commercial preparation, Photofrin [2], which does not contain boron. This photosensitizer has been shown to localize preferentially in glioma relative to normal brain tissue. Moreover, reports of PDT as a treatment for animal and human glioma have been encouraging, and a photosensitizer that is more tumor selective than HpD or Photofrin would have great clinical benefits.

In this study, our results showed the positive efficacy of PDT using H_2OCP in a colony-forming assay (Fig. 3) and in tumorigenesis of in vitro pre-treated cells in Fisher rats (Fig. 4). Additionally, H_2OCP accumulated within the glioma cells to a significantly higher extent than BPA or BSH ($P < 0.05$), and was retained inside the cell to approximately the same extent as BSH (Fig. 2). Based on these findings, we postulated that H_2OCP could be applied to both PDT and BNCT for treatment of glioma tumors. In the fluorescence microscopy experiment, although the cells in the bright field image showed evidence of cytotoxic damage (Fig. 5A), the cytotoxicity of H_2OCP in dark conditions was not observed, even at double the concentration of H_2OCP used in the fluorescence microscopy experiment. Therefore, we considered that the damage to the cells in the bright field image was most probably due to technical complications related to irradiation with the laser during imaging, rather than to the cytotoxic effects of H_2OCP itself. Furthermore, H_2OCP was shown to be taken up by F98 rat glioma cells (Fig. 5B–D). Therefore, our results suggest that H_2OCP can be used intraoperatively for photodynamic diagnosis (PDD) and fluorescence-guided resection of brain tumors.

Pre-operative administration of a boronated porphyrin has a number of advantages in the clinical setting. As noted previously, boronated porphyrins are useful in PDD and in fluorescence-guided resection of brain tumors during surgery. Using fluorescence-guided resection of such tumors during surgery, the resection rate can be augmented, with expected further improvements in patient prognosis [25]. In addition, boronated porphyrins can be used with intra-operative PDT and post-operative BNCT. Although the initial results with commonly used photosensitizers for PDT such as Photofrin (or its unpurified form HpD) were very encouraging, treatment failures did occur, mainly due to the limited penetration of light into the brain. In cases with deep lesions, PDT alone may be inadequate to achieve complete tumor treatment, and it would be preferable in such cases to use BNCT as a supplementary treatment, with boron-containing porphyrin as a photosensitizer. Fairchild et al. [26] reported that thermal and epithermal neutrons are transported to a depth of approximately 10 cm in fact, BNCT has been shown to treat deep lesions. Since boronated porphyrins can be effective for BNCT as boron delivery agents while retaining their photosensitizer ability, the limited penetration of light can be overcome using a combination of BNCT and PDT for the treatment of human gliomas.

ACKNOWLEDGMENTS

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Asialoerythropoietin attenuates neuronal cell death in the hippocampal CA1 region after transient forebrain ischemia in a gerbil model

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Background and purpose: Systemic administration of high-dose recombinant human erythropoietin (rhEPO) is known to attenuate ischemic injury. However, high-dose rhEPO might aggravate ischemic lesions by increasing blood viscosity because of its erythropoietic effects. Asialoerythropoietin (asialoEPO), an EPO derivative with an extremely short plasma half-life, has considerably lesser erythropoietic effect than that of naive EPO. We attempted to determine whether asialoEPO exerts the same neuroprotective effect as naive EPO in a gerbil transient forebrain ischemia model.

Methods: Transient occlusion of both the common carotid arteries was performed in 23 adult gerbils. The drugs (asialoEPO or rhEPO, 10 U/g bodyweight) or phosphate-buffered saline (PBS) were injected intraperitoneally at three times (3 hours before, immediately after, and 24 hours after the ischemic insult). Learning and retention tests were performed on days 6 and 7, respectively, and histological analyses were performed on day 7.

Results: Animals treated with asialoEPO and rhEPO showed significant neurological improvement compared to the PBS-treated animals. The number of viable neurons in the CA1 field of the rhEPO-treated (103.57 ± 27.90 cells/mm) and asialoEPO-treated (144.99 ± 34.87 cells/mm) animals was higher than that of the PBS-treated animals (19.53 ± 3.79 cells/mm). Terminal dinucleotidyltransferase-mediated UTP end labeling-positive cells were significantly lower in the rhEPO-treated (33.40 ± 8.13 cells/mm) and asialoEPO-treated (29.28 ± 14.91 cells/mm) animals than in the PBS-treated animals (76.67 ± 8.14 cells/mm). AsialoEPO treatment did not have any effect on erythropoiesis.

Conclusion: Multiple dosing of asialoEPO, like EPO, could protect the hippocampal CA1 neurons from ischemic damage without affecting erythropoiesis.

Keywords: Asialoerythropoietin, Brain ischemia, Apoptosis, Neuroprotection

Introduction

Primarily, erythropoietin (EPO) is a hematopoietic cytokine that regulates red blood cell production. It is about 35 kDa sialoglycoprotein produced by the fetal liver and the adult kidney.[1] EPO acts synergistically with several growth factors to cause maturation and proliferation.[2] The tissue expression studies revealed that EPO mRNA was detected in the spleen, testis and brain, and up-regulated in a hypoxia environment.[3] Endogenous EPO and functional EPO receptors were detected in the central nervous system.[4] Many reports have shown that exogenous EPO can bring out the neuroprotective effect in animal models of stroke.[5–8] In 2002, the 'Göttingen EPO-stroke trial' was carried out and demonstrated that the patients who were administrated recombinant human EPO (rhEPO) intravenously (total dose 100 000 U/person) were neurologically improved significantly compared to the control group without notable adverse events.[9,10] However, to attain the neuroprotective effect, supra-pharmacological doses are necessary because of the obstruction of the blood brain barrier.[11] Problematically, multiple high-dosing of rhEPO will increase the red cell mass and activate the platelet aggregation that might predispose another thrombotic event such as venous thrombosis and cardiac stroke for the patient with an ischemic stroke.[12] These problems give us pause in applying the high-dose rhEPO for patients with brain ischemia. Asialoerythropoietin (asialoEPO) is a derivative agent generated by total enzymatic desialylation of rhEPO.[13] Due to the short plasma half-life, asialoEPO little affects the erythroid precursor cells in bone mallow.[14]

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Brines *et al.* suggested that systemic administration of asialoEPO showed neuroprotective activity in *in vivo* study.[15] Also, we reported the effectiveness of high-dose pulses of asialoEPO in behavioral and histopathological neuroprotection after cerebral infarction, as well as EPO, using a rat middle cerebral artery occlusion model (submitted for publication). The goal of this study is to determine whether the systemic administration of asialoEPO is efficacious in protecting the vulnerable neurons against transient forebrain ischemia without adverse events that EPO might incite.

Materials and methods

Preparation and characterization of asialoEPO

The rhEPO was purified to homogeneity from a conditioned medium of CHO cells transfected with a cDNA clone for human EPO, as described previously.[13] Various partially or fully desialylated EPO were prepared by neuraminidase digestion of the hormone with a specific activity of 2.2×10^5 IU/mg protein *in vivo*. Four test tubes containing 5 mg EPO (1.0 mg/ml) were added to 1.5 ml 0.5 M sodium acetate, pH 5.5, and 50 pl 0.1 M calcium acetate. The four reaction mixtures were added to 25, 62.5, 250, and 1250 mU neuraminidase (Streptococcus sp., Seikagaku Kogyo Co., Tokyo, Japan), adjusted to 7.8 ml with 0.1 M sodium acetate, pH 5.5, in an iced-water bath, and incubated at 37 °C. At fixed time intervals (0.5, 1, 2, and 3 hours), 1.55 ml samples were withdrawn from each reaction mixture and transferred to test tubes containing 200 µl 10% trifluoroacetic acid. Desialylated derivatives were passed through a reverse-phase high-performance liquid chromatography column (Vydac, protein C4, 4.6 mm × 250 mm) equilibrated with 30% acetonitrile in 0.1% trifluoroacetic acid. The derivatives were eluted with a linear gradient of 30–80% acetonitrile for 20 minutes at a flow rate of 1.0 ml/min. The derivatives were lyophilized and dissolved in 780 µl 10 mM sodium phosphate, pH 7.4, and stored at −80 °C until use.

Animals

Adult male Mongolian gerbils (weighing 60–70 g; Japan SLC, Inc., Shizuoka, Japan) were housed in a laboratory animal center air controlled room set at 22 ± 1°C. Light was provided on a 12 hours light–dark cycle. Procedures were conducted in conformity with institutional guidelines in compliance with national and international laws and policies.

Induction of ischemia

Occlusion of the bilateral carotid arteries

To produce forebrain ischemia, occlusion of the common carotid arteries was performed as follows.[16,17] The animals were anesthetized with 4% isoflurane for induction and 2% isoflurane for maintenance delivered through oral intubation, and underwent mechanical ventilation. The animals were ventilated with 100% oxygen with 2% isoflurane at a rate of 120 breaths/minute and with an inspiratory/expiratory fraction of 1 : 1 and a tidal volume of 0.35 mL. A midline neck incision was carried out and isolated both common carotid arteries, subsequently occluded with microaneurysmal clips (Sugita Aneurysm Clip 07-940-81, closing force 128–134 g; Mizuho Ikakogyo Co., Ltd, Tokyo, Japan) for 3 minutes. We kept rectal temperatures at 37.0 ± 0.2°C while clamping the common carotid arteries by using a heating pad and a heating lamp. To confirm the reduction of cerebral blood flow during the insult, we used a transcranial laser doppler blood flow meter (TBF-LC1; Unique Medical Co., Ltd, Tokyo, Japan) and measured the cerebral blood flow before and during the insult. All procedures were carried out in 20 minutes. The animal that had finished surgery in a short time also waited for the extubation until 20 minutes passed.

Treatment protocols

To evaluate neurological and histological analysis, 24 gerbils were divided into three groups. They were assigned to receive rhEPO [total dose 30 U/g body weight (b.w.), n=8], asialoEPO (total dose 30 U/g b.w., n=8), or phosphate-buffered saline (PBS) with 0.1% bovine serum albumin (n=8). Each volume of injection was 3.2 µl/g b.w. One gerbil in the asialoEPO treatment group was omitted because its cerebral blood flow apparently did not decrease during the ischemic insult. Animals were injected with the drug intraperitoneally 3 hours before (10 U/g b.w.), just after (10 U/g b.w.), and 24 hours after the insult (10 U/g b.w.); therefore, the total dose of asialoEPO and rhEPO was 30 U/g b.w. respectively. Surgeries were performed by a person blind to the experimental conditions.

Estimation of learning ability by passive avoidance task

On the sixth day after forebrain ischemia, each gerbil was submitted to a passive avoidance task, which is an index of learning and retention. We performed a modified multitrial passive avoidance test.[18] The equipment consisted of a box divided by a guillotine door into two compartments (light box: 25 cm length × 25 cm width × 30 cm height; dark box: 28 cm length × 29 cm width × 30 cm height) in which the floor had a grid of stainless steel rods. The removable floor was cleaned and adjusted immediately before every trial. In the beginning, for the learning test, each animal was placed in the light and safe compartment. When they stepped into the dark compartment, they received an electric foot-shock through the grid floor (0.6 mA, 3 seconds). Just after the electroshock, they were taken out of the dark box and returned to the safe compartment. When they re-entered the dark compartment, they received a

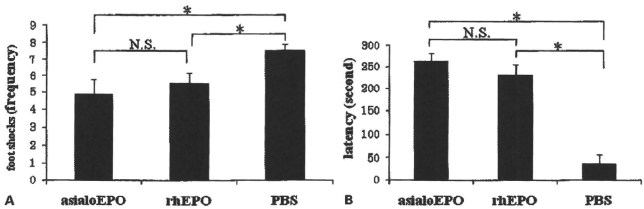

Figure 1 Neurological assessment was performed with a multi-trial passive avoidance test after rhEPO, asialoEPO, and PBS treatments. Passive avoidance testing was carried out 6 days post-ischemic insult, and the subsequent retention test was performed 24 hours later. (A) The number of foot-shocks; (B) response latency. Each column with a vertical bar represents the mean ± standard error. *$P < 0.05$, significantly different from the corresponding PBS-treated ischemic group with Mann–Whitney U test. N.S. means no significant difference.

foot-shock again. This training session continued for 10 minutes, and the frequency of foot-shocks was counted. 24 hours later, we performed a retention test. Animals were placed in the light compartment and the latency to enter the dark compartment was recorded up to a maximum of 300 seconds.

Histopathological study of hippocampal CA1 region

After the passive avoidance tasks, each gerbil was deeply anesthetized with an inhalation of halothane, and perfused transcardially with 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4). The brains were removed from the skulls and placed in the 4% paraformaldehyde for 24 hours. The tissue block contained in the hippocampus area was divided and embedded in paraffin. Coronal sections (5-μm-thick) were taken at the level of the hippocampus using a microtome and stained with 1% cresyl violet for microscopic observation. The total number of neurons with intact morphological appearance was counted in the entire CA1 region and non-intact neurons were excluded. The cellular density (cells/mm) was calculated to compare the effect of therapy. Terminal dinucleotidyltransferase-mediated UTP end labeling (TUNEL) staining was processed to estimate the neuronal apoptosis. Examinations were performed by an observer blind to the treatment of the animals.

Estimation of the effect on the hematopoietic system of administrating rhEPO and asialoEPO to gerbils

To assess the effect on the hematopoietic system, 15 gerbils which received the same experiment protocol were divided into three groups (rhEPO, $n=5$; asialoEPO, $n=5$; PBS, $n=5$), and hemoglobin levels were measured at day 0 (before drug injection) and at day 14 using HemoCue Hb 201+ Analyzer (HemoCue®). Furthermore, for the bone marrow response study, an additional 10 gerbils were classified into three groups and received rhEPO ($n=4$), asialoEPO ($n=3$) and PBS ($n=3$). The animals were sacrificed 3 days later, and their bone marrow and blood samples were collected. The percentage of reticulocyte in the blood and that of erythroblast in the bone marrow were measured at the central clinical laboratory in Osaka Medical College.

Statistics

Values were expressed as the mean ± standard error. Statistical analysis was performed with Dr. SPSS II for windows. The effect of asialoEPO and rhEPO on the foot-shocks, response latency, CA1 neuronal density, and TUNEL-positive cells density were evaluated by Mann–Whitney's U test. The hemoglobin data was evaluated by the Wilcoxon signed-rank test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Effects of rhEPO and asialoEPO on the passive avoidance task

In the learning trial on day 6, the PBS-injected group received 7.5 ± 0.91 foot-shocks. Treatments with rhEPO and asialoEPO decreased the frequency of shocks (rhEPO: 5.5 ± 0.68 foot-shocks, $P < 0.05$; asialoEPO: 4.9 ± 0.40 foot-shocks, $P < 0.05$) (Fig. 1A). In the retention study, both rhEPO and asialoEPO treatments extended the time to enter the dark compartment (rhEPO: 230.6 ± 25.63 seconds; asialoEPO: 263.6 ± 27.18 seconds) in comparison with PBS treatment (37.0 ± 19.20 seconds) (Fig. 1B). Statistically there was no difference in foot-shock frequency in the training session and latency in the retention study between the rhEPO and the asialoEPO treatment groups.

Effects of rhEPO and asialoEPO on neuronal density

To evaluate the effects of rhEPO and asialoEPO for ischemic damage by 3 minutes transient forebrain ischemia, we measured the CA1 neuronal density in the hippocampus 7 days after the insult (Fig. 2). In the asialoEPO- and rhEPO-treated groups, the neuronal density was 144.99 ± 34.87 cells/mm and 103.57 ± 27.90 cells/mm respectively. These treated groups had significantly decreased neurodegeneration compared to the PBS-administered gerbils (19.53 ± 3.79 cells/mm) (asialoEPO versus PBS: $P < 0.05$; rhEPO versus PBS: $P < 0.05$).

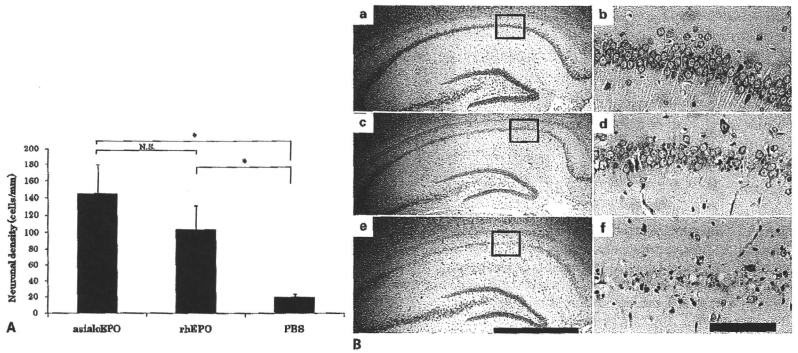

Figure 2 (A) Neuroprotective properties of asialoEPO and rhEPO. Neuronal density in the hippocampal CA1 region after asialoEPO, rhEPO, and vehicle-treatments. AsialoEPO-injected group was 144.99 ± 34.87 cells/mm (versus PBS-injected group, *$P < 0.05$) and rhEPO-injected group was 103.57 ± 27.90 cells/mm (versus PBS-injected group, *$P < 0.05$). (B) Representative photomicrographs of hippocampal sections stained with cresyl violet. (a) asialoEPO treatment group, (c) rhEPO treatment group, (e) PBS treatment group, (b, d, and f) high magnification corresponding to (a, c, and e). [Bar=1.0 mm (a, c, and e) and 0.1 mm (b, d, and f)].

Effects of EPO and asialoEPO on the number of TUNEL-positive neurons in the hippocampal CA1 field

The number of apoptotic CA1 neurons after the different treatments for 3 minutes transient forebrain ischemic gerbils was examined by TUNEL staining 7 days after the ischemic insult (Fig. 3)

The count of TUNEL-positive cells in the PBS-treated group was 76.67 ± 8.14 cells/mm.

The rhEPO and asialoEPO treatments precluded delayed neuronal cell death after ischemia. The TUNEL-positive cells were 32.14 ± 14.92 cells/mm in the asialoEPO-treated group and 33.40 ± 8.13 cells/mm in the rhEPO-treated group (asialoEPO versus PBS: $P < 0.05$; rhEPO versus PBS: $P < 0.05$).

Hemoglobin responses to administered EPO and asialoEPO

In the rhEPO treatment group, the hemoglobin level was elevated at day 14 (14.95 ± 0.35 mg/dl) ($P < 0.05$, versus at day 0, 13.48 ± 0.23 mg/dl) (Fig. 4). AsialoEPO did not elevate hemoglobin levels at day 14 (13.18 ± 0.70 mg/dl) in comparison with day 0 (13.38 ± 0.20 mg/dl). And the PBS treatment group also did not show significant elevation at day 14 (13.67 ± 0.31 mg/dl) compared with day 0 (13.40 ± 0.20 mg/dl).

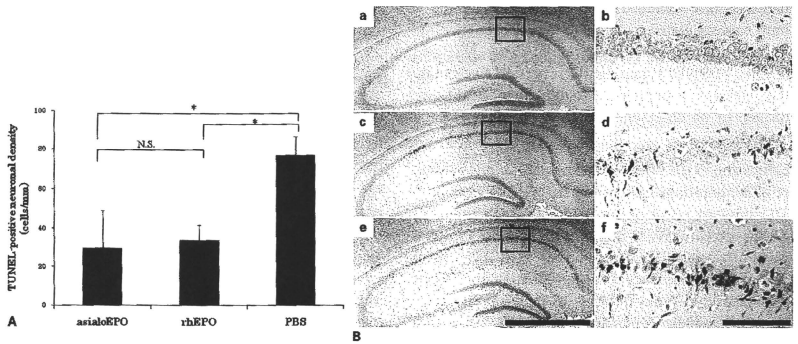

Figure 3 (A) TUNEL staining of the hippocampal CA1 region of ischemic gerbils. AsialoEPO and rhEPO reduced neuronal cell apoptosis at 7 days after ischemia. TUNEL-positive cells density detected in AsialoEPO-injected group was 27.50 ± 39.32 cells/mm (versus vehicle-injected group, *$P < 0.05$). RhEPO-injected group also decreased the TUNEL-positive cells [33.40 ± 23.00 cells/mm (versus vehicle-injected group, *$P < 0.05$)]. Vehicle-injected group was 76.67 ± 23.02 cells/mm. (B) Representative photomicrographs of hippocampal sections stained with TUNEL. (a) asialoEPO treatment group; (c) rhEPO treatment group; (e) PBS treatment group; (b, d, and f) high magnification corresponding to (a, c, and e). [Bar = 1.0 mm (a, c, and e) and 0.1 mm (b, d, and f)].

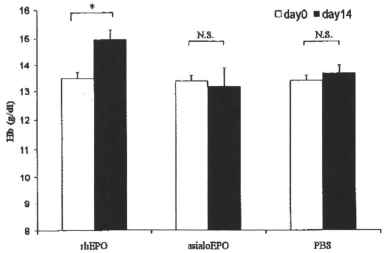

Figure 4 Effects of rhEPO, asialoEPO, and PBS on the hemoglobin level in gerbils. Blood was collected at days 0 and 14. The white bar indicates day 0 and the black bar indicates day 14. RhEPO significantly increased the blood hemoglobin level on day 14 [*$P < 0.05$, day 0 (13.48 ± 0.23 mg/dl) versus day 14 (14.95 ± 0.35 mg/dl)], but asialoEPO [$P > 0.05$, day 0 (13.38 ± 0.20 mg/dl) versus day 14 (13.18 ± 0.70 mg/dl)] and PBS [$P > 0.05$, day 0 (13.40 ± 0.20 mg/dl) versus day 14 (13.67 ± 0.31 mg/dl)] did not change it.

Bone marrow responses to rhEPO and asialoEPO

The ratio of erythroblast in the rhEPO treatment group (41.1 ± 2.5%) was significantly elevated more than in the asialoEPO (15.6 ± 3.1%) and PBS treatment groups (16.7 ± 2.8%). Blood reticulocyte percentage in the rhEPO treatment group (5.9 ± 0.6%) was increased more than those in the asialoEPO (2.6 ± 0.4%) and PBS (2.7 ± 0.5%) treatment groups statistically (Fig. 5). On the other hand, asialoEPO did not elevate erythroblast and reticulocyte in comparison with PBS treatment.

Discussion

Our results clearly demonstrated that intraperitoneally administration of asialoEPO (total dose 30 U/g b.w.) improves learning and memory function in ischemic gerbils (Fig. 1), and we confirmed the neuro-protective effects in histological study (Figs. 2 and 3). Furthermore, in contrast to rhEPO, asialoEPO did not stimulate erythropoiesis, the same as PBS (Figs. 4 and 5). The efficacy of neuro-protection was not different between asialoEPO and rhEPO. In the circulation, asialoEPO is captured rapidly by the galactose-binding protein of the hepatic cells.[13] Despite its short plasma half-life, exogenous asialoEPO appeared promptly within the cerebrospinal fluid at concentrations within the *in vitro* neuroprotective range.[15] Unlike erythropoiesis, it is sufficient to elicit the neuroprotective effect for a short period.[19] Actually, in the middle cerebral infarction model, asialoEPO-injected intravenously bolus (5 U/g b.w.) reduced infarct volume by 50%.[15] Wang *et al.* had shown the effect of asialoEPO on the neonatal hyopoxia-ischemia model.[20] To our knowledge, our current study is the first report that asialoEPO can attenuate ischemic injury for the forebrain ischemia in the adult gerbil model. Unlike the focal ischemia model, the transient forebrain ischemia model is an approximate condition of a re-perfused brain after a cardiac arrest. As we have shown in this study, in the transient forebrain ischemia, one of the mechanisms of EPO-mediated neuro-protection is the strong inhibition of neuronal apoptosis. It was reported that the EPO receptor first activates Janus kinase 2. The activated Janus kinase 2 mediates the phosphorylation of phosphoinositide 3 kinase, mitogen-activated protein kinase, and nuclear factor-κB.[7,8,21] These secondary signal transmitters lead to the up-regulating of the anti-apoptotic proteins such as the B-cell leukemia/lymphoma (BCL) family.[22] Caspase activation is suppressed directly by protein kinase B/Akt in the downstream of phosphoinositide 3 kinase, and indirectly by the process of suppressing the Bcl-2 associated protein.[8] Our result also showed that the number of TUNEL-positive cells in the hippocampal CA1 field was suppressed by exogenous asialoEPO and rhEPO ($P < 0.05$; versus PBS treatment group). In addition to the prevention of apoptosis, EPO is known to have anti-inflammatory, anti-oxidant, angiogeneic, neurogenic, and neurotrophic effects in *in vitro* and *in vivo* study.[6–10]

In conclusion, our study found that the multiple high-dosing schedule of asialoEPO was effective in improving cognitive functions after transient forebrain ischemia in gerbils and its therapeutic gain

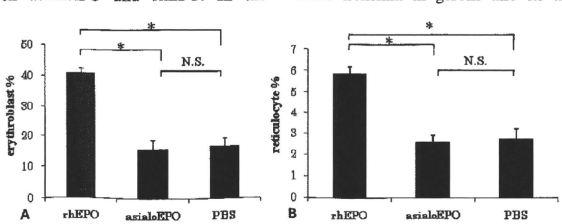

Figure 5 Bone marrow responses were evaluated 3 days after drug administration. (A) The percentage of erythroblast in the bone marrow was elevated by rhEPO (41.1 ± 2.5%) statistically. ($P < 0.05$; versus vehicle-injected group, 16.7 ± 2.8%) Otherwise asialoEPO (15.6 ± 3.1%) did not elevate erythroblast in comparison with PBS ($P > 0.05$). (B) Reticulocyte in the blood increased in the rhEPO treatment group (5.9 ± 0.6%) ($P < 0.05$; versus PBS treatment group, 2.7 ± 0.5%). AsialoEPO (2.6 ± 0.4%) did not change the percentage of reticulocyte ($P > 0.05$; versus PBS treatment group).

compared favorably with that of naive EPO. The histological analyses strongly suggested that the reduced neuronal apoptosis in the CA1 field of hippocampus contributed to symptomatic recovery after ischemic insult. Moreover, asialoEPO did not induce apparent erythropoiesis, unlike with naive EPO, which seems to show the safety advantage of asialoEPO for clinical usage. AsialoEPO may be beneficial for patients with cerebral ischemia, especially in those who are suffering from transient brain ischemia resulting from sudden cardiopulmonary arrest. In this study, animals received ischemic insult with premedication. Further study is needed to determine whether asialoEPO can protect the neuron in the post-ischemic condition.

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悪性脳腫瘍に対する最新放射線治療とその成績
—放射線治療における外科治療の役割—

宮 武 伸 一

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宮 武 伸 一

Modern Radiotherapy for Malignant Brain Tumors including the Role of Surgery in Radiotherapy

by

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BNCT has a unique concept for cell biological targeting. BNCT is a binary approach composed of a boron compound and a neutron beam. If we can accumulate boron compounds selectively to tumor tissue, neutron α reaction will occur only within the tumor cells, which is followed by tumor cell death with minimal hazardous effects for normal tissues. We introduce here the indication, clinical results of BNCT for newly diagnosed GBM and recurrent malignant meningiomas. For deep-seated tumors, instilling air into the tumor cavity is useful to obtain deeper neutron flux penetration. In addition, we also introduce how to treat radiation necrosis in the brain. Surgical removal of the necrotic foci and some medical treatments such as anticoagulants and bevacizumab, anti-VEGF antibody, are useful for this treatment.

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Key words : bevacizumab, BNCT, glioblastoma, malignant meningioma, radiation necrosis

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はじめに

悪性脳腫瘍，ことに悪性グリオーマは，その浸潤性発育のゆえに手術のみでは根治が不可能に近い腫瘍である．最近，テモゾロミド（TMZ）の出現により，化学療法の役割が再認識されている．多施設間の randomized study でグリオブラストーマ（GBM）の生存期間中央値（MST）が 2.5 カ月延長したこと[17]は確かに大きな進歩であり，TMZ+X 線外照射のいわゆる Stupp レジメンが，現在のところ，GBM に対する世界標準治療となっている．しかしながら，裏を返せば，TMZ の併用でも MST の延長は 2.5 カ月にすぎず，化学療法の限界を示すものともいえる．

一方で，悪性グリオーマの補助治療のうち，最も大きな役割を果たしているのは，放射線治療である．影治ら[6]は，第 24 回日本脳神経外科コングレス総会において，過去の報告を詳細にレビューし，悪性グリオーマの標準的放射線治療として，X 線による 1 日 1 回，1 回線量 2 Gy，総線量 60 Gy の分割照射を，MRI での T2 高信号域を照射野とする局所照射が標準的治療として妥当であることを紹介している．多数例の検討により，この標準的放射線治療による MST が 9〜12 カ月という報告が多い[9][20]．また，この治療による画像上の奏効率は 23% と報告されている[1]．少なくとも，新規治療法はこの標準

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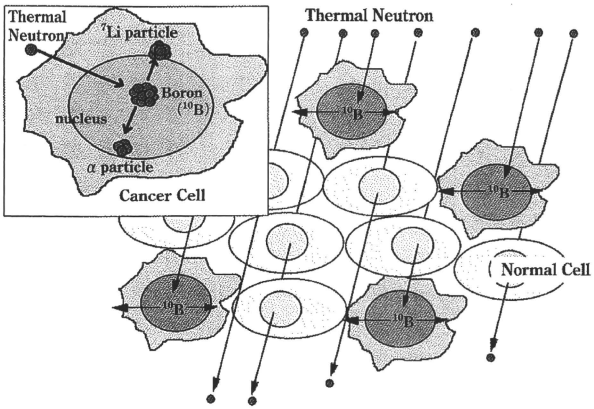


Fig. 1　Principle of BNCT

的治療を上回る成績を残さなければ新しい標準的治療となりえない．

2002年以来，われわれは京都大学原子炉実験所との共同研究を展開し，85例の悪性脳腫瘍症例に腫瘍選択的粒子線治療であるホウ素中性子捕捉療法（Boron Neutron Capture Therapy：BNCT）を実施してきた．ようやくTMZ導入前の新規診断GBMでの生存解析が可能となり，いまだpreliminaryではあるが，その治療成績をまとめることができるようになった．本稿ではBNCTの特徴，適応，成績を紹介し，併せて本治療を前提とした外科治療の役割，ならびに副作用として避けて通ることの難しい放射線壊死に対するわれわれの治療法を紹介する．

ホウ素中性子捕捉療法（Boron Neutron Capture Therapy：BNCT）とは

BNCTは原理上腫瘍に対する細胞選択的照射が可能な唯一の放射線治療法である．ホウ素（^{10}B）化合物を投与し，その後，熱中性子もしくは熱外中性子を照射する．ホウ素化合物自体には細胞毒性はなく，また中性子の殺細胞効果もごく小さいが，ホウ素同位体^{10}B原子核は中性子を捕獲し，きわめて線エネルギー付与（粒子が1μm運動する間に周囲に付与するエネルギー：keV/μm）の高いヘリウム原子核（α粒子）とリチウム反跳核をそれぞれ，9μmと4μmいう，細胞1個に相当する距離に放出し，その細胞を破壊する細胞選択的な粒子線治療ともいえる（Fig. 1）[2]．すなわち殺細胞効果はホウ素中性子捕獲反応の生じた細胞に限局され，近隣の細胞には影響を及ぼさない．そこで，ホウ素化合物を腫瘍に選択的に集積できれば，腫瘍選択的な細胞破壊が可能となる．

BNCTの概念は古く，1930年代にすでに提唱されており[19]，1950年代には悪性グリオーマに対して，実際に治療が施行されたが，その結果は満足すべきものではなかった[3]．その原因は深部への中性子線量の不足，腫瘍と正常脳間のホウ素化合物の濃度比および腫瘍内の絶対濃度が十分でないことが挙げられる．これらの問題を解決すべく，われわれはいくつかの改良を行っている．中性子の深部到達性を高めるべく，熱外中性子の利用を開始した．これにより，非開頭での照射が可能となった．次いで，集積機序の異なる2種類の化合物BSH（borocaptate）とBPA（boronophenylalanine）の併用を行っている．BSHは破綻した血液脳関門より受動的に腫瘍組織に移行し，BPAは亢進した蛋白代謝を利用して能動的に腫瘍組織に蓄積される．また，フッ素ラベルしたBPAをトレーサーとして利用することにより，PETにより腫瘍内および脳内BPA濃度が推測され，治療の適応決定および照射線量がsimulationできる．

悪性グリオーマに対するBNCTの治療効果

Fig. 2に再発GBM症例のBNCT前後の画像を供覧する．48時間の経過で造影域の70%が消失している[7]．経験したすべての症例で画像上の改善効果を認め[11]，かつ

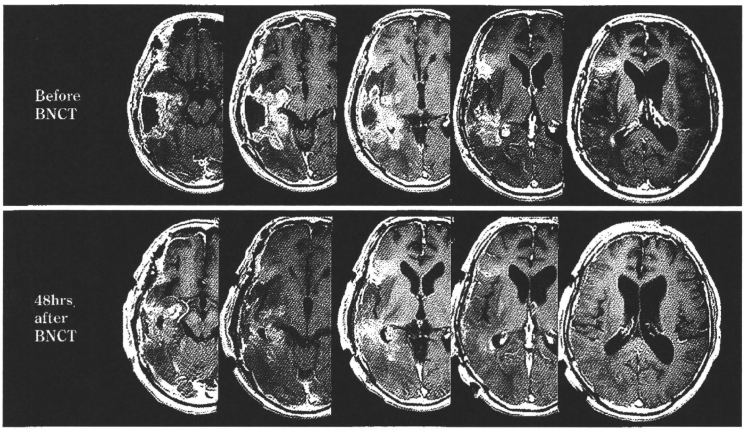


Fig. 2 Early effects of BNCT for recurrent GBM
Seventy % of ehanced mass disappeared in 2 days.

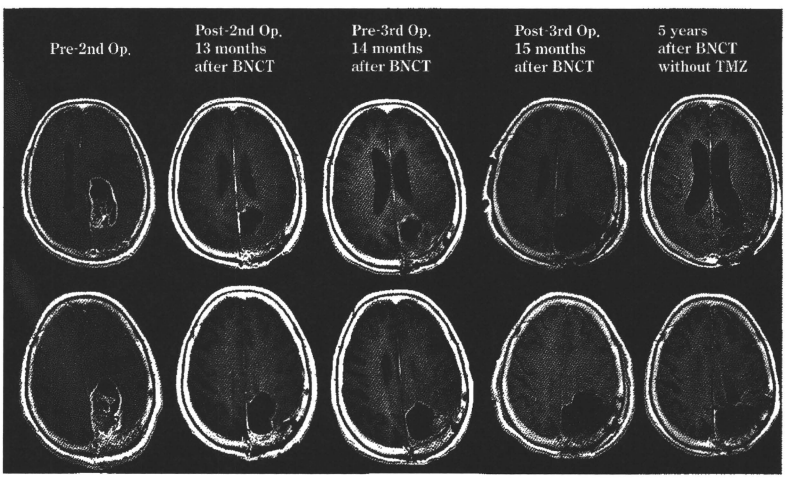


Fig. 3 Periodic change in a newly diagnosed GBM patient after BNCT

多くの症例で BNCT 後に pseudoprogression を経験している[14]．このように，再発例に対しても治療効果を認めたが[7)11)]，前治療として X 線の分割照射が選択されていると，放射線壊死の可能性が高くなり，また，これを避けるため線量不足になる傾向がある．放射線壊死については後述する．

次いで，新規診断 GBM に対する治療効果を紹介する．代表例を **Fig. 3** に供覧する．この症例は左頭頂葉に存在

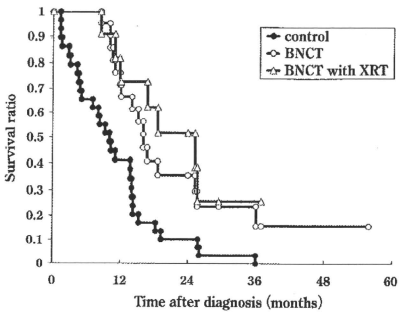
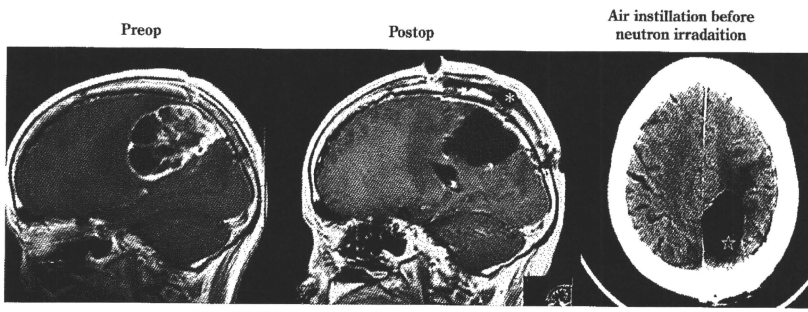


Fig. 4 Survival benefit of newly diagnosed GBM patients treated by BNCT
Closed circle, open circle and open triangle show the overall survaival of historical control, BNCT-treated group and BNCT with XRT-treated groups, respectively.



Air instillation	with	without
Minimum tumor dose (Gy-Eq)	26.9	18.9
Maximum brain dose (Gy-Eq)	9.07	12.7

Fig. 5 Effects of air instillation in tumor-removed cavity
＊：Ommaya's reservoir，☆：air

する最大径 7 cm の GBM であり，近医で生検術のみ行われ，当院に紹介となった．開頭術にて gross total resection を行い，後述の空気置換の後，BNCT を施行．その後，深部線量の不足およびホウ素化合物の不均一分布を補うため 30 Gy の X 線外照射を追加した．

TMZ などの化学療法は追加せず，経過観察を続けていた．BNCT 13 カ月までは何ら症状も画像上増悪もなく経過したが，14 カ月後に造影域の再燃，軽度麻痺の出現を経験し画像上再発の可能性もあり，再開頭により造影域を摘出した．組織は放射線壊死のみであり，腫瘍再発はなく，その後も壊死に対する治療を 2 年続けたのみであり，5 年を経過した現在も再発はない．この症例には再発を疑った段階で 2 回目の BPA-PET を施行し，病変部でのトレーサーの取り込みの低下を確認しており，この症例以降，腫瘍再発と放射線壊死の鑑別に本 PET を活用している[10]．

2007 年までに経験した新規診断 GBM に対する BNCT の治療成績を **Fig. 4** に供覧する．当施設で経験した historical control 27 例，化学療法未施行の BNCT 治療群 21 例，BNCT プラス外照射 11 例の MST はそれぞれ 10.3，15.6，23.5 カ月であり，コントロールと比較して有意な成績を達成することができた[8]．Yamamoto ら[21]もわれわれと同様の治療成績を報告している．TMZ 抜きでは GBM の治療が倫理的に許されない現在，「BNCT+X 線外照射+TMZ」の多施設共同試験を展開中であり，近い将来この成績を公開し，標準治療化の第一歩としたい．

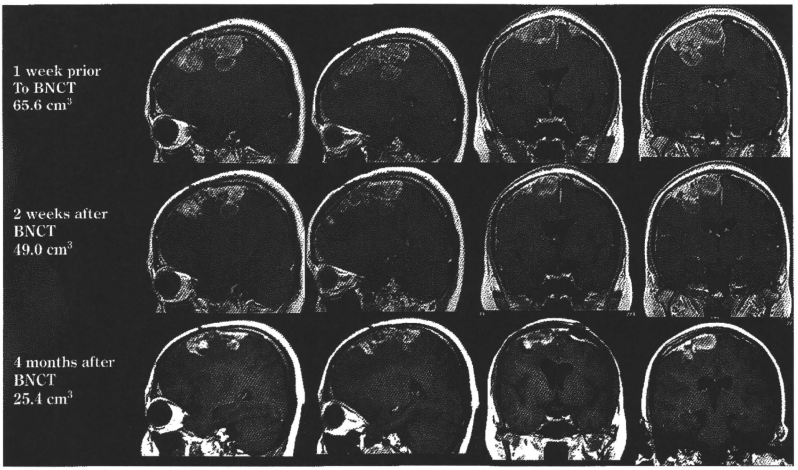


Fig. 6 Concept of BNCT

Effects of BNCT on a recurrent malignant meningioma.
The tumor volume doubled for one month, while waiting for BNCT, which induced hemiparesis. One week after BNCT, she could walk again

また，再発 GBM にも BNCT を積極的に適応し，他の治療では達成できない，再治療後の MST 11 カ月という成績を示している（データ未提示）[15]．再発症例では腫瘍最深部が頭皮より 5 cm 以内であれば，追加切除なしに照射を行っているが，これより深い症例には再摘出のうえ，空気置換を行った後，BNCT を施行している．再発症例ではすでに 60 Gy の X 線外照射が行われており，空気置換により深部腫瘍線量を増加させ，正常脳への吸収線量を減じることがその目的である．しかしながら，BNCT といえど再発例に適応すると，放射線壊死が大きな問題となる．後述のように放射線壊死の治療が奏効すれば再発症例に対する治療成績はさらに伸ばせるものと期待している．

BNCT を前提とした外科治療

残念ながら，GBM では手術による治癒は望めない．また，BNCT にも欠点があり，いかに深部深達性に優れた熱外中性子を使用しても，脳表より 6 cm を超えるような大きな腫瘍に対しては，腫瘍最深部には十分な中性子束の付与は期待しがたい．深部腫瘍に十分な吸収線量を付与しようとすると，正常脳の吸収線量も増加する．そこで Fig. 2 で紹介した患者に用いたわれわれの工夫を紹介する．大型の悪性グリオーマでは腫瘍を可及的に摘出後，腔内に Ommaya's reservoir を設置している（Fig. 5）．中性子照射直前に摘出腔内の髄液を reservoir より排除し，ここを空気で置換した．この操作により腫瘍最深部には空気置換なしに比較して，1.4 倍の吸収線量の付与が可能となり，良好な治療経過に結びついたものと推論している[16]．この症例以降，大型の腫瘍にはこの空気置換を行っている．

悪性髄膜腫に対する BNCT

全髄膜腫のうち数％を占める悪性髄膜腫（WHO grade 3）は GBM 同様きわめて予後不良である．われわれは 15 例の難治性悪性髄膜腫に BNCT を適応し，全例に画像上の改善を認めている[12]．代表例を Fig. 6 に供覧する．5 度の手術，5 回の SRS で制御できなかった悪性髄膜腫であるが，BNCT 直後から歩行障害の改善を認め，腫瘍体積の著減を経験した[18]．ただしこの症例は 2 年後に対側への伸展および全身への転移で亡くなっている．亡くなった症例の多くが全身転移および脳脊髄腔内の播種であり，悪性神経膠腫での播種と同様，解決できていない

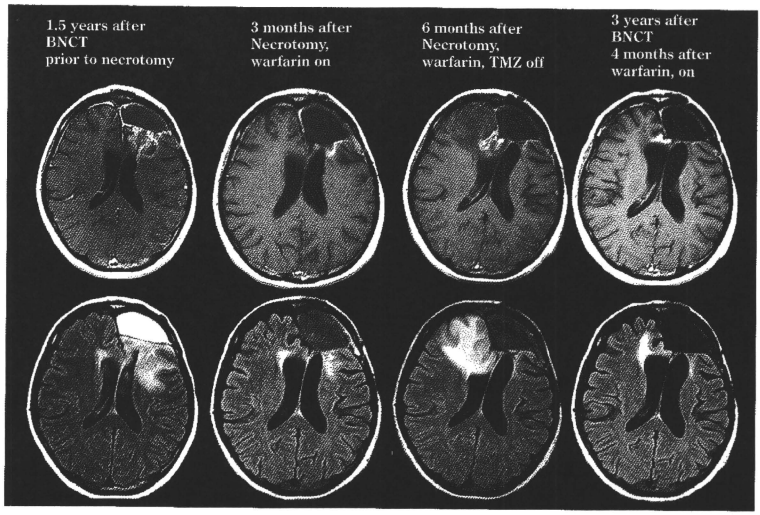


Fig. 7 Effects of surgical removal and anticoagulant therapy on radiation necrosis in the brain

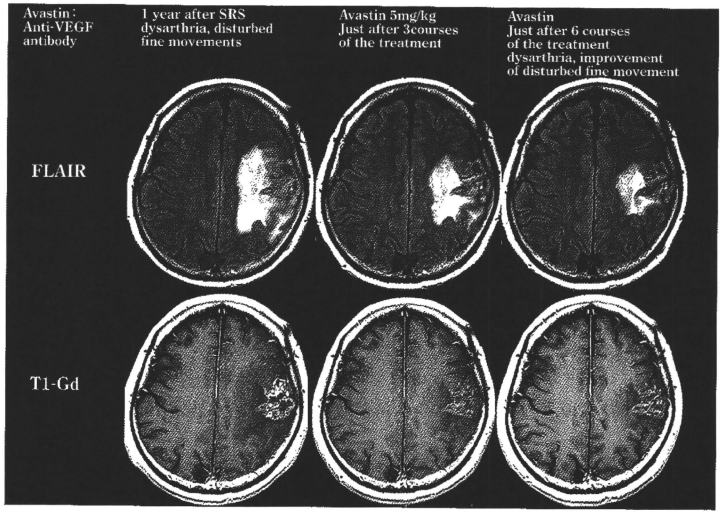


Fig. 8 Effects of bevacizumab on radiation necrosis

大きな問題である[12].

脳放射線壊死に対する外科治療も含めた積極的な治療

BNCT，粒子線治療，強度変調放射線治療，定位放射線治療（SRS）などの高線量放射線治療は，確実に悪性脳腫瘍の生命予後を改善しているが，高線量放射線治療の宿命として脳放射線壊死は避けて通れない大きな問題である．細胞選択的粒子線治療であるBNCTといえども，先行するX線照射歴のある再発症例ではほぼ不可避である．また，造影MRIなどの通常の画像診断では腫瘍進展と放射線壊死との鑑別も困難であり，われわれはBNCTに用いているBPA-PETを両者の鑑別に使用している[10]．この検査により放射線壊死が疑われれば，まず，抗凝固療法等内科的治療を考慮する[4]．この治療に反応せず，症候性となった場合は積極的な壊死巣除去を行っている．また，この壊死巣除去の手術に際して摘出範囲の決定には5-ALAによる蛍光ガイドが有用である[13]．壊死巣除去および抗凝固療法の有効例を供覧する（**Fig. 7**）．この症例では壊死巣除去後も抗凝固療法を続けていたが，妊娠を希望されたため，催奇形性のあるワーファリンおよびTMZを中断しところ，壊死巣の再燃を認め，再度ワーファリンのみ再開し，画像，症状とも落ち着いている．

ただ，症候性放射線壊死に対しても手術による壊死巣摘出が症状の増悪をきたす恐れがある場合や，抗凝固療法が奏効しない症例も多い．最近抗VEGF抗体であるベバシズマブ（商品名アバスチン）が放射線壊死に有効であるとの報告が発表され[5]，われわれも手術不能例には適応している．代表例を**Fig. 8**に供覧する．左運動野に存在する転移性脳腫瘍に対してSRSによる放射線治療後に発症した症候性放射線壊死に対して，5 mg/kgのベバシズマブを2週ごと6回投与した症例の経過である．放射線壊死であるという診断さえ正しければ本治療により，全例改善している．なぜ，壊死巣除去やベバシズマブが浮腫の軽減に有効であるかは現在投稿中であり，別稿で明らかにしたい．また放射線壊死に対するベバシズマブの投与は適応外使用にあたり，現在高度医療として厚生労働省に申請中であることを付記しておく．

おわりに

腫瘍選択的粒子線治療であるBNCTの原理，適応，膠芽腫，悪性髄膜腫に対する治療成績，さらには放射線壊死に対する治療戦略を紹介した．TMZを併用した，新規診断GBMに対する多施設共同研究を展開中であり，より客観的な成績評価が可能となるものと期待する．また，サイクロトロン型小型加速器はすでに完成しており，まもなくこれによる治験を開始する予定である．これが予定どおり稼働すれば，原子炉を必要としない院内BNCTが可能となるものと期待される．

謝　辞

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要　旨

悪性脳腫瘍に対する最新放射線治療とその成績
—放射線治療における外科治療の役割—

宮武　伸一

悪性脳腫瘍，ことにグリオーマに対する放射線治療は標準的治療として，1日1回2Gy，総線量60GyのX線分割照射が確立されている．しかしながら，グリオブラストーマに話を限ると，術後この標準的放射線治療を行っても，生存期間中央値（MST）は12カ月であり，夢の新薬といわれたテモゾロミドを併用してもMSTを2.5カ月延長するにすぎず，満足すべき状況とはいえないのが現状である．

第30回日本脳神経外科コングレス総会PS1-1「悪性グリオーマ治療の進歩」では，最新の放射線治療として，陽子線，炭素線などの粒子線治療の特徴と適応ならびに腫瘍選択的粒子線治療であるホウ素中性子捕捉療法（BNCT）の特徴と治療成績，ならびに放射線壊死の治療について紹介を行った．またコングレス総会では「放射線治療における外科治療の役割」という副題をいただいたので，BNCTを前提とした手術の工夫および放射線壊死に対する外科治療を紹介した．本稿では，限られた誌面の都合もあり，BNCTをmainに紹介し，放射線治療における外科治療の役割を述べる．

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新時代の脳腫瘍学

—診断・治療の最前線—

V. 脳腫瘍の治療

脳腫瘍の放射線療法

中性子捕捉療法

川端信司　　宮武伸一

脳腫瘍の放射線療法

中性子捕捉療法

Neutron capture therapy

川端信司　宮武伸一

Key words：悪性神経膠腫，加速器，膠芽腫，ホウ素中性子捕捉療法，PET

はじめに

悪性神経膠腫の治療上最大の難題となるのは，正常脳組織に浸潤性に存在する腫瘍細胞であり，これを治療の標的としなければ予後の改善は見込めない．腫瘍の画像上の造影域を手術ですべて摘出しえても，周囲脳の浸潤細胞からの再発は免れず，治療には放射線・化学療法を組み合わせることが必須となる．

画像診断を基に治療医が治療計画を行う定位的照射は，開頭手術における治療計画と同様，浸潤部からの再発や正常脳の損傷が問題となる．ホウ素中性子捕捉療法(boron neutron capture therapy: BNCT)は，局所高線量による放射線治療という性格を有しながら，腫瘍を細胞レベルで標的とし，正常脳に浸潤した腫瘍細胞をも選択的に治療できるという‘細胞選択的粒子線治療’であり，画期的な治療法として注目される[1]．

1. BNCTの原理・背景

ホウ素の同位体である^{10}B(ホウ素-10)が中性子と核反応を生じ，そこから生じたヘリウム原子核(α粒子)とリチウム(Li)反跳核により腫瘍細胞を破壊する‘ホウ素中性子捕捉療法(BNCT)’の理論は，1936年に既に米国のLocherにより提唱されていた．この反応は非常に弱いエネルギーの中性子で得られ，しかも生じるこれらの粒子の飛程がほぼ腫瘍細胞1つ分に相当するため，腫瘍選択性のある^{10}B化合物をあらかじめ患者に投与しておき，化合物が腫瘍に十分集積し，かつ正常脳・血中の濃度が低下した時点で患部に中性子を照射すれば，腫瘍細胞のみが選択的に破壊されるわけである(**図1**)．中性子の発見が1932年であり，そのわずか4年後には既に治療への応用が期待されていたが，大量の中性子線や腫瘍選択性のホウ素化合物を必要とし，当時は夢のような治療であった．

2. BNCTの臨床

a. 新規診断悪性神経膠腫に対する臨床研究

BNCTの原理が提唱されて以後，臨床応用へ向けた開発研究が進められた．その結果，1951年には医療用原子炉(ブルックヘブン国立研究所(BNL)研究炉，米国)が作られ，1953年から脳腫瘍患者に対するBNCTが開始された．BNLおよびその後のマサチューセッツ工科大学炉(MITR)での臨床研究は1961年に終了し，当時のホウ素化合物が腫瘍選択性に乏しかったこと，熱中性子線の深達性が悪いことなどから，血中ホウ素濃度が高く，正常組織の障害が生じた[1]．その後改良が行われ，米国では単剤のホウ素化

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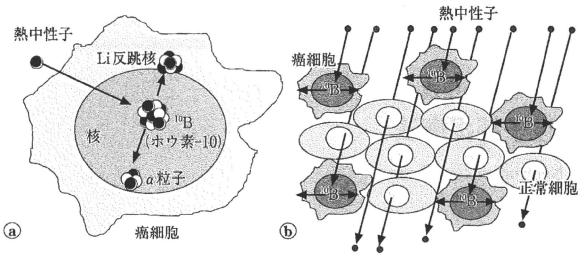

図1　ホウ素中性子捕捉療法（BNCT）における細胞選択的照射の概念図

BNCTでは，腫瘍選択性を有するホウ素-10（^{10}B）化合物を投与し，低エネルギーの中性子を照射することで，^{10}Bが中性子と核反応を生じ，そこから生じたヘリウム原子核（α粒子）とLi反跳核で，腫瘍細胞を選択的に破壊する（a）．ホウ素化合物が選択的に腫瘍に十分集積し，かつ正常脳・血中の濃度が低下した時点で患部に中性子を照射すれば，腫瘍のみが浸潤部においても選択的に破壊され，正常細胞は温存される（b）．

合物（boronophenylalanine：BPA）を用い，組織深達性で勝る熱外中性子を用いた非開頭照射が1999年まで行われたが，生存期間は13-15カ月と治療効果はわずかであり，中性子照射線量の増加を試みたところ，生存期間が延長したが深刻な中枢神経合併症が生じ[2,3]，現在米国でのBNCTは困難となっている．

欧州においては，これまでにオランダ，チェコでのBSH（sodium borocaptate）を用いた臨床試験，スウェーデン，フィンランドでのBPAを用いた臨床試験などがある．注目すべきは最近スウェーデンのグループが行ったBPAの投与量増量試験（900 mg/kg）であり，これによってより均一に高濃度のホウ素を腫瘍に集積させる試みである．2001-03年に本手法で新規診断膠芽腫を治療し，生存期間中央値（MST）が17.7カ月（n=29）と，BNLの成績（BPA 250-330 mg/kg，MST 12.8カ月，n=53）[2]に比較して有意に良好であった[4]．また，BNCTにテモゾロミドを併用することで，治療成績が向上することも示している．

我が国でも1968年から日本原子力研究開発機構（JAEA）などでBNCTが行われるようになり，Nakagawaらは149例の神経膠腫に対しBSH単剤による開頭術中中性子照射（1968-95年）を行い，膠芽腫の平均生存期間は21.3カ月と報告している[5]．著者らが利用する京都大学原子炉実験所（KURRI）では，1990年から悪性神経膠腫に対する治療が行われたが，多くの再発例を含みながらも，3年生存率で20％以上と従来の治療に比べ約2倍に向上した[6]．しかし，当時の熱中性子では，深部での十分な線量が得られず，更なる改善が必要であると考えられていた．

現在までに臨床研究で用いられてきたホウ素化合物は，BSHとBPAのみであり，従来は単剤で使用されてきた．BSHは分子量が大きいが，1分子あたりに多量のホウ素が含まれ，BPAはフェニルアラニン骨格を有し，アミノ酸代謝の活発な腫瘍細胞に高集積を示す．BSHは血液脳関門を通過しない点，BPAは腫瘍とコントラストがあるものの正常脳にも分布する点，および細胞周期に依存しやすい点など一長一短がある[7]．

著者らの施設では2002年から，熱外中性子を用いた非開頭BNCTにこれら2種類のホウ素化合物を併用することで両者の欠点を克服する試みを開始し[8,9]，新規診断膠芽腫のMSTは15.6カ月（n=23）と，それまでの施設コントロールを有意に上回った（**図2**）．2004年以降著者らは，BPAの増量（700 mg/kg）および20-30 GyのX線分割外照射をBNCTに組み合わせ，MSTが