウ素デリバリー法として，大きく 2 つの方法に分けられる．1 つは，リポソーム内にホウ素薬剤を封入する方法である．この方法は，一般的なリポソームを用いた DDS を応用するものであり，BSH などのホウ素化合物を封入する．もう 1 つの方法は，リポソーム膜にホウ素を埋め込む方法である．この方法では，リポソーム内にさらに抗がん剤などの薬剤を封入することができるため，化学療法との複合治療が期待できる．様々なリガンドをリポソーム膜に結合させることにより，能動的に標的がん細胞に取り込ませるような機能をリポソームに持たせることが可能となってきた．

4-1. ホウ素薬剤内封型リポソーム　ホウ素薬剤内封リポソーム型 BDS の最初の報告は，柳衛らによる anti-human CEA (carcinoembryonic antigen) モノクローナル抗体を結合させた BSH 内封イムノリポソームであった．AsPC-1（ヒト膵臓がん）細胞を移植したヌードマウスを用いた実験では，BSH 封入イムノリポソームを投与し中性子照射したマウスにおいて，腫瘍増殖が 50% 以下に抑えられることを見い出した．[12,13] Hawthorne らは，DSPC（distearoyl phosphatidylcholine）とコレステロールを用いて，様々なホウ素イオンクラスターを封入したリポソームを報告している．[14,15]

ホウ素封入リポソームを細胞選択的にかつ能動的に取り込ませるために葉酸，[16] 細胞増殖因子の 1 つである EGF（上皮細胞増殖因子），[17] トランスフェリン，[18,19] EGFR モノクローナル抗体（Cetuximab）[20] などのリガンドを表面に導入したリポソームの開発研究が行われてきた．Table 1 にこれまで報告された主なホウ素薬剤内封アクティブターゲティングリポソームに関する内封ホウ素薬剤，リポソーム表面修飾リガンドとその分子標的についてまとめた．

4-2. ホウ素脂質型リポソーム　多面体構造のホウ素クラスターイオンを封入したリポソームを用いることで，高濃度 BDS が達成できる可能性が示されてきた．使用されているホウ素封入リポソームは非常に高いイオン濃度であり高浸透圧的な溶液であることから，これ以上の高濃度化は困難であると同時に，このような条件下でのリポソーム膜安定性の問題が生じている．実際に，われわれはリポソーム膜からの内封ホウ素イオンクラスターの漏出を確認している．また，ホウ素イオンクラスターのリポソームへの封入効率の低さが問題となってきた．そこでわれわれは，リポソームの脂質二分子膜に着目した．リポソームの脂質二分子膜は，分子間相互作用により自己集合化しているため密度が高く，この二分子膜へホウ素分子を導入できれば，非常に高濃度でホウ素をデリバリーできると考えられる．さらに，リポソーム膜内にホウ素を導入させることで，リポソーム内に抗がん剤など様々な薬剤が封入できることから，BNCT と化学療法の複合治療が可能となる．われわれはリポソームの二分子膜を形成しているリン脂質の骨格に着目し，Fig. 3 に示すように水溶性部位であるホスファチジルコリン部位にホウ素原子 9 個と炭素原子 2 個からなるかご状のホウ素イオンクラスター（*nido* 型カルボラン）を導入した二本鎖ホウ素イオンクラスター脂質 1 を設計し合成に初めて成功した．[21] 合成したイオン性ホウ素クラスター脂質 1 は安定なリポソームを形成することが電子顕微鏡などで確認されたが，実際に腫瘍移植マウスを用いた *in vivo* 実験では，ホウ素濃度で

Table 1. Ligand-conjugated Boron-encapsulated Liposomes for Active Targeting

Boron compound	Ligand	Molecular target	Ref.
BSH	anti-human CEA[a]	CEA	12, 13
SPD-5	folic acid	folic acid receptor	16
WSP1	EGF[b]	EGF receptor	17
BSH	TF[c]	TF receptor	18
$Na_2B_{10}H_{10}$	TF	TF receptor	19
$Li_2B_{12}H_{12}$	Cetuximab	EGF receptor	20

[a] Carcinoembryonic antigen. [b] Epidermal growth factor. [c] Transferrin.

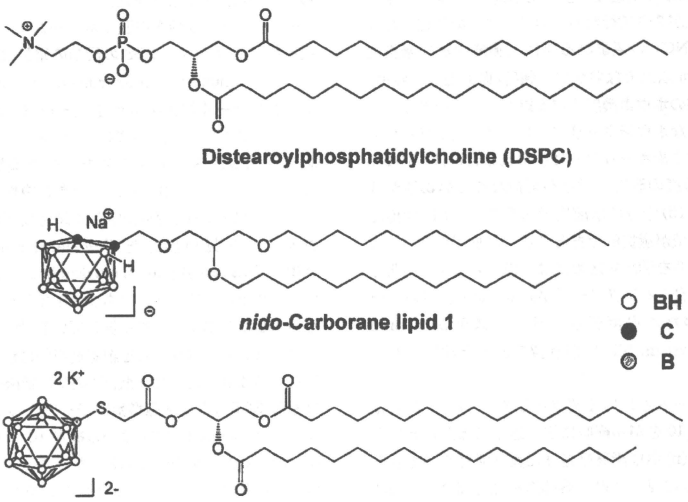

Fig. 3. Structures of a Phospholipid and Boron Lipids

7 mg/kg 投与において中性子照射後に顕著な延命効果がみられたものの，14 mg/kg 投与した場合に重篤な急性毒性がみられた．[22]

われわれはこのかご状の *nido* 型カルボランクラスターが毒性に深く係わっていると考えた．そこで，*nido* 型カルボランの代わりに，実際の臨床で用いられている BSH を導入した二本鎖ホウ素イオンクラスター脂質 DSBL（distearoylboron lipid）を再設計した．このホウ素 12 個からなる 20 面体のホウ素イオンクラスター，dodecaborate 構造を有する BSH は，先にも述べたように脳腫瘍患者の BNCT に使用されており，イオンクラスターであることから高水溶性であり，低毒性で非常に代謝が早く，血中での半減期は数時間であることが分かっている．しかし，有機合成を考えた場合，この dodecaborate は無機イオンクラスターであり，有機分子への導入は困難であった．われわれは BSH が有するチオール（-SH）基を手がかりに有機分子を導入することにした．BSH は高水溶性無機イオン化合物でありほとんどの有機溶媒に溶けないが，ナトリウム塩からテトラメチルアンモニウム塩に変えることで高極性非プロトン性溶媒であるアセトニトリルに溶けることが分かった．このことを利用して，アセトニトリル溶媒中で BSH のチオール基のアルキル化反応を行うことで，BSH の有機分子導入に成功した．[23,24] 合成した dodecaborate 脂質は，脂溶性部位に生体リン脂質と同じ立体構造を有しており，リンカー部位にエステル基を有し，dodecaborate 骨格と S を介して結合している．

5. ホウ素脂質型リポソームの BNCT 効果

5-1. ホウ素脂質のリポソーム化　このようにして合成したホウ素クラスター脂質 DSBL に対し，DSPC，コレステロールを X : 1-X : 1 で X の値を 0 -0.5 の範囲で混合し，逆相蒸発（REV）法を用いてリポソームを調製した．エクストルーダーを用いて，100 nm にサイジングした．得られたリポソームのリン脂質とホウ素の濃度を ICP-AES 法を用いて定量し，各々モル比に換算した．Figure 4 に示すように形成したリポソーム膜内の DSBL/DSPC 比は，調整した混合比に比例していることが分かった．さらに，得られたリポソームの粒子径並びにゼータ電位を Table 2 に示したが，DSBL の混合比にかかわらず粒子径は 100 nm 前後に分布しており，ゼータ電位は DSBL により負に大きく帯電していることが分かった．また，DSBL（25%）のリポソームに関して，透過型電子顕微鏡測定を行ったところ，直径 100 nm の二分子膜構造を形成していることが分かった．

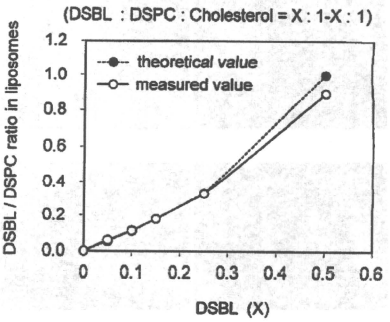

Fig. 4. Composition of DSBL and DSPC in Boronated Liposome Membranes

Boronated liposomes were prepared from DSBL, DSPC, and cholesterol (molar ratio is X:1-X:1).

Table 2. Particle Size, Polydispersity Index, and Zeta Potential of DSBL Liposomes

DSBL X value	Particle size (nm)[a]	Polydispersity index[a]	Zeta potential (mV)
0	94.8±0.62	0.050±0.003	−2.4
0.05	100.5±0.17	0.033±0.011	−42.8
0.1	95.6±1.01	0.022±0.011	−45.7
0.15	99.0±0.20	0.029±0.010	−42.5
0.25	102.0±0.44	0.048±0.005	−45.8
0.5	104.3±0.61	0.034±0.014	−46.7

[a] Data are expressed as mean±S.E.M.

5-2. Colon 26 細胞への取り込み機構　蛍光脂質（PKH67）を用いてホウ素リポソームを蛍光標識し，マウス大腸がん細胞（colon 26）に 37℃ で 3 時間インキュベートした．その結果，Fig. 5(A) に示すように細胞質に取り込まれていることが分かった．一方，この蛍光標識ホウ素リポソームを細胞のエンドサイトーシスが起こらない条件であるアジ化ナトリウム存在下，4℃ で同様に 3 時間インキュベートしたところ，Fig. 5(B) に示すように細胞膜表面に存在しているものの細胞質には取り込まれていないことから，ホウ素リポソームはエンドサイ

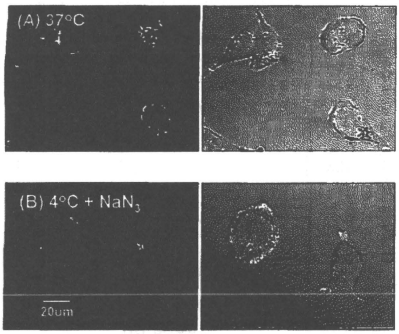

Fig. 5. Intracellular Localization of PKH-labeled Boronated Liposomes

(A) PKH67-labeled DSBL-25% liposomes were incubated at 37°C for 3 h in colon 26 cells, and visualized under a fluorescent confocal microscope. (B) The cells were incubated at 4°C for 3 h with PKH67-labeled DSBL-25% liposomes in the presence of NaN_3 (1 mM).

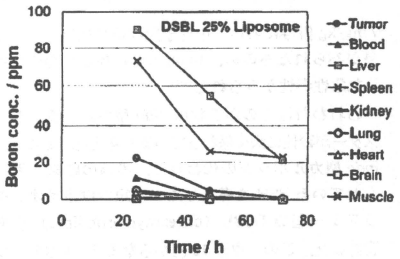

Fig. 6. Time Course of Biodistribution of DSBL-25% PEG Liposomes (20 mg B/kg) Injected into Tumor-bearing Mice (Balb/c, female, 6 weeks old, 14–20 g) *via* the Tail Vein

トーシスを経由して細胞内に取り込まれていることが明らかとなった.[25]

5-3. 腫瘍移植マウスを用いたホウ素リポソームの体内挙動と BNCT 効果 細網内皮系によるリポソームの取り込みを避けるため，調製したリポソームには，PEG 2000 を結合した DSPE（distearoyl phosphatidylethanolamine）を脂質に対して 10% 用いた．DSBL-25% を含むホウ素リポソームを用いて，担がんマウスを用いて体内挙動を調べた．Colon 26 細胞を移植した BALB/c マウス（生後 6 週間，16–18 g）にホウ素リポソームをホウ素濃度で 20 mg/kg 尾静脈投与した．投与 24–72 時間後に各臓器を分画し，ホウ素濃度を ICP-AES にて測定した．Figure 6 に示すように，脾臓・肝臓では非常に高いホウ素蓄積がみられ，腫瘍内ホウ素蓄積量は，投与 24 時間後にホウ素濃度で 23 ppm であった．

つぎに，Colon 26 細胞を左太腿部に移植した BALB/c マウスにホウ素リポソームをホウ素-10 濃度で 20 mg/kg 投与し，24 時間後にマウスの左太腿部に中性子照射した．BNCT では，標的部位へのホウ素デリバリーと中性子照射のダブルターゲティング治療法であるため，脾臓・肝臓のようにホウ素が高濃度で集積しても，中性子照射をしない限り細胞毒性を示さない．中性子照射は独立行政法人日本原子力研究開発機構東海研究開発センターの原子炉 JRR4 において行い，30 分間照射後（1.8×10^{12} neutrons/cm^2），経過を 2 週間観察した．本照射実験に用いたマウス照射装置を Fig. 7 に示す．本装置では原子炉運転中にセットしたマウス 16 匹を遠隔で移動させることができ，合計 32 匹のマウスを 1 度に照射することができる．その結果，Fig. 8 に示すように，中性子照射のみのマウス及び中性子照射していないマウス群では，腫瘍が成長したのに対し，ホウ素リポソームを投与し中性子照射したマウス群では，照射後 1 週間で腫瘍の萎縮がみられ，2 週間後も腫瘍の成長を顕著に抑制した.[26] 実際に，腫瘍が完全に消失したマウスも数匹みられた．このことから，ホウ素脂質を用いたリポソームによる BDS は，BNCT に対して非常に有効な手法となる可能性が示唆された．

6. 今後の展望

BNCT のためのホウ素キャリアーの開発には，いわゆるナノモルレベルで薬理効果が要求される抗がん剤のようなドラッグデザインではなく，ミリモルレベルで投与できるのに十分な低毒性であり，なおかつ腫瘍細胞に集積することが必要とされる．BNCT において 1950 年代に開発された BSH，BPA という 2 剤以外には，まだ臨床応用されたホウ素薬剤は残念ながら登場してない．現在小型加速器 BNCT の研究が進められ，保険適応型放射線治療法を目指して臨床研究が進められている．この QOL の高い本治療法の適応疾患拡大のためにも，次世代 BNCT に向けた腫瘍部位へのデリバリー効

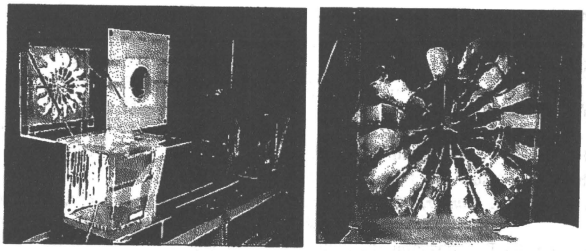

Fig. 7. Neutron Irradiation Apparatus at Japan Atomic Energy Agency Reactor (JRR4)

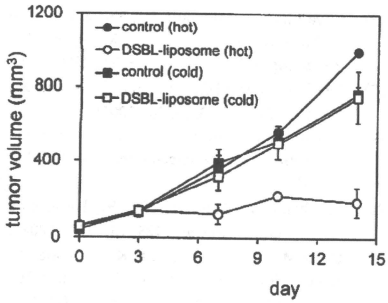

Fig. 8. Tumor Volume in Mice

Mice (Balb/c, female, 6 weeks old, 14–20 g) bearing colon 26 solid tumor with thermal neutron irradiation (hot) for 30 min (1.8×10^{12} neutrons /cm^2) and without irradiation (cold) were examined. The irradiation was performed at 24 h after i.v. injection of DSBL-25% PEG liposomes (20 mg B/kg).

率の高い BDS の開発が望まれる.

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REFERENCES

1) Barth R. F., Coderre J. A., Vicente M. G., Blue T. E., *Clin. Cancer Res.*, **11**, 3987–4002 (2005).
2) Locher G. L., *Am. J. Roentgenol.*, **36**, 1–13 (1936).
3) Soloway A. H., Hatanaka H., Davis M. A., *J. Med. Chem.*, **10**, 714–717 (1967).
4) Hatanaka H., *J. Neurology*, **209**, 81–94 (1975).
5) Snyder H. R., Reedy A. J., Lennarz W. J., *J. Am. Chem. Soc.*, **80**, 835–838 (1958).
6) Mishima Y., Ichihashi M., Hatta S., Honda C., Yamamura K., Nakagawa T., *Pigment Cell Res.*, **2**, 226–234 (1989).
7) Imahori Y., Ueda S., Ohmori Y., Kusuki T., Ono K., Fujii R., Ido T., *J. Nucl. Med.*, **39**, 325–333 (1998).
8) Kato I., Ono K., Sakurai Y., Ohmae M., Maruhashi A., Imahori Y., Kirihata M., Nakazawa M., Yura Y., *Appl. Radiat. Isot.*, **61**, 1069–1073 (2004).
9) Miyatake S., Kawabata S., Kajimoto Y., Aoki A., Yokoyama K., Yamada M., Kuroiwa T., Tsuji M., Imahori Y., Kirihata M., Sakurai Y., Masunaga S., Nagata K., Maruhashi A., Ono K., *J. Neurosurg.*, **103**, 1000–1009 (2005).
10) Suzuki M., Sakurai Y., Hagiwara S., Masuna-

ga S., Kinashi Y., Nagata K., Maruhashi A., Kudo M., Ono K., *Jpn. J. Clin.Oncol.*, **37**, 376–381 (2007).

11) Aihara T., Hiratsuka J., Morita N., Uno M., Sakurai Y., Maruhashi A., Ono K., Harada T., *Head Neck*, **28**, 850–855 (2006).

12) Yanagie H., Tomita T., Kobayashi H., Fujii Y., Takahashi T., Hasumi K., Nariuchi H., Sekiguchi M., *Br. J. Cancer*, **63**, 522–526 (1991).

13) Yanagie H., Tomita T., Kobayashi H., Fujii Y., Nonaka Y., Saegusa Y., Hasumi K., Eriguchi M., Kobayashi T., Ono K., *Br. J. Cancer*, **75**, 660–665 (1997).

14) Shelly K., Feakes D. A., Hawthorne M. F., Schmidt P. G., Krisch T. A., Bauer W. F., *Proc. Natl. Acad. Sci. USA*, **89**, 9039–9043 (1992).

15) Feakes D. A., Shelly K., Knobler C. B., Hawthorne M. F., *Proc. Natl. Acad. Sci. USA*, **91**, 3029–3033 (1994).

16) Pan X. Q., Wang H., Shukla S., Sekido M., Adams D. M., Tjarks W., Barth R. F., Lee R. J., *Bioconjug. Chem.*, **13**, 435–442 (2002).

17) Kullberg E. B., Carlsson J., Edwards K., Capala J., Sjoberg S., Gedda L., *Int. J. Oncol.*, **23**, 461–467 (2003).

18) Maruyama K., Ishida O., Kasaoka S., Takizawa T., Utoguchi N., Shinohara A., Chiba M., Kobayashi H., Eriguchi M., Yanagie H., *J. Control. Release*, **98**, 195–207 (2004).

19) Masunaga S., Kasaoka S., Maruyama K., Nigg D., Sakurai Y., Nagata K., Suzuki M., Kinashi Y., Maruhashi A., Ono K., *Int. J. Radiat. Oncol. Biol. Phys.*, **66**, 1515–1522 (2006).

20) Pan X., Wu G., Yang W., Barth R. F., Tjarks W., Lee R. J., *Bioconjug. Chem.*, **18**, 101–108 (2007).

21) Nakamura H., Miyajima Y., Takei T., Kasaoka S., Maruyama K., *Chem. Commun.*, 1910–1911 (2004).

22) Miyajima Y., Nakamura H., Kuwata Y., Lee J.-D., Masunaga S., Ono K., Maruyama K., *Bioconjug. Chem.*, **17**, 1314–1320 (2006).

23) Lee J.-D., Ueno M., Miyajima Y., Nakamura H., *Org. Lett.*, **9**, 323–326 (2007).

24) Nakamura H., Lee J.-D., Ueno M., Miyajima Y., Ban H., *Nanobiotechnol.*, **3**, 135–145 (2007).

25) Mudhakir D., Akita H., Tan E., Harashima H., *J. Control. Release*, **125**, 164–173 (2008).

26) Ueno M., Ban H. S., Nakai K., Inomata R., Kaneda Y., Matsumura A., Nakamura H., *Bioorg. Med. Chem.*, **18**, 3059–3065 (2010).

リポソームを用いる中性子捕捉治療

ダブルターゲティング：薬物と医療機器テクノロジーの融合

上野　学・潘　鉉承・中村浩之*

Liposomal neutron capture therapy

The cell-killing effect of boron neutron capture therapy (BNCT) is due to the nuclear reaction of two essentially nontoxic species, boron-10 (^{10}B) and thermal neutrons, whose destructive effect is well observed in boron-loaded tissues. Therefore, the high accumulation and selective delivery of ^{10}B into the tumor tissue are the most important requirements to achieve efficient neutron capture therapy for cancer. BNCT has been applied clinically for the treatment of malignant brain tumors, malignant melanoma, head and neck cancer, and hepatoma using two boron compounds : sodium borocaptate (^{10}BSH) and L-*p*-boronophenylalamine (L-^{10}BPA). These low molecule compounds are easily cleared from the cancer cells and blood, therefore, high accumulation and selective delivery of boron compounds into tumor tissues are most important to achieve effective BNCT and to avoid damage of adjacent healthy cells. Recently, much attention has been focused on liposomal drug delivery system as an attractive intelligent technology of targeting and controlled release of ^{10}B compounds.

In this review, recent development of liposomal boron delivery system is summarized.

低エネルギーの熱中性子はそれ自体では人体には無害であるが，ホウ素10と核反応を起こし生成するリチウムとヘリウム(α線)の有するエネルギーは，細胞を殺傷するのに充分なエネルギーである．したがって，あらかじめホウ素をがん細胞のみに送達することができれば副作用のきわめて少ない治療法となる．これを利用するのがホウ素中性子捕捉療法(BNCT：boron neutron capture therapy)である．そのためホウ素をいかに高濃度かつ選択的にがん細胞へ送達することが重要となる．BNCTは^{10}BSHとL-^{10}BPAの2剤を用いて脳腫瘍，悪性黒色腫，頭頸部腫瘍，肝臓がん治療などの治療に臨床用されてきた．しかし，治療効果の向上や適応疾患拡大のためには，より効率的なホウ素デリバリーシステムの開発が望まれている．そのような状況下，リポソームを用いるホウ素デリバリーシステムが注目されている．

本稿では，筆者らが進めてきたホウ素脂質リポソームによるホウ素デリバリーシステムを中心に紹介する．

*Manabu Ueno · Hyun Seung Ban · Hiroyuki Nakamura**

key words : boron neutron capture therapy (BNCT), boron delivery system (BDS), boronated liposome, boron lipids

次世代放射線療法としての中性子捕捉療法

近年，わが国の疾病構造は感染症などの急性疾患が減少した反面，がんや循環器病，糖尿病などの生活習慣病が増加しており，そのなかでも予期せぬがんの発症は多くの国民の潜在的健康不安として存在している．実際に，年間に30万人以上ががんで亡くなっており，最も解決の迫られている疾病の一つとなっている．がん治療は外科的手術，放射線療法，化学療法の複合的療法が主となっているが，近年サイバーナイフやガンマナイフ，重粒子線療法などの技術の進歩により放射線治療を受ける患者は年間18万人に上る．しかし放射線治療では照射範囲の正常組織損傷の問題が常に存在しており，化学療法では強い抗がん作用の反面，全身的な副作用との戦いを強いられている．そのなかで化学療法と放射線療法の両方の原理を上手く利用したホウ素中性子捕捉療法(BNCT：boron neutron capture therapy)が注目されている[1,2]．

BNCTの概念は，1936年にLocherによってはじめて提唱された[3]．比較的エネルギーの低い熱中性

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