一方，Hawthorne らも，最近同様な二本鎖ホウ素イオンクラスター脂質 **3** を開発しているが，彼らのホウ素リポソームは投与ホウ素濃度 6 mg/kg で 72 時間以内にマウスの急性毒性がみられたことを報告している．[29]

2-2-4. 低毒性二本鎖ホウ素イオンクラスター脂質の開発　このように，二本鎖ホウ素イオンクラスター脂質は安定なホウ素リポソームを形成し，腫瘍へも効率よく集積することが分かった．残る問題は毒性である．われわれは，このホウ素リポソームの毒性は，二本鎖ホウ素イオンクラスター脂質の水溶性部位である *nido* 型カルボランによるものではないかと考ええた．そこで，より低毒性で非常に代謝が早く実際の臨床で用いられている BSH に注目し，Fig. 19 のような硫黄置換型 undecahydrododecaborate を有する次世代ホウ素イオンクラスター脂質 **8** 及び **9** を設計した．[30] この脂質は，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(**8**)又はカルバメート基(**9**)を有し，undecahydrododecaborate 骨格と S を介して結合している．これらの二本鎖ホウ素イオンクラスター脂質を Fig. 20 に示すような合成スキームで合成に成功した．そのリポソーム安定性を調べたところ，Fig. 21 に示すように，ジパルミトイル型ホウ素脂質の場合，全脂質の 75% まで加えても血清中で安定なリポソームを形成することが分かった．また，正常マウスへの投与ホウ素濃度 20 mg/kg では急性毒性はみられなかった．現在，実用化に向けて研究を進めている．

また，最近 Gabel らは Fig. 22 に示すように窒素置換型 undecahydrododecaborate 骨格を有する二本鎖ホウ素イオンクラスター脂質の開発に成功しており，この脂質からリポソーム形成を Cryo-TEM に

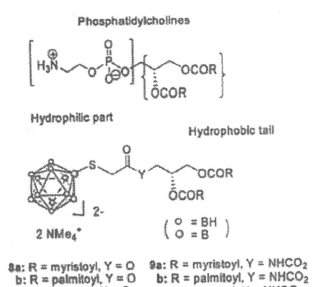

Fig. 19. Design of Advanced Boron Lipidsbased on Biomimetic Composition of Phosphatidylcolines

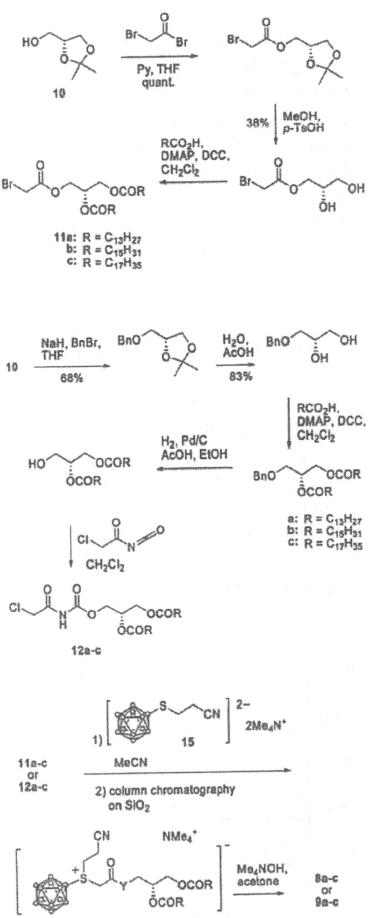

Fig. 20. Synthesis of the Boron Lipids **8** and **9**

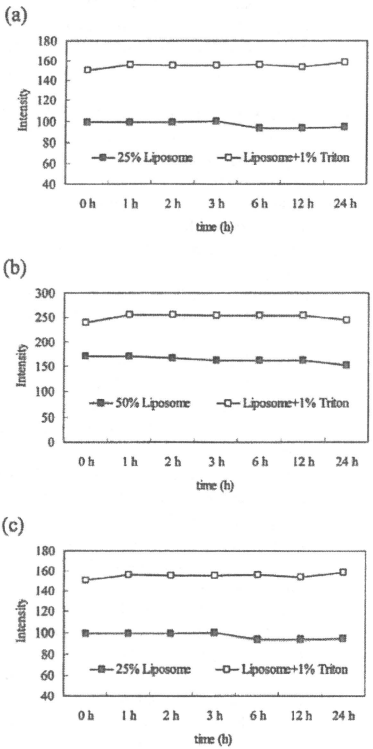

Fig. 21. Time-dependent Fluorescence Intensities of Calcein-encapsulated Liposomes Composed of **8b** with Various Ratios ((a) X=0.25, (b) =0.5, (c) =0.75) in FBS

Fluorescence intensity is plotted on the vertical axis and incubation time is plotted on the horizontal axis. The black plots show the fluorescence intensity of the FBS solution containing liposomes and the white plots show that of the solution after destruction of liposomes by the addition of Triton X-100.

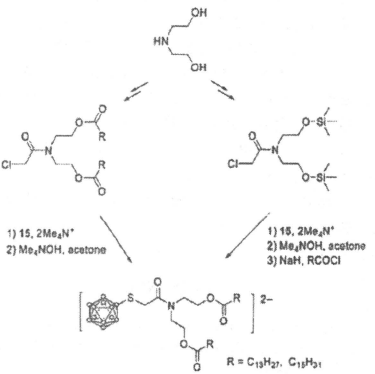

Fig. 22. Synthesis of Boron Lipids

よって確認している．彼らのホウ素リポソームも同様，細胞レベルで毒性は 5.6 mM と低いことが報告されている．[31]

3. 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．そのために，ここ十数年で低毒性小分子ホウ素化合物の開発だけでなく，リポソームを用いた BDS の開発が盛んに研究されてきた．本稿では，ホウ素のリポソーム内封型 BDS とホウ素脂質を用いたリポソーム膜導入型 BDS について紹介した．また，本稿では紹介できなかったが，リポソーム膜の安定性には，リン脂質やホウ素脂質の特性だけでなくコレステロール含有率が重要であることに着目し，このコレステロールにホウ素を導入することで，リポソーム膜に集積させようというアプローチについても研究されている．[32-34] 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．核燃料の問題から現在 BNCT に適応できる小型加速器の開発が精力的に行われている．熱中性子源が原子炉から加速器に移行できれば都市部病院併設型加速器による BNCT が可能となり，将来放射線療法の一般的治療法の 1 つになるであろう．そのためにも治療効果の高い BDS の開発が期待される．

謝辞 二本鎖ホウ素イオンクラスター脂質の開発に関する研究に当たり，日夜研究に励んでくれた学習院大学大学院自然科学研究科化学専攻の宮島祐

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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) Soloway A. H., Tjarks W., Barnum B. A., Rong F-G., Barth R. F., Codogni I. M., Wilson J. G., *Chem. Rev.*, **98**, 1515–1562 (1998).
3) Locher G. L., *Am. J. Roentgenol.*, **36**, 1–13 (1936).
4) Farr L. E., Sweet W. H., Robertson J. S., Foster C. G., Locksley H. B., Sutherland D. L., Mendelsohn M. L., Stickley E. E., *Am. J. Roentgenol. Radium Ther. Nucl. Med.*, **71**, 279–293 (1954).
5) Hatanaka H., Nakagawa Y., *Int. J. Radiat. Oncol. Biol. Phys.*, **28**, 1061–1066 (1994).
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., Tamura Y., Kawabata S., Iida K., Kuroiwa T., Ono K., *Meurosurgery*, **61**, 90–91 (2007).
10) Suzuki M., Sakurai Y., Hagiwara S., Masunaga S., Kinashi Y., Nagata K., Maruhashi A., Kudo M., Ono K., *Jpn. J. Clin. Oncol.*, **37**, 376–381 (2007).
11) Mumtaz S., Ghosh P. C., Bachhawat B. K., *Glycobiology*, **1**, 505–510 (1991).
12) Vaage J., Mayhew E., Lasic D., Martin F., *Int. J. Cancer*, **51**, 942–948 (1992).
13) Yanagie H., Tomita T., Kobayashi H., Fujii Y., Takahashi T., Hasumi K., Nariuchi H., Sekiguchi M., *Br. J. Cancer*, **63**, 522–526 (1991).
14) 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).
15) Shelly K., Feakes D. A., Hawthorne M. F., Schmidt P. G., Krisch T. A., Bauer W. F., *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 9039–9043 (1992).
16) Feakes D. A., Shelly K., Knobler C. B., Hawthorne M. F., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 3029–3033 (1994).
17) Pan X. Q., Wang H., Shukla S., Sekido M., Adams D. M., Tjarks W., Barth R. F., Lee R. J., *Bioconjugate Chem.*, **13**, 435–442 (2002).
18) Antony A. C., *Annu. Rev. Nutr.*, **16**, 501–521 (1996).
19) Allen T. M., Brandeis E., Hansen C. B., Kao G. Y., Zalipsky S., *Biochim. Biophys. Acta*, **1237**, 99–108 (1995).
20) Kullberg E. B., Carlsson J., Edwards K., Capala J., Sjöberg S., Gedda L., *Int. J. Oncol.*, **23**, 461–467 (2003).
21) Ishida O., Maruyama K., Tanahashi H., Iwatsuru M., Sasaki K., Eriguchi M., Yanagie H., *Pharm. Res.*, **18**, 177–180 (1997).
22) 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).
23) 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**, 1523–1527 (2006).
24) Pan X., Wu G., Yang W., Barth R. F., Tjarks W., Lee R. J., *Bioconjug. Chem.*, **18**, 101–108 (2007).
25) Feakes D. A., Shelly K., Hawthorne M. F., *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 1367–1370 (1995).
26) Nakamura H., Miyajima Y., Takei T., Kasao-

ka T., Maruyama K., *Chem. Commun.*, 1910–1911 (2004).
27) Miyajima Y., Nakamura H., Kuwata Y., Lee J.-D., Masunaga S., Ono K., Maruyama K., *Bioconjug. Chem.*, **17**, 1314–1320 (2006).
28) http://nano.cancer.gov/news_center/nanotech_news_2006-09-05a.asp
29) Li T., Hamdi J., Hawthorne M. F., *Bioconjug. Chem.*, **17**, 15–20 (2006).
30) Lee J.-D., Ueno M., Miyajima Y., Nakamura H., *Org. Lett.*, **9**, 323–326 (2007).
31) Hustus E., Awad D., Hohnholt M., Schffran T., Edwards K., Karlsson G., Damian L., Gabel D., *Bioconjug. Chem.*, **18**, 1287–1293 (2007).
32) Feakes D. A., Spinler J. K., Harris F. R., *Tetrahedron*, **55**, 11177–11186 (1999).
33) Thirumamagal B. T. S., 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., *Bioconjug. Chem.*, **17**, 1141–1150 (2006).
34) Nakamura H., Ueno M., Lee J.-D., Ban H. S., Justus E., Fan P., Gabek D., *Tetrahedron Lett.*, **48**, 3151–3154 (2007).

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Synthesis and characterization of polar functional group substituted mono- and bis-(*o*-carboranyl)-1,3,5-triazine derivatives

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Abstract—Synthesis, structural characterization, and biological activity studies of *o*-carborane-substituted 1,3,5-triazines (**9**–**12**) containing polar functional groups such as methoxyethyl and *t*-butoxycarbonylmethyl amine units are described. De-methylation of di(methoxyethyl)amine functionalized triazines **9** and **10** resulted in the production of di(hydroxyethyl)amine derivatives **13** and **14**. NMR (^{1}H and ^{13}C) and X-ray crystallographic studies confirmed the structures derived from the sequential *o*-carborane substitution on the 1,3,5-triazine core. Preliminary in vitro studies revealed that compounds **9**, **10**, **13**, and **14**, despite their low cytotoxicity, accumulated at high levels in B-16 melanoma cells.

1,3,5-Triazines are a class of nitrogen-containing heterocyclic compounds with remarkable chemical stability.[1] The stability of these compounds along with their anti-tumor activities has led to their utilization in several specialized biomedical applications.[2] As a surrogate for 1,3,5-triazine, 2,4,6-tris(*N*-methyl-*N*-hydroxymethyl-amino)-1,3,5-triazine (known as trimelamol) was proposed as a potent anti-tumor agent.[3] The 1,3,5-triazine ring has three distinct nucleophilic centers,[4] making it possible to attach various functional groups to the ring by simple nucleophilic substitution reactions at each of the cyanyl chloride (–N=C–Cl) units.[5] It has been demonstrated that *o*-carboranyl anions can function as nucleophiles[6] to facilitate substitution on the carbon atoms of 1,3,5-triazine. Given this behavior, and our previous success[7] in sequentially incorporating *o*-carboranyl units to 1,3,5-triazine, in the present work we sought to utilize the triazine core as a template for the production of potential boron neutron capture therapy (BNCT) agents. For a compound to have potential as

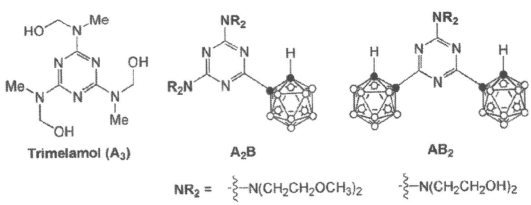

Figure 1. A_2B and AB_2 triazine systems.

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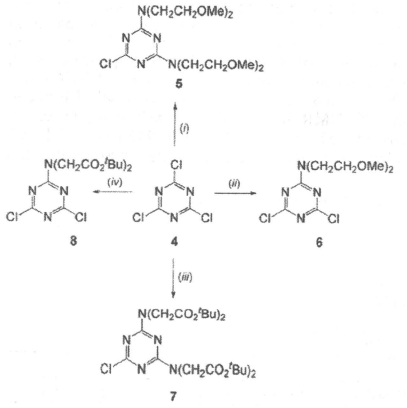
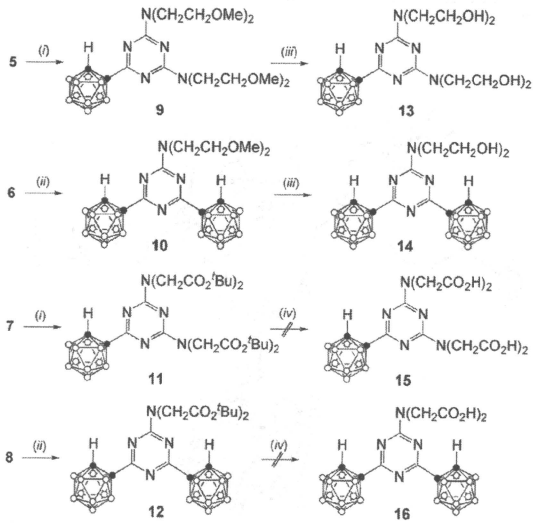


Scheme 1. Reagents and conditions: (i) $HN(CH_2CH_2OCH_3)$ (2 equiv), $(i\text{-Pr})_2EtN$ (2 equiv), THF, rt; (ii) $HN(CH_2CH_2OCH_3)$, $(i\text{-Pr})_2EtN$, THF, −10 °C; (iii) $HN[CH_2CO_2C(CH_3)_2]$ (2 equiv), $(i\text{-Pr})_2EtN$ (2 equiv), THF, rt; (iv) $HN[CH_2CO_2C(CH_3)_2]$, $(i\text{-Pr})_2EtN$, THF, −10 °C.

a BNCT agent, it should be water-soluble, have low cytotoxicity, and take up boron in cancer cells.[8] Due to the lipophilic character of the *o*-carboranyl unit,[9] the introduction of a second functional group into the *o*-carboranyl triazine that endows the molecule with water solubility is highly desirable. The fact that trimelamol, which contains three hydroxyl methyl moieties, is a water-soluble bioactive agent[3] suggests that introducing one or more hydroxyalkyl units to the *o*-carboranyl triazine may enhance its solubility in aqueous solution. As shown in Figure 1, conversion of the second functional group of *o*-carboranyl triazine to a hydroxyethyl group yielded a molecule 10–100 times more soluble in water than previously reported A_2B-type molecules without a polar functional group (7.24×10^{-6} (mol/mL) (av.)),[10] where A and B represent the aminoalkyl- and *o*-carboranyl substituents of the triazine, respectively.

To incorporate polar groups into the triazine system, we first attempted to prepare hydroxyethyl- and hydroxycarbonylmethyl amine surrogates. Thus, a series of mono- and bis-substituted precursors (**5–8**) containing di(methoxyethyl)- and di(*t*-butoxycarbonylmethyl)-amine functional groups was prepared by the reaction of compound **4** with di(methoxyethyl)- and di(*t*-butoxycarbonylmethyl)amine, respectively, in 1:1 and 1:2 stoichiometry (Scheme 1).[11]

When lithiated *o*-carborane was reacted with precursors **5–8** in 1:1 or 1:2 stoichiometry, the corresponding mono- and di-substituted *o*-carboranyl triazines (**9–12**) were formed in 12–80% yield (Scheme 2).[12] Finally, the desired free alcohol species **13** and **14** were prepared in

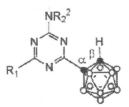


Scheme 2. Reagents and conditions: (i) Lithio-*o*-carborane (1 equiv), THF, −78 °C to rt; (ii) lithio-*o*-carborane (2 equiv), THF, −78 °C to rt; (iii) BBr_3, CH_2Cl_2; (iv) CF_3CO_2H.

57–71% yield by reacting **9** and **10** with BBr_3, respectively.[13] On the other hand, the free acid forms of **15** and **16** were not obtained when we attempted the de-alkylation of **11** and **12** under trifluoroacetic acid conditions; rather, it appeared that **11** and **12** were easily decomposed under acidic conditions.[13]

Selected physical and spectroscopic properties of *o*-carboranyl-1,3,5-triazine derivatives **9–14** are listed in Table 1. The presence of the *o*-carboranyl ring was confirmed by the characteristic absorption bands at around 2563–2606 cm^{-1} assignable to B–H bonds in the infrared spectra. In the ^1H NMR spectra of **9–14**, signals diagnostic for methylene protons of NCH_2 were observed at around δ 3.56–4.25. Key signals detected in the ^{13}C NMR spectra of **9–14** include resonances at around δ 57.2–61.5 (*C*-β), 59.0–69.9 (NCH_2), 72.4–75.7 (*C*-α), and 163.4–175.6 (triazine ring). Sequential

Table 1. Summary of selected physical and spectral properties of the *o*-carboranyl-1,3,5-triazine derivatives **9–14**



R_1 = Carboranyl, $N(CH_2CH_2OCH_3)$, $N(CH_2CH_2OH)_2$
R_2 = $N(CH_2CH_2OCH_3)$, $N(CH_2CH_2OH)_2$

No.	Compound	Mp[a] (°C)	Yield[b] (%)	IR (B–H)	NMR ($^1H/^{13}C$)				
					$C(NCH_2)$	$C(OCH_2)$	C(triazine)	C(α)	C(β)
1	**9**	97–98	18	2584	3.57 (m) 49.0	3.84 (m) 69.9	163.4, 167.5	72.7	4.5 (s) 59.0
2	**10**	120–122	80	2563	3.56 (t) 49.0	3.83 (t) 69.9	163.5, 167.5	72.8	4.42 (s) 59.0
3	**11**	104–106	12	2606	4.20 (d) 50.5 (d)		164.9, 166.5	73.9	4.43 (s) 56.2
4	**12**	102–104	72	2582	4.24 (s) 51.2		164.4, 167.2	72.4	4.36 (s) 56.2
5	**13**	106–107	54	2600	3.81 (t) 51.6 (d)	3.87 (t) 59.0	164.0, 167.3	73.5	5.28 (s) 57.6
6	**14**	108–110	71	2600	3.81 (t) 51.6 (d)	3.87 (t) 59.0	164.0, 167.3	73.5	5.28 (s) 57.6

[a] Melting points are uncorrected.
[b] Purified yields.

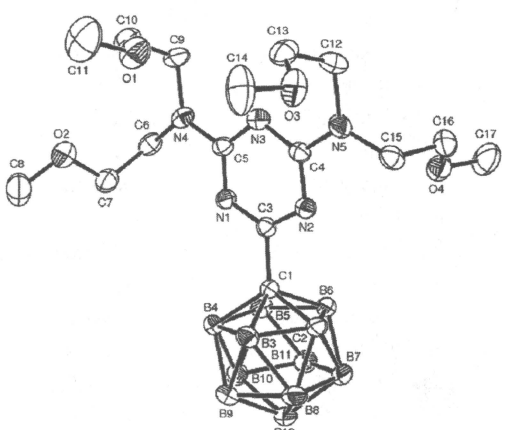


Figure 2. Molecular structure of compound **9**. The thermal ellipsoids are drawn at the 30% probability level.

mono- and bis-substitutions of *o*-carboranyl cages to the triazinyl center were further authenticated through X-ray structural studies of **9** and **10**, respectively (Figs. 2 and 3). The crystal structures determined on the basis of X-ray diffraction data corresponded well with the conformations derived from the NMR spectra.

Taking into consideration the three essential requirements for BNCT precursors—good water solubility, low cytotoxicity, and high boron uptake—**9**, **10**, **13**, and **14** appear to be good candidate molecules (see Table 2).[8] It has been noted that trimelamol shows high cytotoxicity in addition to its high anti-tumor activity. However, four structurally related compounds exhibit low cytotoxicity,[14] with IC_{50} values (the half maximal inhibitory concentration) in the range of 4.49×10^{-5}–6.54×10^{-5} M. Among the series, A_2B systems were more soluble in water than AB_2 systems. Furthermore, as the amino functional group was converted to a more polar substituent with a hydroxyethyl group, the water solubility increased to 5.18×10^{-4}(mol/mL) for **13**, which is about two orders of magnitude higher than the solubilities we observed for A_2B-type molecules with alkylamino functional groups in our previous work.[10] All four compounds prepared in the present work (**9**, **10**, **13**, and **14**) were found to accumulate markedly in B-16 melanoma cells when compared to BPA (*p*-boronophenylalanine). We found no direct correlation between water solubility and boron uptake for this series. We attribute the small variation in boron uptake among the four compounds to their similar lipophilic characters, even when a polar functional group was introduced in the A_2B system such as in **13**.

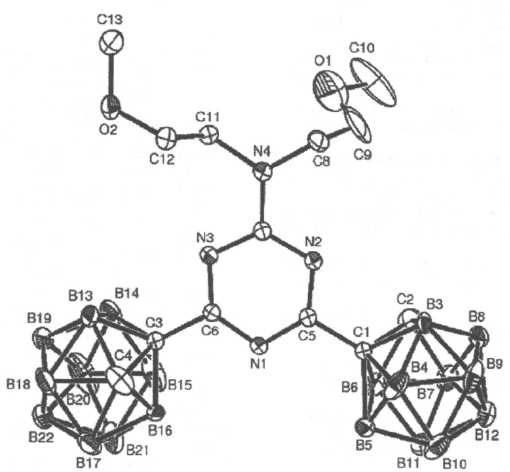


Figure 3. Molecular structure of compound **10**. The thermal ellipsoids are drawn at the 30% probability level.

Table 2. Cytotoxicity (IC_{50}) of the test compounds toward B-16 cells and boron uptake

Compound		B-16[a] (IC_{50}) M	Boron uptake[b] (μg B/10^6 cells)		Water solubility (mol/mL)
1	**9**	4.49×10^{-5} (± 0.30)	2.55	± 0.84	3.27×10^{-6}
2	**10**	6.54×10^{-5} (± 0.07)	2.01	± 0.37	1.51×10^{-6}
3	**13**	4.71×10^{-5} (± 0.33)	1.81	± 0.81	5.18×10^{-4}
4	**14**	4.75×10^{-5} (± 0.11)	2.16	± 2.56	1.03×10^{-5}
	BPA	4.49×10^{-5} (± 0.30)	0.083	± 0.012	

[a] B-16: B-16 melanoma cells.

[b] Boron uptake by B-16 cells was determined using the ICP-AES method (see Ref. 15). Briefly, cells were cultured in Falcon dishes (90 mm∅) until they grew to fill the dishes (~3.0×10^6 cells/dish). Cells were then incubated for 3 h with Eagle–MEM medium containing one of the test compounds (boron concentration: 10.8 ppm). After 3 h, the cells were washed three times with PBS(—) and processed for the determination of the boron concentration by ICP-AES. Each experiment was carried out in triplicate.

Electronic supplementary information (ESI) available: experimental details and spectral data for **9**, **10**, **11**, **12**, **13** and **14**, X-ray crystallographic data for **9** and **10** (CCDC No. 655714 and 655715).

Acknowledgments

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References and notes

1. Chen, J.; Cai, D.; Jin, W.; Wu, F.; Chen, X. *J. Appl. Polym. Sci.* **2006**, *102*, 1291.
2. (a) Sączewski, F.; Bułakowska, A. *Eur. J. Med. Chem.* **2006**, *41*, 611; (b) Januszko, A.; Kaszynski, P.; Drzewinski, W. *J. Mater. Chem.* **2006**, *16*, 452; (c) Brzozowski, Z.; Sączewski, F. *Eur. J. Med. Chem.* **2002**, *37*, 709; (d) Azev, Y.; Slepukhina, I.; Gabel, D. *Appl. Radiat. Isot.* **2004**, *61*, 1107.
3. Coley, H. M.; Brooks, N.; Phillips, D. H.; Hewer, A.; Jenkins, T. C.; Jarman, M.; Judson, I. R. *Biochem. Pharm.* **1995**, *49*, 1203.
4. (a) Gulevskaya, A. V.; Maes, B. U. W.; Meyers, C. *Synlett* **2007**, 71; (b) Maheswari, P. U.; Modec, B.; Pevec, A.; Kozlevčar, B.; Massera, C.; Gamez, P.; Reedijk, J. *Inorg. Chem.* **2006**, *45*, 6637; (c) Shastin, A. V.; Godovikova, T. I.; Korsunskii, B. L. *Chem. Heterocycl. Compd.* **2003**, *39*, 624.
5. (a) Mooibroek, T. J.; Gamez, P. *Inorg. Chim. Acta* **2007**, *360*, 381; (b) Meier, H.; Karpuk, E.; Holst, H. C. *Eur. J. Org. Chem.* **2006**, 2609.
6. Valliant, J. F.; Guether, K. J.; King, A. S.; Morel, P.; Schaffer, P.; Sogbein, O. O.; Stephenson, K. A. *Coord. Chem. Rev.* **2002**, *232*, 173.
7. Lee, C.-H.; Lim, H.-G.; Lee, J.-D.; Lee, Y.-J.; Ko, J.; Nakamura, H.; Kang, S. O. *Appl. Organomet. Chem.* **2003**, *17*, 539.
8. (a) Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950; (b) Soloway, A. H.; Tjarks, W.; Barnum, A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
9. (a) Reynolds, R. C.; Campbell, S. R.; Fairchild, R. G.; Kisliuk, R. L.; Micca, P. L.; Queener, S. F.; Riordan, J. M.; Sedwick, W. D.; Waud, W. R.; Leung, A. K. W.; Dixon, R. W.; Suling, W. J.; Borhani, D. W. *J. Med. Chem.* **2007**, *50*, 3283; (b) Ogawa, T.; Ohta, K.; Yoshimi, T.; Yamazaki, H.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3943; (c) Goto, T.; Ohta, K.; Suzuki, T.; Ohta, S.; Endo, Y. *Bioorg. Med. Chem.* **2005**, *13*, 6414.
10. Unpublished results.
11. *6-Chloro-2,4-bis[di(2-methoxyethyl)amino]-1,3,5-triazine (5)*: To a stirred solution of cyanuric chloride **4** (1.84 g, 10 mmol) and *N,N*-diisopropylethylamine (2.58 g, 20 mmol) in 30 mL of THF at −10 °C was added di(2-methoxyethyl)amine (2.66 g, 20 mmol) via a syringe. The reaction temperature was maintained at −10 °C for 1 h, after which the reaction mixture was warmed slowly to room temperature. The mixture was then stirred for an additional 12 h, after which it was quenched with distilled H_2O (50 mL). The crude product was then extracted with diethyl ether (30 mL × 2). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated in vacuo to give 3.62 g (96%) of **5**. Mp 94–95 °C. ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 6H), 3.58 (t, J = 5.5 Hz, 4H), 3.86 (t, J = 5.5 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 48.6, 59.0, 70.1, 164.8, 169.9. *Compound* **6**: Yield. 91%. Mp 54–56 °C. ^{1}H NMR ($CDCl_3$) δ 3.32 (s, 12H), 3.57 (t, J = 5.5 Hz, 8H), 3.85 (t, J = 5.5 Hz, 8H). ^{13}C NMR ($CDCl_3$) δ 48.6, 58.9, 70.1, 164.8, 169.9. *Compound* **7**: Yield. 96%. Mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 36H), 4.15 (d, J = 45.8 Hz, 8H). ^{13}C NMR ($CDCl_3$) δ 28.11–28.15, 50.0–50.2, 81.9–82.0, 165.4, 168.3–168.5, 169.3. *Compound* **8**: Yield. 94%. Mp 65–66 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H), 4.23 (d, J = 47.2 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 28.1, 50.0–50.2, 81.9, 165.4, 168.3–168.5, 169.3.
12. *6-(o-Carboran-1-yl)-2,4-bis[di(2-methoxyethyl)amino]-1,3,5-triazine (9)*: To a stirred solution of *o*-carborane (1.44 g, 10 mmol) in 30 mL of THF at −78 °C was added 2.5 M *n*-BuLi (4.0 mL, 10 mmol) via a syringe. A solution of compound **5** (3.78 g, 10 mmol) in THF was slowly added to the reaction flask at −78 °C, and the reaction temperature was maintained at −78 °C for 1 h. The reaction mixture was then warmed slowly to room temperature, stirred for an additional 12 h, and quenched with distilled H_2O (30 mL). The crude product was then extracted with diethyl ether (30 mL × 2). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated in vacuo. Product **9** was isolated by flash column chromatography (ethylacetate/hexane 1:8) in 18% yield (0.86 g). Mp 97–98 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2584. ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 6H), 3.34 (s, 6H), 3.57 (m, J = 5.1 Hz, 8H), 3.84 (m, J = 5.1 Hz, 8H), 4.50 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 49.0, 56.1, 59.0, 69.9, 72.7, 163.4, 167.5. Compound **10**: Yield. 80%. Mp 120–122 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2563. ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 6H), 3.56 (t, J = 5.5 Hz, 4H), 3.83 (t, J = 5.5 Hz, 4H), 4.41 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 49.0, 56.1, 59.0, 69.9, 72.7, 163.4, 167.5. Compound **11**: Yield. 12%. Mp 104–106 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2606, ν (C–H) 1747. ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 18H), 1.45 (s, 18H), 4.15 (d, J = 45.8 Hz, 8H), 4.43 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.1, 50.2–50.5, 56.2, 73.9, 82.1, 164.9, 166.5, 168.4–168.5. *Compound* **12**: Yield. 72%. Mp 102–104 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2582, ν (C–H) 1743. ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 18H), 4.24 (s, 4H), 4.36 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.1, 51.2, 56.2, 72.4, 83.3, 164.4, 167.2, 167.9.
13. *6-(o-Carboran-1-yl)-2,4-bis[di(2-hydroxyethyl)amino]-1,3,5-triazine (13)*: To a stirred solution of compound **9** (2.43 g, 5 mmol) in 20 mL of THF at −10 °C was added BBr_3 (5.01 g, 20 mmol) via a syringe. The reaction temperature was maintained at −10 °C for 30 min, after which the reaction mixture was warmed slowly to room temperature. After stirring for an additional 2 h, the reaction was quenched with distilled H_2O (50 mL). The crude product was extracted with diethyl ether (50 mL × 2). The organic layer was washed with H_2O and then dried in vacuo. Product **13** was isolated by flash column chromatography (ethylacetate/hexane 1:2) in 54% yield (1.16 g). Mp 106–107 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2600. ^{1}H NMR (acetone-d_6) δ 3.81 (t, J = 5.1 Hz, 8H), 3.87 (t, J = 5.7 Hz, 8H), 5.28 (s, 1H). ^{13}C NMR (acetone-d_6) δ 51.2–51.6, 57.6, 59.0, 73.5, 164.0, 167.3. *Compound* **14**: Yield. 71%. Mp 108–109 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2600. ^{1}H NMR (acetone-d_6) δ 3.81 (t, J = 5.05 Hz, 4H), 3.87 (t, J = 5.7 Hz, 4H), 5.28 (s, 1H). ^{13}C NMR (acetone-d_6) δ 51.2–51.6, 57.6, 59.0, 73.5, 164.0, 167.3.

Compounds **15** and **16**: A trifluoroacetic acid (8 mL) solution containing compound **11** (1.41 g, 2 mmol) or **12** (1.22 g, 2 mmol) was stirred for 24 h at room temperature and then dried in vacuo. However, we did not obtain the de-alkylated compounds.

14. Asayama, S.; Mizushima, K.; Nagaoka, S.; Kawakami, H. *Bioconjugate Chem.* **2004**, *15*, 1360.
15. Tietze, L. F.; Bothe, U.; Griesbach, U.; Nakaichi, M.; Hasegawa, T.; Nakamura, H.; Yamamoto, Y. *ChemBioChem* **2001**, *2*, 326–334.

Synthesis and evaluation of a novel liposome containing BPA-peptide conjugate for BNCT

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Abstract

We aimed at securing sufficient concentrations of ^{10}B in BNCT by developing a new drug delivery system. We have designed and developed a novel lipid analog and succeeded in using it to develop the new boron component liposome. It consisted of three different kinds of amino acid derivatives and two fatty acids, and could react directly with the peptide synthesized first on resin by Fmoc solid-phase synthesis. In this study, lipid analog conjugated with HIV-TAT peptide (domain of human immunodeficiency virus TAT protein) and boronophenylalanine (BPA) was synthesized and successfully incorporated into liposomes.

Keywords: boron neutron capture therapy (BNCT), boron delivery system (BDS), liposome, boronophenylalanine (BPA) . HIV-TAT

1. Introduction

Boron neutron capture therapy (BNCT) is a tumor-selective radiation modality which depends on a sufficient cellular uptake of Boron (^{10}B) followed by irradiation with a beam of thermal or epithermal neutrons. ^{4}He and ^{7}Li particles are produced during the neutron capture reaction and damage DNA, which leads to cell killing. Regarding BNCT, the short radiation range of ^{4}He and ^{7}Li particles is decisive for the distribution of ^{10}B. Thus, successful treatment of cancer by BNCT requires the selective delivery of relatively large amounts of ^{10}B compound to malignant cells. The estimated boron concentration required for effective therapy is in the range of 20–30 μg ^{10}B per g tissue. However there have been no ideal boron compounds that fulfill the conditions of low toxicity, water solubility, and low distribution in normal tissue. Therefore, we aimed at securing sufficient concentrations of ^{10}B in BNCT by developing a new drug delivery system.

2. Materials and methods

2.1. Synthesis of lipopeptide

Lipopeptide conjugated with HIV-TAT peptide and boronophenylalanine (BPA) was synthesized on TGS-RAM resin by the Fmoc solid-phase synthesis method using an automatic peptide synthesizer (Shimadzu PSSM-8 Peptide Synthesizer Simultaneous Multiple) (**Figure 1**).

Tryptophan residue was added at the N-terminus of HIV-TAT peptide as a fluorescence probe. BPA was coupled arbitrarily. Then, Fmoc-AEEA (9-fluorenylmethoxycarbonyl-8-amino-3,6-dioxaoctanoic acid, linker domain), 11 Fmoc-Asp-OtBu (hydrophilic domain), and Fmoc-Dap(Fmoc)-OH (glycero mimic domain) were coupled sequentially. Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), N-hydroxybenzotriazole (HOBt), and N-methyl morpholine (NMM) were used, respectively, for the peptide coupling reaction with 1.0, 1.0, and 1.5 equivalents based on amino acids. Fmoc amino acid and alkyl chain were used for resin in an equivalent of the excess of 7 and 6, respectively. Each coupling reaction was carried out for 30 min. The last condensation reaction with palmitic acid was carried out in a manual mode with the reaction progress checked by a ninhydrin test. De-protection and cleavage of resin were accomplished with a cleavage cocktail (10 mg/mL of 2-methylindole containing trifluoroaceticacid /H_2O/thioanisole/1,2-ethanedithiol/ethylmethyl sulfide/phenol = 82/5/5/3/2/3) for 16 hours at room temperature, then precipitated by adding a large amount of diethyl ether. After the drying procedure, we got a purpose thing.

2.2. Preparation of liposome

The lipid mixture prepared using the constant ratio was dissolved in organic solvent. It was prepared by the

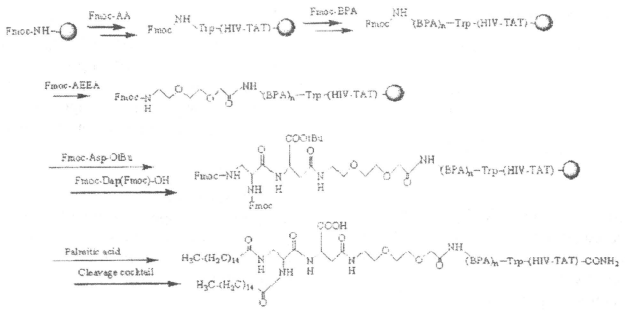

Figure 1. Synthesis of the HIV-TAT and BPA peptide conjugated lipid analog

conventional lipid-film method. The resulting liposomes were extruded through polycarbonate membrane using an extruder, yielding the peptide-modified liposome.

2.3. Gel Filtration Chromatography

The prepared liposome was subjected to size exclusion chromatography, which separated the liposome, micelle and monomolecule fractions, and the content of the lipopeptide was determined (**Figure 2**). The liposome was then measured using the fluorescence of the tryptophan residue of the lipopeptide.

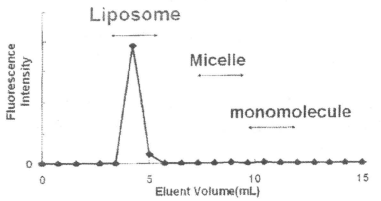

Figure 2. Gel Filtration Chromatography of liposome, micelle and monomolecule

3. Results

3.1. Identification of lipopeptide

In the case of n=5 (B5-TAT), the C8-column used for high performance liquid chromatography (HPLC) analysis showed the main peak (retention time at 13.6 min) accompanied by the existence of some impurities (**Figure 3a**). However, the synthesis of conjugated lipopeptide attached to palmitoyl chain as an anchor domain proceeded very smoothly. HPLC analysis showed almost one peak, and ESI-TOFMS (electrospray ionization mass spectroscopy) showed *m/z* 3504 of the dehydration peak as an exact mass of *m/z* 3522. In addition, ^{1}H NMR analysis (JEOL JMN-AL400) also showed the structure of the lipopeptide; for example, the molar ratio of the TAT-peptide and palmitoyl moiety showed the correct proton ratio of the ortho position on the tyrosine residue (2H as a characteristic signal of the peptide at 6.62 ppm) to the methyl signal at 0.81 ppm in the alkyl chain end (6H as a characteristic signal of the lipid) using an integration value of ^{1}H NMR spectra (**Figure 3b**). The overall yield of the lipopeptide was greater than 70% based on the molar ratio of the amino group on TGS-RAM resin.

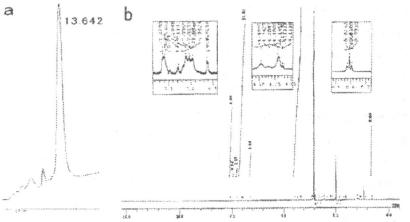

Figure 3. Identification of HIV-TAT peptide and BPA conjugated lipid analog B5-TAT. (a) C8-HPLC, (b) ^{1}H NMR

3.2. Incorporated ratio of lipopeptide

The incorporation of lipopeptide into the liposome was examined. The methods are showed in Figure 2. Synthesized lipopeptide was incorporated into the liposome effectively. The incorporated ratios of lipopeptide to liposome are summarized in Table 1.

Table 1 Liposome formation and lipopeptide incorporated into liposome

Lipopeptide	Theoretical lipopeptide ratio (mol %)	incorporated ratio (%)
BS-TAT	5	66.1
	10	73.4

4. Discussion

We synthesized a new peptide lipid containing multiple BPA components and a TAT domain for use in a boron-containing liposome which can encapsulate a boron compound in its internal water phase. The peptide lipid can be efficiently incorporated into liposomes that are 100 nm in diameter.

HIV-TAT was first developed from reverse transcriptase of HIV. It is a kind of protein transduction domain[3] which can introduce intracellular protein, deoxyribonucleic acid and macromolecular-containing liposome. *Yagi et al.* reported an *in vitro* anti-tumor effect of DOX encapsulated by TAT-modified liposome in 2007.[1] The TAT-conjugating liposome facilitated an *in vitro* gene expression as well as in vivo expression when the same liposome was locally injected[4].

Active targeting against tumor cells using TAT have been evaluated; however, there is no previous report involving a boron-containing TAT liposome or compound.

A sufficient concentration of boron is necessary for successful BNCT. Thus, a material with high boron content generally has an advantage.[2] *Nakamura et al.* developed a double-stranded boron cluster in 2004.[5] In the present study, the peptide lipid synthesized contains only 1 to 5 boron in a single molecule. However, our peptide lipid allows the number of boron to be increased up to n=12 or n=15.

In general, the hydrophilic charge of BSH in a boron-containing liposome has certain difficulty in encapsulating more BSH in the internal water phase of the liposome itself. There has been no previous report involving encapsulated BSH in the internal water phase within a boron liposome. Our peptide modification liposome of the hydrophilic charge is aspartic acid, and it shows high performance in terms of film stability and has a potential advantage in encapsulating BSH in the liposome in which the lipopeptide conjugate BPA.

Further investigation is needed to determine the *in vitro* and *in vivo* toxicity and the boron introduction efficiency.

5. Conclusions

We succeeded in synthesizing a lipopeptide containing boron. This lipopeptide could be incorporated into the liposome effectively. After toxicity testing, these liposomes will be administered to the cells or in vivo as a new BDS candidate.

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References

1. Yagi, N.; Yano, Y.; Hatanaka, K.; Yokoyama, Y.; Okuno, H. *Biomed. let.* **2007,** *17. 2590-2593.*
2. Maruyama, K.; Ishida, O.; Kasaoka, S.; Takizawa, T.; Utoguchi, N.; Shinohara, A.; Chiba, M.; Kobayashi, H.; Eriguchi, . *J. Controlled. Release.* **2004,** *98,195-207.*
3. Green, M.; Loewenstein, P. M., *Cell* **1998,** *55, (6), 1179-1888.*
4. Torchilin, V. P.; Levchenko, T. S.; Rammohan, R.; Volodina, N.; Papahadjopoulos-Sternberg, B.; D'Souza, G. G. M., *Proc. Natl. Acad. Sci.U.S.A.* **2003,** *100, (4), 1972-1977.*
5. Lee J.-D. ; Ueno, M.; Miyajima, Y.; Nakamura, H. *Org. let.* **2007,** *9, 323-326.*
6. Wadia, J. S.; Stan, R.V.; Dowdy, S.F., *Nat. Med.* **2004,** *10, (3), 310-315*
7. Eric, L. S.; Steven, F. D., *Pharm. Res.* **2004,** *V21, (3), 389-393.*
8. Fretz, M. M.; Mastrobattista, E.; Koning, G. A.; Jiskoot, W.; Storm, G., *Int. J. Pharm.* **2005,** *298, (2), 305-309.*
9. Kageji, T.; Nagahiro, S.; Otersen, B.; Gabel, D.; Nakaichi, M.; Nakagawa, Y. *J. Neurooncol.* **2002,** *59, (2), 135-142.*

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Mini-review

Boron neutron capture therapy for glioblastoma

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Abstract

Boron neutron capture therapy (BNCT) theoretically allows the preferential destruction of tumor cells while sparing the normal tissue, even if the cells have microscopically spread to the surrounding normal brain. The tumor cell-selective irradiation used in this method is dependent on the nuclear reaction between the stable isotope of boron (^{10}B) and thermal neutrons, which release α and ^{7}Li particles within a limited path length (~9 μm) through the boron neutron capture reaction, $^{10}B(n,\alpha)\,^{7}Li$. Recent clinical studies of BNCT have focused on high-grade glioma and cutaneous melanoma; however, cerebral metastasis of melanoma, anaplastic meningioma, head and neck tumor, and lung and liver metastasis have been investigated as potential candidates for BNCT. To date, more than 350 high-grade gliomas have been treated in BNCT facilities worldwide. Current clinical BNCT trials for glioblastoma (GBM) have used the epithermal beam at a medically optimized research reactor, and *p*-dihydroxyboryl-phenylalanine (BPA) and/or sulfhydryl borane $Na_2B_{12}H_{11}SH$ (BSH) as the boron delivery agent(s). The results from these rather small phase I/II trials for GBM appear to be encouraging, but prospective randomized clinical trials will be needed to confirm the efficacy of this theoretically promising modality. Improved tumor-targeting boron compounds and optimized administration methods, improved boron drug delivery systems, development of a hospital-based neutron source, and/or other combination modalities will enhance the therapeutic effectiveness of BNCT in the future.

Keywords: Glioma; BNCT; Radiation; Cancer

1. Introduction

The glioblastoma (GBM), a common type of a radio- and chemo-resistant malignant brain tumor in adults, shows rapid tumor growth and wide microscopic invasion to the surrounding normal brain tissue. Despite the improvements in diagnostic modality and the use of intensive multimodal therapies that include surgery, radiotherapy and chemotherapy, there have been rather small survival benefits for patients with GBM, which continues to have a median survival time (MST) of less than one year, and even in emerging therapeutic modalities for selected patients, MST is generally less than 2 years. Investigations have revealed the presence of microscopic invading cells at distances of 2–3 cm or even further from the main tumor mass that can be clinically identified by contrast enhancement area on a magnetic resonance image (MRI), and that are found in the microsurgical field during surgical operation. Extensive surgical resection or high-dose

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radiation therapy sufficient to cover microscopic invasion into the healthy brain tissue inevitably leads to some degree of post-therapeutic neurological deterioration. Consequently, 80–90% of GBM recur locally, indicating the need for more intensive and tumor-selective therapy [1–3]. Recently, image-guided surgery utilizing fluorescence with 5-aminolevulinic acid, neuronavigation and intraoperative MRI has enabled more complete resections of contrast-enhancing tumor [4,5]. Concomitant and adjuvant use of temozolomide with a standard photon radiotherapy has demonstrated a significant survival advantage compared to the radiotherapy alone with minimal additional toxicity: the MST was 14.6 months with temozolomide plus radiotherapy and 12.1 months with radiotherapy alone [6].

Several randomized trials have demonstrated a significant improvement of survival time by post-operative fractionated photon radiation at a total dose of 45–60 Gy [7–12]. Among many dose-escalation studies, some small case series found favorable results, which involved dose-escalation mainly in a main tumor mass using an additional stereotactic radiosurgery or other conformal radiotherapy [13–17]. A dose of 90 Gy in accelerated fractionation with photon and proton irradiation almost completely prevented central recurrence, extending the MST of GBM patients treated by this modality to 20 months. However, recurrence occurred in areas immediately peripheral to the 90 Gy volume, mostly in the 70–80 Gy volume, and radiation necrosis also frequently occurred [13]. Therefore, there is urgent need of a method that can deliver high-dose radiotherapy to an extended target area encompassing the microscopic invasion while avoiding radiation necrosis.

Boron neutron capture therapy (BNCT) is the unique high-dose tumor-selective radiotherapy for cancer treatment. BNCT theoretically allows the preferential destruction of ^{10}B-loaded tumor cells, while sparing the normal tissue without ^{10}B, based on the a nuclear reaction between ^{10}B and thermal neutrons, which release high linear energy transfer (LET) α and ^{7}Li particles through the boron neutron capture reaction, $^{10}B(n,\alpha)\,^{7}Li$ (Fig. 1). The boron neutron capture reaction provide the tumor-selective dose (boron dose), and the other non-selective dose components consist of the proton recoils due to fast neutrons, $^{1}H(n,n')p$, 0.54 MeV protons from the nitrogen capture reaction, $^{14}N(n,p)^{14}C$, the γ ray arising from contamination in the primary beam, and 2.2 MeV, the prompt γ rays from the hydrogen capture. Recent clinical studies of BNCT have focused on high-grade glioma [18] and cutaneous melanoma [19,20], malignant meningioma [21,22], head and neck tumor [23], and lung and liver tumors [24,25] as potential candidates for BNCT. Single session of BNCT seems to be more or at least equally effective as conventional fractionated photon radiation (e.g. 60 Gy by 30 fractions for GBM). However, the procedure is one of the most complex of all anti-tumor treatments and the effectiveness of this therapy is highly dependent on neutron and boron distributions. So far, only a simple-shaped, one direction neutron beam is available in exclusive research reactors. This article provides a review of the clinical trials for BNCT of GBM, and discusses future prospects for this treatment.

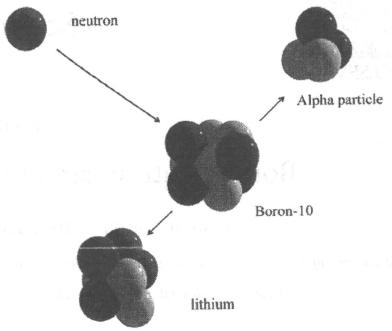

Fig. 1. BNCT is based on the nuclear reaction between ^{10}B and thermal neutrons, which release high linear energy transfer (LET) α and ^{7}Li particles through the boron neutron capture reaction, $^{10}B(n,\alpha)\,^{7}Li$. The effectiveness of BNCT is highly dependent on selective boron-10 accumulation in tumor cells as well as sufficient thermal neutrons even at depth.

2. Clinical studies

2.1. Thermal neutron beam era

The first theoretical account of the biological effects and therapeutic possibilities of BNCT was published by Locher [26]. Early clinical trials of BNCT for brain tumors were conducted by Farr et al. [27,28] at the Brookhaven National Labora-

tory (BNL) and by Sweet et al. [29,30] at the Massachusetts Institute of Technology (MIT) in the 1950s and 1960s. Thermal beams were combined with boron delivery agents, such as borax ($Na_2B_4O_71OH_2O$), *p*-carboxy-phenylboronic acid, and sodium phenyldodecaborate ($Na_2B_{10}H_{10}$). To overcome the poor penetrability of thermal neutrons, BNCT was combined with craniotomy to irradiate the brain directory [31]. Neither the early trials at BNL nor those at MIT achieved a significant improvement in survival over the conventional photon beam therapy in use at the time. In their retrospective analysis, Slatkin et al. postulated that the disappointing results may have been due to inadequate numbers of neutrons delivered to the tumor because of the poor penetration property of the thermal neutron beam employed, radiation damage to the cerebral vasculature attributable to the considerable variation of blood boron level among patients, and the insufficient tumor selectivity of boron delivery agent used [32].

In a study by Hatanaka and Nakagawa, more than 200 patients with high-grade glioma were treated with BSH-mediated intraoperative BNCT (IOBNCT). The results showed that the IOBNCT treatment was more effective than conventional photon beam therapy. The median survival time of GBM and anaplastic astrocytoma (AA) were 22.9 and 64.8 months, respectively. Among the subgroup of 103 high-grade glioma patients, 2 patients with glioblastoma and 4 with AA lived more than 10 years. However, reevaluation of a small subgroup of 12 of the 103 patients according to Curran's recursive partitioning analysis [33] revealed that there were no differences in their survival data compared to those of age-matched controls [34].

2.2. *Epithermal neutron beam era; beginning of BNCT without craniotomy*

A major advantage of the epithermal beam over the thermal beam relates to the enhanced dose delivery to the deep-seated lesion based on the improved penetrability in the first several centimeters of irradiated volume before epithermal neutrons are moderated and converted into thermal neutrons, generating the $^{10}B(n,\alpha)$ reaction. The epithermal neutron beam is currently being employed in clinical trials worldwide, as shown in Table 1 [18,35–44]. In a phase I/II clinical trial at BNL and Harvard/MIT,

Table 1
BNCT clinical trials using an epithermal neutron beam for newly-diagnosed GBM

Facility	No. of pts.	Boron drug (infusion)	Normal brain dose peak/ave.	Median survival	Other procedures
BMRR, BNL [18,42,43] 1994–1999	53	BPA: 250–330 mg/kg (2 h)	8.4–14.8/1.8–8.5 Gy(w)	13 M for early 37 pts, 14.8 M for one field group	1–3 fields beam size 10 or 12 cm
MITR-II, MIT [41] 1996–1999	20	BPA: 250–350 mg/kg (1–1.5 h)	8.7–16.4/3.0–7.4 Gy(w)	13 M	1–3 fields beam size 15 cm
JRR-4, JAEA [35] 1998–2004	7	BSH: 100 mg/kg (1 h)	20.3–36.0[a]/2.3–8.1 Gy(w)	20.7 M	Intraoperative irradiation 1 field beam size 10 or 12 cm
HFR, Petten [36] 1997–	26	BSH: 100 mg/kg/min	8.6–11.4 Gy (boron physical dose)	13.2 M for 10.4 Gy cohort	4 fractions 1 or 2 fields beam size 12 cm
FiR1, Helsinki [39] 1999–	18	BPA: 290–400 mg/kg (2 h)	8–13.5/3–6 Gy(w)	15.0 M	2 fields beam size 11 or 14 cm
Studsvik Medical AB, Sweden [37] 2001–	17	BPA: 900 mg/kg (6 h)	7.3–15.5/3.3–6.1 Gy(w)	18 M	1 or 2 fields
LVR-15, NRI Rez [40] 2001–	5	BSH: 100 mg/kg (1 h)	14.2 >/2 Gy(w)	–	–
KUR, KURRI [38] 2002–	27	BPA: 250–700 mg/kg (1–6 h), BSH: 100 mg/kg (1 h)	15 Gy(w) >/–	–	Local photon radiation or fractionated extended local photon radiation for selected cases
JRR-4, JAEA 2005–	8	BPA: 250 mg/kg (1 h) BSH: 100 mg/kg	13 Gy(w) >/–	–	Fractionated extended local photon radiation (30 Gy)

BNL, Brookhaven National Laboratory; MIT, Massachusetts Institute of Technology; JAEA, Japan Atomic Energy Agency; NRI Rez, Nuclear Research Institute Rez; KURRI, Kyoto University Research Reactor Institute.

[a] The peak dose of the tissue adjacent to post-surgical cavity during intraoperative irradiation.

an epithermal beam was used in BNCT to enable an external beam treatment by the closed-cranium method [18]. In these clinical trials, BPA was used as a boron delivery agent. An epithermal beam has also been employed in European clinical trial (EORTC-Study 11961) at the High Flux Reactor (HFR) in Petten that used fractionated closed-cranium BNCT and BSH as a boron delivery agent [36]. Although both trials were primarily phase I safety and dose-escalation studies, the clinical trials at BNL and Harvard/MIT completed in 1999 provided detailed data on normal brain tolerance and survival benefit. MST for the 18 GBMs from the Harvard/MIT trial was 13 months, and was comparable, even as a single session of BNCT, with that of historical controls treated with conventional external beam photon therapy [18,33,41]. The majority of the central nervous system toxicities (CNS) were acute, self-limiting, and primarily related to a temporal increase in intracranial pressure. A tumor volume of more than 60 cm^3 was associated with a higher incidence of grade 3–4 symptoms. In the BNL dose-escalation study, the MST for the first 38 of 53 subjects was 13 months. However, retrospective analysis revealed that the later subgroup, which showed an increased average brain dose (ABD) and an increased number of irradiation fields, had a higher incidence of CNS toxicity and did not compare favorably with the historical controls. Median times to progression from histopathological diagnosis following one, two and three field BNCT were 34.5, 28.1, and 18 weeks, respectively. MST following one, two and three field BNCT were 14.8, 12.1, and 11.9 months, respectively. Two of the seven subjects received an ABD of 8 Gy-Eq or above, using three fields, and had grade 3 CNS toxicity [42]. An ABD of 6.2 Gy-Eq was associated with 50% incidence of somnolence [43]. In the EORTC-Study 11961, BNCT was carried out in 4 fractions on 4 consecutive days and BSH were administered enough to keep the average boron concentration over the 4 fractions at 30 ppm. Beginning at the starting dose at 8.6 Gy as boron dose, four dose-escalation cohort were treated. A single field with 12 cm collimator was used for the first 5 patients and 2 fields were used for later subjects. A better MST of 13.2 months was recorded in the third dose-escalation cohort [36].

The clinical teams at the Japan Research Reactor-4 (JRR-4) employed an epithermal neutron beam into the BSH-mediated IOBNCT [35,44,45]. Among 16 newly diagnosed GBMs treated by the clinical team at the University of Tokushima and the University of Tsukuba, 2 subjects were irradiated with a pure epithermal beam and 14 subjects were irradiated with a mixed thermal–epithermal beam at 12 h following the 1 h intravenous infusion of BSH (250 mg/kg). Single field and either a 10 or a 12 cm-collimator was minimized by an LiF shielding device at the craniotomy window, resulting in a relatively low ABD of approximately 5 Gy-Eq. In 7 GBMs from the University of Tsukuba underwent IOBNCT, and the MST and the median PFS for the small series were 22.6 months and 12.0 month, respectively [35].

2.3. Modification of BNCT with an epithermal neutron beam

Since the addition of epithermal neutron beam irradiation to BNCT for the treatment of high-grade gliomas, a sufficient number of thermal neutrons have been delivered to most unilateral tumors. However, once the tumor volume reduction on MRI was achieved, local recurrences occurred in the majority of the subjects treated with the external beam BNCT, while both local and distant recurrence occurred in the subjects receiving IOBNCT [35,36,42]. These observations imply that the boron concentrations in the infiltrating tumor and the main tumor mass are insufficient and heterogeneous to a certain degree. Therefore, the main key to successful BNCT for GBMs is a homogeneous preferential accumulation of boron-10 in the main tumor mass as well as in the infiltrating tumor cells. Preclinical data suggest that a longer infusion time may improve this situation [46–48]. In an *in vitro* study, the accumulation of BPA appeared to be more cell-cycle dependent than the accumulation of BSH, but cell-cycle dependency in the accumulation of BPA became minimal over the long-term infusion [48]. In the *in vivo* rat 9L gliosarcoma model, up to 6-h infusion of BPA increased the tumor boron-10 concentration, maintaining a constant tumor-to-blood ratio of 3.7:1 [46]. In a quantitative microlocalization study, extending the time of BPA administration improved the B-10 distribution, with 6-h intravenous infusion of BPA at a dose rate of 250 mg/kg/h resulting in a nearly 90% higher boron-10 concentration in infiltrating tumor cells compared to 2- and 3-h infusions [47].

The long-term infusion method has been applied in the Swedish clinical trial since 2001. BPA administration at a dose of 900 mg/kg over 6 h has been

well tolerated, and the interim median survival time for the first 17 GBMs was 18 months [37]. In this protocol, neutron irradiation has been performed using 2-fields and an average brain dose (ABD) ranging from 3 to 6 Gy(w).

The combination of BPA and BSH was first applied to clinical use by Miyatake and associates with the intention of minimizing the heterogeneous boron-10 distribution based on the different mechanisms by which BPA and BSH accumulate in tumor cells [38]. In principle, ^{18}F-labeled positron emission tomography (PET) was followed by BNCT to calculate the L/N ratio of BPA-mediated boron-10. BSH (5 g/body) and BPA (250 mg/kg) were administrated intravenously 12 and 1 h before epithermal neutron beam irradiation, respectively. Regardless of the pre-BNCT tumor volume, improvements on neuroimages were recognized both at the initial (46.4%) and at follow-up (58.5%) assessments. More than 50% of the contrast-enhanced lesions disappeared in 8 of the 13 patients during the follow-up period. In the following series, the total amount of BPA was increased to 700 mg/kg, infused over 6 h to yield a more homogeneous boron distribution.

In the Finnish trial conducted by Helsinki University Central Hospital (HUCH) and the Technical Research Center of Finland (VTT), 290 mg/kg of BPA infused over 2 h was used in the first 12 GBMs with two irradiation fields [39]. The BPA dose to subsequent GBMs was escalated from 330 ($n = 1$) to 360 mg/kg ($n = 3$), 400 ($n = 3$), and 450 mg/kg ($n = 3$). These BPA doses with a planning target volume (PTV) dose ranging from 30 to 72 Gy(w) achieved a median survival of 15 months with no dose-limiting toxicity encountered. Moreover, the 7 GBMs that recurred after conventional photon irradiation of 50–60 Gy showed a favorable response to reirradiation with BNCT using 290 mg/kg of BPA infused over 2 h, in which protocol the PTV dose ranged from 25 to 36 Gy(w) and the average whole brain dose ranged from 2 to 4 Gy(w).

Although a number of studies have reported on the efficacy of multimodal treatment for brain tumors, there have been relatively few investigations on combinations between BNCT and other modalities. Because of the relatively low normal brain dose, BNCT allows additional photon radiation sufficient to maintain a total normal brain dose below the tolerance limit. An *in vivo* study revealed that a photon radiation boost after BNCT could significantly enhance the survival of F98 glioma- and MRA 27 melanoma-bearing rats after administration of BPA and BSH [49]. Clinically, the photon boost has been used in recent protocols in order to ensure a sufficient BNCT dose to deep-seated lesions [38]. Since 2005, new protocol with a combination of fractionated, extended local photon radiation and BPA- and BSH-mediated BNCT was begun at JRR-4. In this protocol, boron-unloaded subpopulations of tumor cells are irradiated only by non-selective components of BNCT radiations and fractionated photon radiation, and an additional boron dose boosts these non-selective doses in the boron-loaded tumor cells. Eight patients with newly diagnosed GBMs were followed up for 17.2 months (mean) after treated by this protocol, six patients are alive at analysis and the MST and the median PFS for the patients are 19.8 and 12.0 months, respectively (unpublished data).

2.4. Optimization of BNCT

Successful BNCT is highly dependent on the selective accumulation of boron-10 in tumor cells. There have been only two boron delivery agents available for clinical BNCT trials for high-grade glioma (Fig. 2), *p*-dihydroxyboryl-phenylalanine (BPA) and the sulfhydryl borane $Na_2B_{12}H_{11}SH$ (BSH). BSH biodistribution studies have suggested that the primary mode of the selective BSH distribution is passive diffusion from blood to tumor tissue *via* the disrupted blood–brain barrier (BBB) [50,51]. The boron concentration in the normal brain with an intact BBB remains minimal, while the tumor boron-10 concentration is related to both the tumor vascularity and the blood boron-10 level. The tumor-to-blood boron concentration ratio ranged from 0.5 to 1.0 in the biodistribution studies using animal brain tumor models [52,53]. Tumor-to-blood boron concentration ratios of 0.5–1.0—a slightly

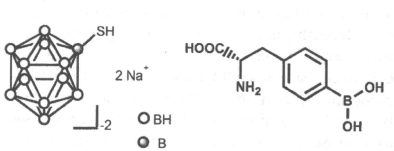

Fig. 2. Currently available boron delivery agent for BNCT. Left: sulfhydryl borane $Na_2B_{12}H_{11}SH$ (BSH); right: *p*-dihydroxyboryl-phenylalanine (BPA).

higher range than in these animal studies—have been reported in human patients treated with BSH-mediated BNCT [51].

BPA, the amino acid analogue, has structural characteristics similar to those of a melanin precursor. The findings that BPA is selectively accumulated *in vivo* and *in vitro* and that it achieves a high tumor control rate after neutron irradiation support the potential application of BPA for clinical BNCT of melanoma. Mishima et al. reported promising clinical results in a pilot study of BNCT for skin melanoma [19,20,54]. In contrast to BSH, BPA is actively transported through the tumor cell membrane due to elevated rate of amino acid transport in proliferating cells [48,55]. Non-proliferating cells escape radiation damage following BNCT. The recent clinical trials have used a combination of BPA and BSH with the intention that these different compounds would accumulate in different subpopulations of tumor cells.

To date, a variety of boron delivery agents have been investigated, including porphyrins [56,57], amino acids [58], polyhedral borane [59], carbohydrates [60], polyamines [61], biochemical precursors [62,63], DNA-binding agents [64,65], antisense agents [66], peptides [67], liposomes [68–70], and monoclonal antibodies [71]. A boron delivery agent for BNCT would be required to fulfill all of the following conditions: (a) non-toxic at a dose of clinically use (b) to achieve at least 10–30 μg ^{10}B/g of tumor boron-10; (c) high tumor/brain and tumor/blood concentration ratios; (d) rapid clearance from blood circulation and normal tissues but persistence in tumor; (e) water solubility; and (f) chemical stability [49,72]. Among the boron delivery agents, BOPP [73], a boronated porphyrin, and GB-10 [59], polyhedral borane $Na_2B_{10}H_{10}$, have been evaluated for their pharmacokinetics in humans. A phase I trial for potential application of BOPP for photodynamic therapy was interrupted because of its general toxicity [73]. These results suggest the need for development of a better boron delivery agent, as well as an improved method of drug delivery. Recently, improvement of the synthesis pathway, and novel agents such as porphyrin–cobaltacarborane conjugates [74] or ^{10}B-enriched carboranyl-containing phthalocyanine [75] have been under examination. GB-10 has been proposed as a boron neutron capture agent for an additional dose in fast neutron therapy. In a trial in which GB-10 was used in 15 lung cancer patients, no toxicity was recorded [59].

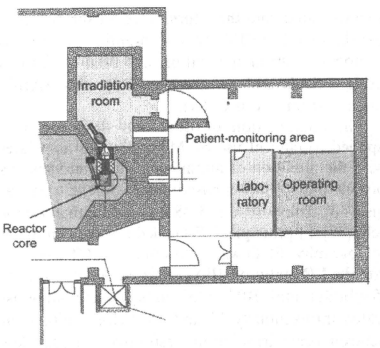

Fig. 3. The general arrangement of the medical irradiation facility of Japan Research Reactor-4, showing the irradiation room located in the basement.

The use of epithermal neutron beams has provided a non-invasive method for clinical BNCT without craniotomy for high-grade glioma, and the encouraging results have motivated further investigations to optimize this unique therapeutic modality. At the present time, clinical BNCT trials have been performed at limited numbers of nuclear reactors that have simple medical facilities (Fig. 3). A prompt gamma-ray analysis (PGA) device, an inductively coupled plasma atomic emission spectroscopy (ICP-AES) device for boron-10 measurement, and an irradiation room are installed in the reactor for BNCT [35]. For dose planning of BNCT, ^{18}F-labeled PET has been employed to calculate the L/N ratio of BPA-mediated boron-10 in some institutions [38,76]. However, because patients must be transferred from and returned to medical centers at varying distances from the reactor facilities, there is need for a hospital-based compact reactor or accelerator-based neutron source [77] that could be constructed in a selected medical center specializing in the treatment of brain tumors.

3. Conclusion

BNCT is an emerging therapeutic modality for cancers which can theoretically allow tumor-selective destruction while sparing normal tissue. The selectivity is highly dependent on the dose from the boron neutron capture reaction, $^{10}B(n,\alpha)\ ^{7}Li$, i.e., the accumulation of boron-10 in tumor cells.

Recent clinical studies of BNCT have focused on the treatment of high-grade gliomas and cutaneous melanomas. More than 350 high-grade gliomas have been treated in BNCT facilities worldwide. Cerebral metastasis of melanoma, anaplastic meningioma, head and neck tumor, and lung and liver metastasis have been investigated as potential candidates for BNCT [21–23]. In the near future, ongoing clinical BNCT trials will disclose the dose-related effectiveness and safety of BNCT, the most suitable method of administrating boron drugs, and the feasibility of combination with photon therapy. The clinical results from the rather small trials performed to date appear to be encouraging. In the future, improvement of the tumor-targeting boron compound, the administration method, and the drug-delivery system, and development of a hospital-based neutron source and/or other combination modalities will enhance the therapeutic effectiveness of BNCT.

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References

[1] L.E. Gasper, B.J. Fisher, D.R. Macdonald, et al., Supratentorial malignant glioma: patterns of recurrence and implications for external beam local treatment, Int. J. Radiat. Oncol. Biol. Phys. 24 (1992) 55–57.

[2] K.E. Wallner, J.H. Galicich, G. Krol, E. Arbit, M.G. Malkin, Malkin MG: patterns of failure following treatment for glioblastoma multiform and anaplastic astrocytoma, J. Radiat. Oncol. Biol. Phys. 16 (1989) 1405–1409.

[3] U. Oppitz, D. Maessen, H. Zunterer, S. Richter, Flentje M: 3D-recurrence-patterns of glioblastomas after CT-planned postoperative irradiation, Radiother. Oncol. 53 (1999) 53–57.

[4] W. Stummer, U. Pichlmeier, T. Meinel, O.D. Wiestler, F. Zanella, H.J. Reulen, Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomized controlled multicentre phase III trial, Lancet Oncol. 7 (2006) 392–401.

[5] C. Nimsky, O. Ganslandt, B. von Keller, R. Fahlbusch, Intraoperative visualization for resection of gliomas: the role of functional neuronavigation and intraoperative 1.5 T MRI, Neurol. Res. 28 (2006) 482–487.

[6] R. Stupp, W.P. Mason, M.J. van den Bent, M. Weller, B. Fisher, M.J.B. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R.C. Janzer, S.K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, G. Cairncross, E. Eisenhauer, R.O. Mirimanoff, Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, N. Engl. J. Med. 352 (2005) 987–996.

[7] M.D. Walker, E. Alexander Jr., W.E. Hunt, et al., Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas: cooperative clinical trial, J. Neurosurg. 49 (1978) 333–343.

[8] M.D. Walker, S.B. Green, D.P. Byar, et al., Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery, N. Engl. J. Med. 303 (1980) 1323–1329.

[9] K. Kristiansen, S. Hagen, T. Kollevold, et al., Combined modality therapy of operated astrocytomas grade III and IV: confirmation of the value of postoperative irradiation and lack of potentiation of bleomycin on survival time: a prospective multicenter trial of the Scandinavian Glioblastoma Study Group, Cancer 47 (1981) 649–652.

[10] M. Sandberg-Wollheim, P. Malmstrom, L.G. Stromblad, et al., A randomized study of chemotherapy with procarbazine, vincristine, and the lomustine with and without radiation therapy for astrocytoma grade 3 and/or 4, Cancer 68 (1991) 22–29.

[11] A.P. Anderson, Postoperative irradiation of glioblastomas. Results in a randomized series, Acta Radio Oncol. Radiat. Phys. Biol. 17 (1978) 475–484.

[12] N.M. Bleehen, S.P. Stennning, A Medical Research Council trial of two radiotherapy doses in the treatment of grades 3 and 4 astrocytoma, Br. J. Cancer 64 (1991) 769–774.

[13] M.M. Fitzek, A.F. Thornton, J.D. Rabinov, M.H. Lev, F.S. Pardo, J.E. Munzenrider, P. Okunieff, M. Bussiere, I. Braun, F.H. Hochberg, E.T. Hedley-Whyte, N.J. Liebsch, G.R. Harsh 4th, Accelerated fractionated proton/photon irradiation to 90 cobalt gray equivalent for glioblastoma multiforme: results of a phase II prospective trial, J. Neurosurg. 91 (1999) 251–260.

[14] M. Tanaka, Y. Ino, k. Nakagawa, M. Tago, T. Todo, High-dose conformal radiotherapy for supratentorial malignant glioma: a historical comparison, Lancet Oncol. 6 (2005) 953–960.

[15] E.C. Nwokedi, S.J. DiBase, S. Jabbour, J. Herman, P. Amin, L.S. Chin, Gamma knife stereotactic radiosurgery for patients with glioblastoma multiforme, Neurosurgery 50 (2002) 41–47.

[16] B.G. Baumert, J. Lutterbach, R. Bernays, J.B. Davis, F.L. Heppner, Fractionated stereotactic radiotherapy boost after post-operative radiotherapy in patients with high-grade gliomas, Radiother. Oncol. 67 (2003) 183–190.

[17] L. Souhami, W. Seiferheld, D. Brachman, E.B. Podgorsak, M. Werner-Wasik, R. Lustig, C.J. Schultz, W. Suse, P. Okunieff, J. Buckner, L. Zamorano, M. Mehta, W.J. Curran, Randomized comparison of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme: report of RadiationTherapy Oncology Group 93-05 protocol, Int. J. Radiat. Oncol. Biol. Phys. 60 (2004) 853–860.

[18] A.D. Chanana, J. Capala, M. Chadha, J.A. Coderre, A.Z. Diaz, E.H. Elowitz, J. Iwai, D.D. Joel, H.B. Liu, R. Ma,