特集2

グリオーマの新しい治療薬 temozolomide（TMZ，テモダール®）の最近の話題

“Pseudoprogression”と「硼素中性子捕捉療法」

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SUMMARY

pseudoprogression（psPD）とは強力な治療の直後に画像上の増悪を一時的に示す現象をさし，neuro-oncologyの分野では最もホットなトピックスである．近年temozolomideと放射線の併用時に悪性グリオーマで起こる変化として注目されているが，我々が悪性脳腫瘍の治療に用いている硼素中性子捕捉療法（BNCT）では，化学療法抜きでこの現象を高率に認めている．また，true progressionとpsPDの鑑別は難しいが，我々はアミノ酸PETの一種であるフッ素ラベルBPAを用いたPETにより鑑別を行っている．psPDの組織所見はほとんどが壊死であり，MIB-1 indexも低値を示し，多くの場合自然軽快し，治療法の変更は必要がなく，経過観察で十分である．またBNCT後のpsPDは単に悪性グリオーマでのみ認められるものではなく，悪性髄膜腫でも高率に認められる．

Key Words
硼素中性子捕捉療法
F-BPA-PET
悪性神経膠腫
pseudoprogression
temozolomide

はじめに

temozolomide（TMZ）の開発，発売以来，世界的にもX線による放射線治療とTMZの併用治療が悪性グリオーマ（MG）の標準治療として行われている[1]．Brandsma等の優れた総説によれば[2]，このレジメンで治療したMGのうち，治療開始後3ヶ月の間に40％の症例で画像上の増悪を認めるが，このうちの半数は組織学的には壊死が主体であり，新たな治療を行わなくても，その病変は不変もしくは縮小をきたすことが多く，“pseudoprogression”（psPD）という概念が紹介されている．

一方，我々は悪性脳腫瘍の治療として腫瘍特異的粒子線治療である硼素中性子捕捉療法（boron neutron capture therapy: BNCT）を第一選択として治療に用いてきた[3,4]．本治療法による腫瘍縮小効果は著しいが，そのうちの多くの症例において，治療数日～数週後の画像では著明な縮小効果を確認したが，その後数ヶ月の経過では造影域ならびに浮腫の再増大をきたし，tumor progression（TP）を疑った．初期の何例かには組織診断確定のための開頭術を追加したが，そのほとんどは壊死で占められていた．本稿ではこの経験を元に，psPDの代表例を提示し，画像上の特徴，TPとの鑑別法，治療法などを紹介する．なお，詳細は既に発表している英文誌をご覧頂きたい[5]．

1344-0128/09/¥100/頁/JCLS

代表症例

症例番号はBNCTで治療した症例に対する通し番号であり，表1の番号に対応する．

【症例5】

新規診断膠芽腫．BNCT後の造影MRIを**図1**，A-Dに示す．BNCT 1ヵ月後に劇的な腫瘍縮小効果を示したが，2ヵ月後には広範な浮腫を伴う造影域の再増大を認め，TPを疑い，開頭術を追加した．**図1F**にその時の組織像を示すが，広範な壊死を認め，活発な腫瘍の増殖は認めなかったので，手術は生検にとどめた．MIB-1indexも1%以下であった．BNCT 10ヵ月後に髄腔内播腫で症例を失ったが，原発治療巣は縮小し，よく制御できている（図1D）．

【症例35】

新規診断膠芽腫．BNCTおよびそれに続く30 GyのX線分割照射で治療した．最近のプロトコールでは新規MGに対して，TP予防のため，X線の分割照射を追加している[9]．治療の経過を**図2, A1-4**に示す．この症例も治療1ヵ月後には顕著な縮小効果を示したが(A-2)，2ヵ月後には再増大を認めた（A-3）．この症例は経過観察のみで，7ヵ月後には腫瘍は縮退したが(A-4)，その後髄腔内播腫で症例を失った．

【症例56】

悪性髄膜腫（WHO grade 3）．数回の手術，X線照射後の再発例．BNCT後の経過を**図2, B1-4**に示す．治療1.5ヵ月後には腫瘍内部の造影が抜け，やや縮小しているが（B-2），2.5ヵ月後には造影域の増大と浮腫の拡大を認めた（B-3）．その後再度腫瘍内部の造影域は縮小した（B-4）．

考　察

2002年に非開頭BNCTを開始して以来，悪性グリオ

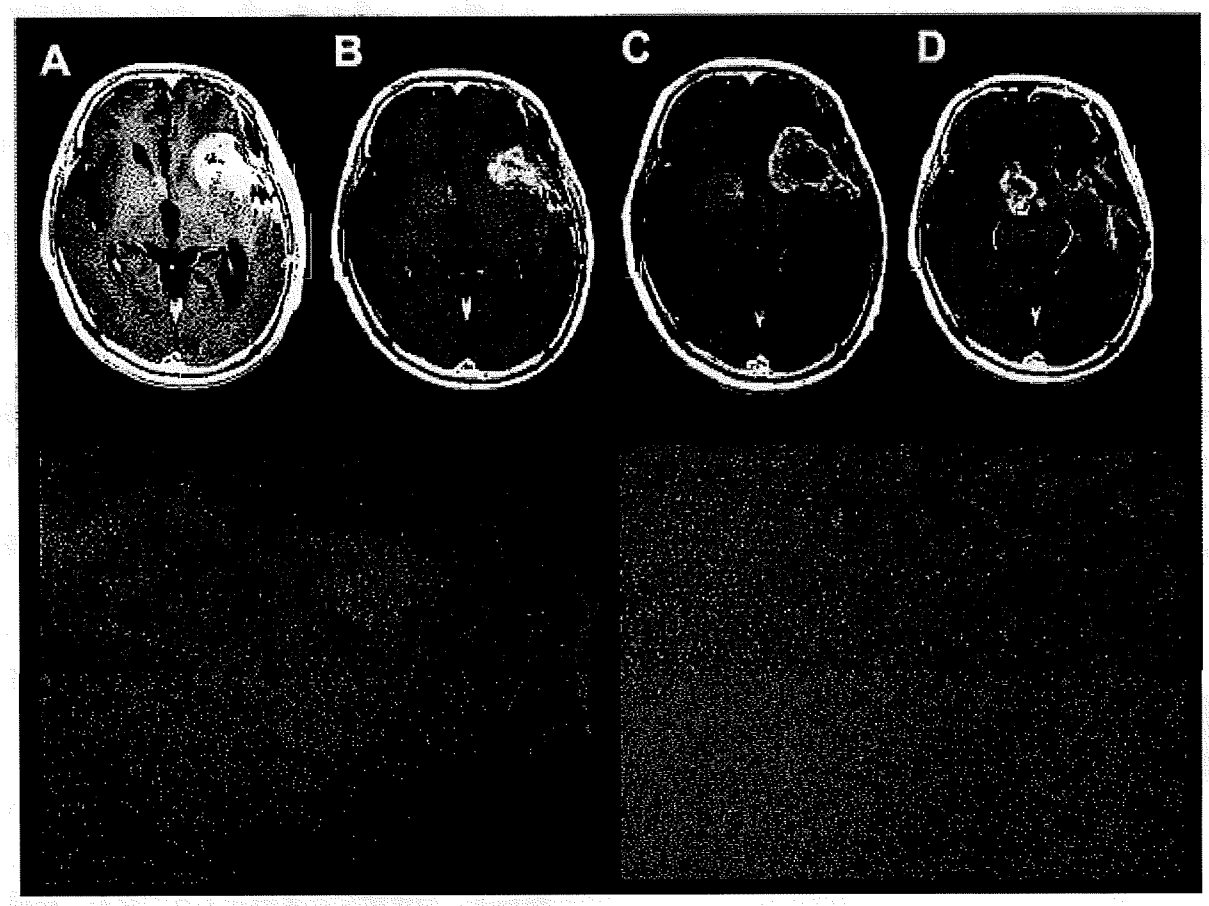

図1　症例5の経時的造影MRI (A-D) と組織所見 (E,F)

A：BNCT前，B：BNCT 5週後，C：BNCT 2ヵ月後，D：BNCT 6ヵ月後，E：初回手術時組織，F：BNCT 3ヵ月後の生検時組織

(p.4カラー図参照)

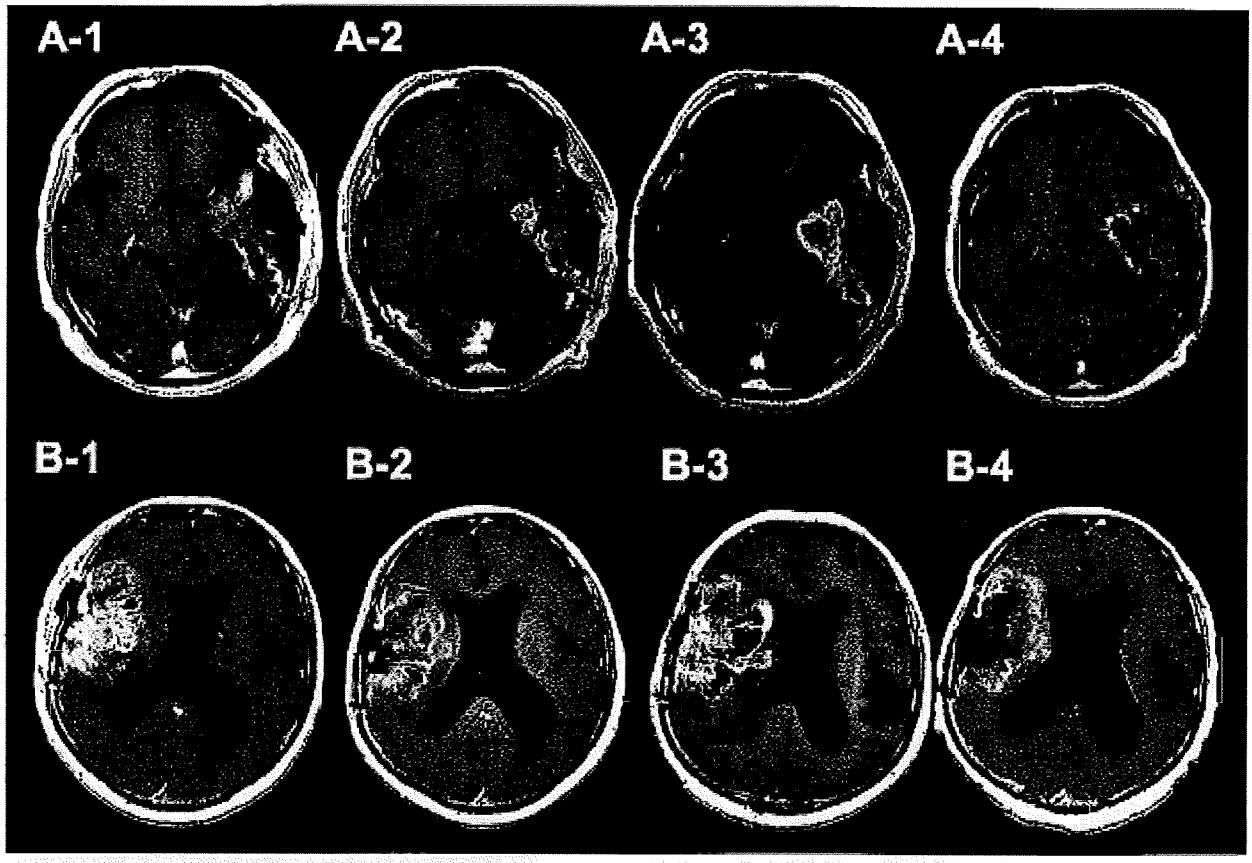

図2　症例35の経時的造影MRI（A-1, 2, 3, 4）．症例56の経時的造影MRI（B-1, 2, 3, 4）
A-1：BNCT前，A-2：BNCT 4週後，A-3：BNCT 2ヵ月後，A-4：BNCT 7ヵ月後．B-1：BNCT前，B-2：BNCT 6週後，B-3：BNCT 10週後，B-4：BNCT 6ヵ月後．

ーマ52例，悪性髄膜腫13例に延べ81回のBNCTを施行してきた．そのうちの多くの症例で，治療開始早期に急激な腫瘍の縮小を認めた後，再増大をきたした．当初はTPを疑い，開頭術による部分摘出や生検術を行ってきたが，ほぼすべてで壊死巣が主体を占る病理所見であった．筆者自体もその病態が把握できていなかったが，2007年にChamberlain MCらがpsPDを発表して以来[7]，我々の症例もこの概念に相当するpsPDと考えている．そこで，我々が経験したBNCTの症例の画像をretrospectiveにreviewしてみた．その結果を表1に示す（文献5より改編）．ここではBNCT 3ヶ月以内に画像上再増大をきたした症例をreviewしている．悪性グリオーマ52例の内12例で，悪性髄膜腫13例の内では3例にpsPDと思われる画像変化を認めた．BNCT全症例の画像が3ヵ月以内のbest timingで得られた訳ではないが，高率にこの現象を認めたことは確かである．

前述のように，これらの症例は当初TPを疑ったが，組織所見はほぼ壊死巣であり，MIB-1 indexも初回手術時より激減していた．そこで，後半の症例では開頭術を適応せず，BNCT前に行っているF-BPA-PETでの再検を行ってみた．表1の2nd PETでのL/N（病変/正常脳）比に示すように，psPDを疑ったすべての症例でBNCT前のL/N比より低下を示していた．よって，その後の経過は観察に留めたところ，画像上は軽快を示した．この観察に先立ち，我々は放射線壊死とTPとの鑑別にこの解析を行っており，経時的なPETの解析により，その診断が可能となることを経験していた[8]．このことが，psPDの診断にも役立ったと考えている．総説(2)によれば，TMZとX線併用後のpsPDでは化学療法の変更は慎むべきであると警告している．よって何らかの方法により，psPDとTPとの鑑別は必要であるが，現時点ではこのF-BPA-PETは極めて有用であると考えている．今ひとつ，psPD

表1　BNCTにおけるPseudoprogression症例一覧

Case	Histology	new or rec	RT[a] Pre-BNCT	RT[a] Post-BNCT	1st PET L/N	2nd PET L/N	Max BNCT (Gy-Eq)	Min BNCT (Gy-Eq)	Explor-ation
Case 1	GBM	rec	60Gy	—	7	—	64.1	34.4	−
Case 4	GBM	new		SRS	—	—	50.6	23.8	+
Case 5	GBM	new		BNCT[b]	7.8	—	108.7	47.4	+
Case 10	GBM	rec	80Gy		2.8	—	48.3	27.2	+
Case 14	GBM	new		BNCT[c]	5.1	—	141	37.1	+
Case 15	AA	rec	60Gy		3.1	—	55.9	33.7	+
Case 35	GBM	new		30Gy	—	—	90.6	61.4	−
Case 39	GBM	rec	60Gy		3.6	1.6	77.1	58	+
Case 46	GBM	new		30Gy	4.8	2	115	63	−
Case 48	GBM	rec	60Gy		3.3	1.7	50.5	49.2	−
Case 51	GBM	new		20Gy	2.6	1.5	64	44.6	−
Case 57	AA	rec	60Gy		4.7	2.1	104.2	44.9	−
Case 33	MM	rec	60Gy+SRS		2.8	1.8	55.1	29.8	−
Case 50	MM	rec	50Gy		3.2	1.9	75.8	18.8	−
Case 56	MM	rec	50Gy		4.4	—	111.5	50.7	−

RT[a]: radiotherapy. BNCT[b]: BNCT was applied 2 times, because he moved during the neutron irradiation at 1st BNCT.
BNCT[c]: BNCT was intentionally applied 2 times.

の特徴を述べると，症例5では，造影域，浮腫の増大により，失語症の増悪を認めたが，他の症例では画像上の増悪に比して，臨床症状の悪化は軽度である印象を受けている．

総説(2)によるとpsPDはintensive treatmentに伴いやすいとされており，かつ，Brandes AA等によると，psPDは予後良好のサインとしている[9]．ここで挙げた症例はすべて化学療法を行っておらず，BNCT単独（一部はX線との併用）の効果と考えられる．また，我々の観察ではpsPDは単に悪性グリオーマにおいてのみ観察されるものではなく，悪性髄膜腫でも観察された．これらの考察は，BNCTの強力な抗腫瘍効果を証明するevidenceと考えられる．

まとめ

psPDはtreatment-related intratumoral necrosis in subacute phaseであり，基本的には経過観察で十分であり，治療法の変更は必要ではない．

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グリオブラストーマに対する治療戦略
—BNCT, PETによる治療効果の検討およびTailor-made化学療法を用いて—

Therapeutic strategy for glioblastoma, using BNCT, amino acid PET and tailor-made chemotherapy

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抄録

グリオブラストーマ（glioblastoma：GBM）に対するわれわれの最近の治療戦略を紹介する．まず，画像上GBMが疑われれば，フッ素ラベルのboronophenylalanine（BPA）をトレーサーに用いたPETを行う．その後，5-ALAガイド下に可及的摘出を試みる．手術摘出標本よりDNAを回収し．MGMT promoterのmethylation-specific PCRを行っておく．術後はPETの情報を基に，硼素中性子捕捉療法を行い，引き続き20～30GyのX線照射を行う．その後，temozolomide（TMZ）による化学療法と造影MRIを中心とした画像上follow-upを行う．画像上造影域の増大が出現した段階で，再度PETを行い，tumor progression（TP）かradiation necrosis（RN）もしくはpseudoprogression（psPD）かの鑑別を行う．TPと判断したときはさらなる治療を行うが，この際のchemotherapyのregimenは先のPCRの結果によって決定する．RNもしくはpsPDと判断すれば，ステロイド等の投与を1カ月程度行い，その反応を見る．保存的療法で改善がなければ壊死巣の除去を考慮する．

Abstract

Here, we demonstrate our recent strategy for the treatment of glioblastoma (GBM). Prior to any treatment, we apply the PET study using F-labeled boronophenylalanine (BPA) as tracer. This is the reference for further changes of the biological activity of the lesions after a couple of treatments. After PET, tbe lesion is removed by 5-ALA-guided surgery. The methylation status of the MGMT promoter of GBM is analyzed by PCR for each tnmor, jnst after the snrgery. Tben the patients are treated with tumor-selective particle radiation, boron neutron capture therapy (BNCT), based on the data of BPA-PET, followed by 20～30Gy X-ray treatment. After this radiation treatment, patients were followed by chemotherapy with temozolomide. When the lesions show increase in size in enhanced MRI, they are re-analyzed by F-BPA-PET. Depending on this analysis, the lesions are judged as tumor progression (TP), pseudoprogression (psPD) or radiation necrosis (RN). If the lesion is judged as TP, further treatment is applied including tailor-made chemotherapy based on methylation status of the MGMT promoter, as

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described above. If the lesion is judged as psPD or RN, some medical treatments including steroids are applied for the lesions.

Keywords··· ⊛ boron neutron capture therapy ⊛ glioblastoma ⊛ MGMT ⊛ positron emission tomography ⊛ radiation necrosis

はじめに

神経膠芽腫（GBM）の予後は不良であり，EORTCの第三相臨床試験においても，temozolomide（TMZ）の併用によりようやく生存期間中央値（MST）が14.5カ月に伸びたにすぎない．その予後不良の最たる原因は浸潤性発育にあり，造影MRIでの造影域を摘出しても根治は不可能であり，何らかのadjuvant therapyの追加に期待せざるを得ない．本稿では最近のわれわれのGBMに対する治療戦略を紹介し，日々GBMの治療に難渋されている方々，また，何より患者の皆さまに少しでもお役に立つことができれば幸甚である．

以下に2007年度以降のわれわれの戦略を述べるが，生存曲線等のデータは2006年までに治療した症例に基づくことをお許しいただきたい．

治療戦略

1…F-BPA-PET

まず，画像上GBMが疑われれば，フッ素ラベルのboronophenylalanine（BPA）をトレーサーに用いたPETを行う．代表的なPET画像を**Fig.1**に示す．もともとF-BPA（^{18}F-labelled boronophenylalanine)-PETは後述の硼素中性子捕捉療法（boron neutron capture therapy：BNCT）を行う際の適応決定，線量評価のツールとして開発されてきた経緯がある[3,8]．トレーサーはBNCTでの治療薬そのものであり，PET上での病変／正常脳（L/N）比をもって治療計画を立てる．**Fig.1**ではBPAが造影域を越えて腫瘍浸潤部に蓄積していることが読み取れる．すなわちBNCTにより，造影域のみならず，腫瘍浸潤部への効果も担保されている．一方で，本PETが腫瘍の動態をよく反映し，以降の治療

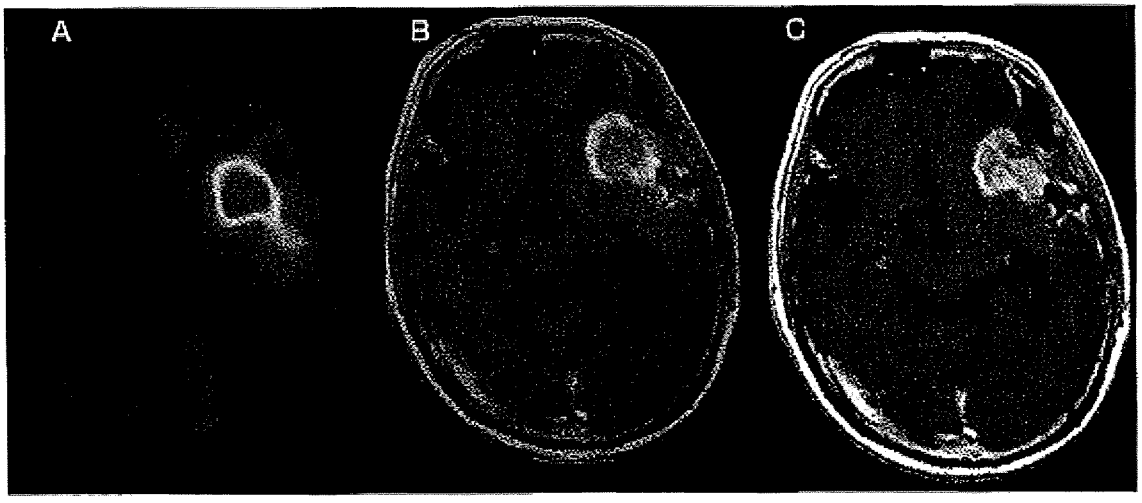


Fig.1 F-BPA-PET of Case 4 (newly diagnosed GBM)
A: F-BPA-PET, B: Fusion image of PET and MRI, C: Gd-enhanced MRI
Lesion/Normal ratio of BPA uptake in this case was 7.8.

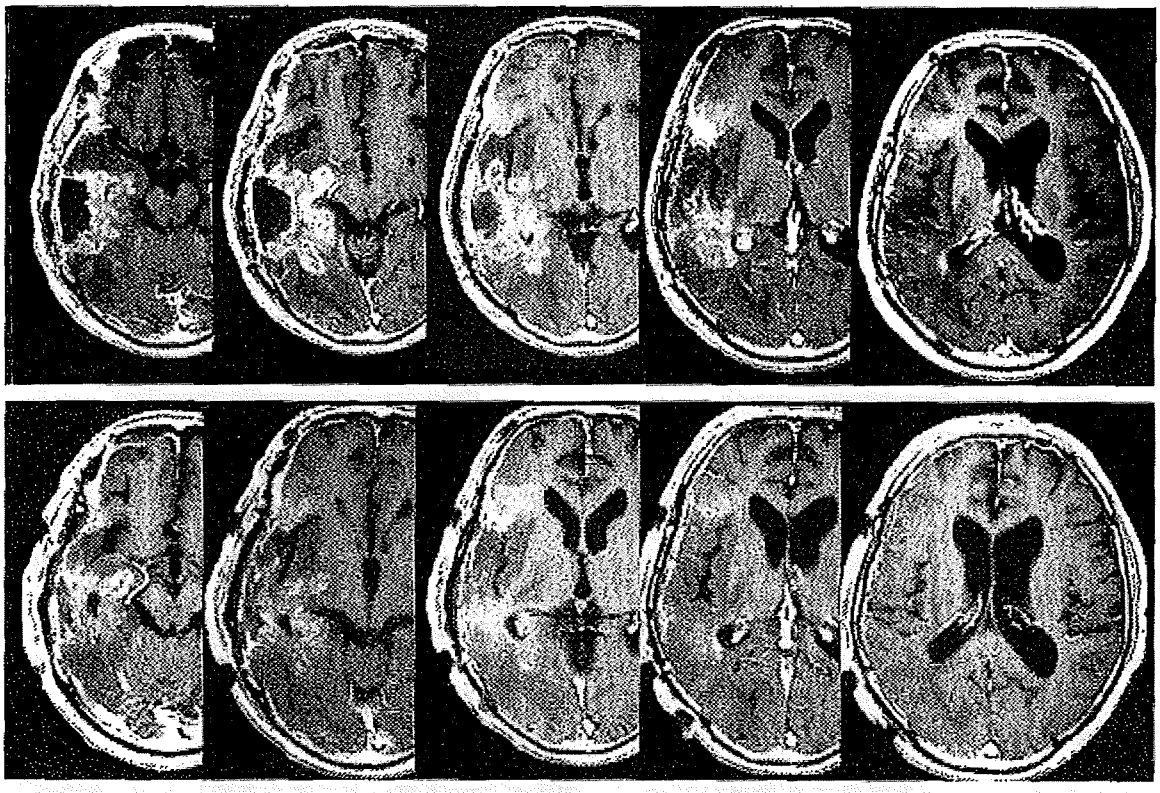


Fig.2 Early Effects of BNCT on recurrent GBM (Case 1)
Upper column shows Gd-MRI taken just prior to BNCT and lower one shows that taken 2 days after BNCT.

の基準を示すことにより，その後の治療の反応性および画像上造影域の増大が出現した段階で，tumor progression（TP）かradiation necrosis（RN）もしくはpseudoprogression（psPD）かの鑑別に役立つことが判明した．これについてはBNCTの紹介後に詳述する．

2…5-ALAによる可及的摘出

紙面の制約により，詳細は拙稿を参照いただきたいが，GBMを安全に摘出するために本法は非常に有用である[7]．

3…硼素中性子捕捉療法（BNCT）

BNCTは腫瘍選択的高線量粒子線治療である．これも紙面の制約上詳細は拙稿を参照いただきたいが，短期間で顕著な腫瘍縮小効果を示す[4,8]．**Fig.2**にわれわれがBSH，BPA併用で経験した最初の症例（GBM再発例）を示す．わずか48時間で70％の造影域が消失した．当初は再発例を対象に治療していたが，画像上の縮小効果が顕著であるため，2004年度以降は新規診断

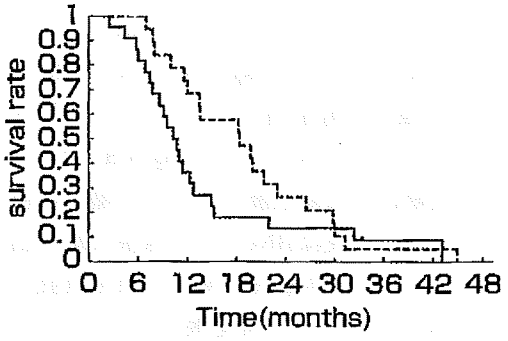


Fig.3 Kaplan-Meier survival curves for recurrent MG cases treated by BNCT
The continuous line shows the survival of all patients after BNCT (n=22). The broken line shows the survival of GBM (on-site histology) after diagnosis of GBM (n=19).

GBMにも積極的にBNCTを応用した．最近，再発悪性グリオーマ（**Fig.3**）および新規診断GBMに対する生存の成績（**Fig.4**）を発表したので[5,10]，ここに示す．再発悪性グリオーマに対しては，recursive partitioning analysisを適

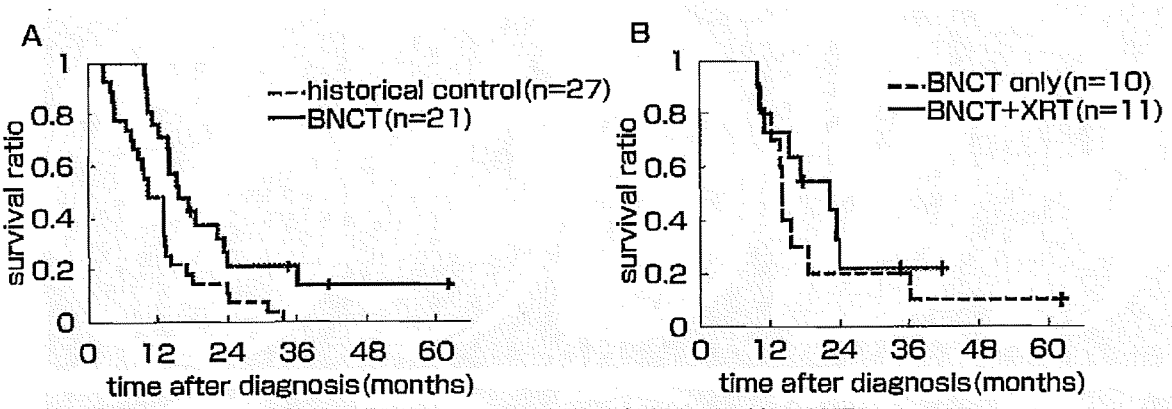


Fig.4 Kaplan-Meier survival curves for newly diagnosed GBM treated by BNCT
Kaplan-Meier survival curves of newly diagnosed glioblastoma patients treated with BNCT.
A: A continuous line represents the survival times of the patients treated with BNCT (protocols 1 plus 2, n = 21), and show an MST of 15.6 months. Four out of 21 cases are still alive. A broken line represents the survival times of our institutional historical controls (surgical removal, XRT and chemotherapy) (n = 27) and show an MST of 10.3 months (log-rank test, p=0.0035).
B: A continuous line represents the survival times of the patients treated with BNCT followed by XRT boost (protocol 2, n=11), and show an MST of 23.5 months. Three out of 11 cases in protocol 2 are still alive. A broken line represents the survival times of the patients treated by protocol 1(n=10), and show an MST of 14.1. There is no statistical significance in the difference in MSTs between these groups.

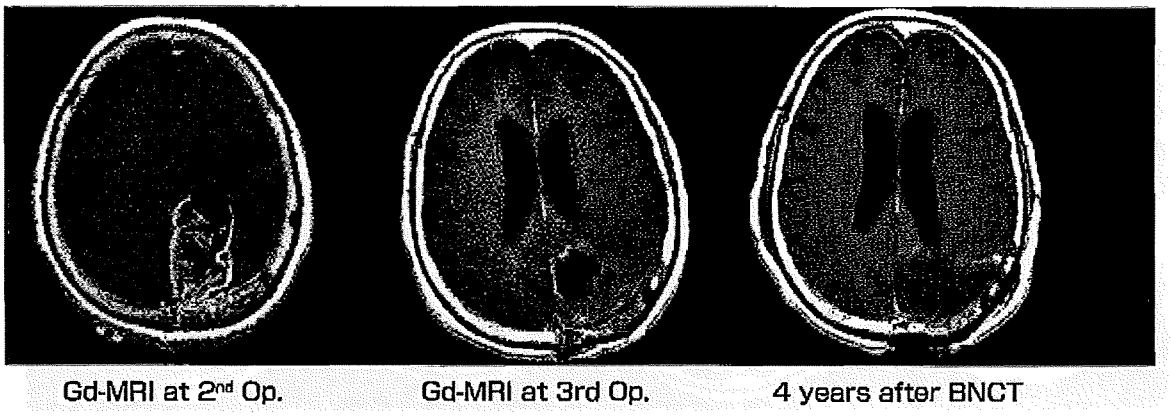
応することにより，本来予後不良群に対しても，BNCTが有意に生存期間延長に寄与できたことを証明した[10]．かつ新規診断GBMに対しても，BNCTはhistorical controlに比して，有意に生命予後を改善し（**Fig.4-A**），BNCTに20～30GyのX線外照射を加えることで，さらに予後を改善することが示された（**Fig.4-B**）[5]．なお，この成績には初期治療として化学療法は施行していない．今後はBNCT，小線量X線の追加とTMZによる化学療法併用での臨床研究を行っていきたい．ただ，再発例ではすでにfull doseのX線が照射され，また，新規診断例でも最近のプロトコルでは20～30GyのX線外照射を加えており，放射線壊死の発生頻度が増加していることも事実である．

4…F-BPA-PETによる病態解析

F-BPA-PETは腫瘍の病態をreal timeに表す．**Fig.5**にTPとRNの鑑別に初めてF-BPA-PETを用いた症例を示す．新規診断GBMに対して，当院で開頭術を行い，BNCTと30GyのX線追加照射で治療した．その後14カ月はCRで経過したが，その後造影域の再燃と軽度麻痺の出現を認めた．TPを疑い再手術を考慮したが，念のため再度BPA-PETを施行したところ，造影域のL/N比は治療前に比べ，顕著に低下していた．造影域を全摘出し，組織をくまなく検索したが，明らかな腫瘍細胞は認めず，RNと診断した．その後，化学療法なしで，4年間再発は認めていない．

この結果を踏まえ，BNCTやその他の高線量放射線治療後に造影域の拡大を示した症例にBPA-PETを適応してTPとRNの鑑別を試みた．治療前のGBMのL/N比は3.5～4.0を示すが，治療後造影域のL/N比が2.0以下であればまず，RNであることが判明した（**Fig.6**）[6]．また，鑑別に悩む症例にはPETを繰り返すことにより，その病態の解析が可能であり，単回の解析よりも病態把握に有用であることが分かった．

次にpsPDの診断に同PETが有用であることを紹介する．psPDとはStuppのregimen等の強力な放射線化学療法後早期に，造影域の縮小後の一時的な再増大のことを表し，最近のneuro-onocologyでの大きなトピックスとなっている[1]．すなわちpsPDなら化学療法の変更は必要



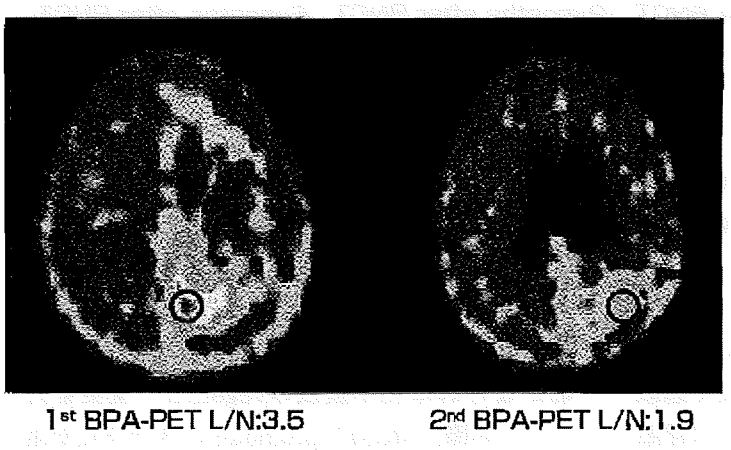


Fig.5 Sequential changes of MRI and F-BPA-PET in Case 26 (Newly diagnosed GBM treated with BNCT followed by XRT)

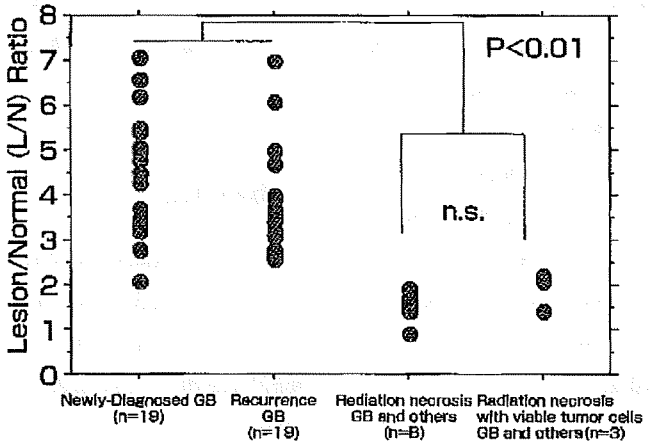


Fig.6 L/N ratio determined by F-BPA-PET
The L/N ratio between GBM (newly diagnosed and recurrent cases) was statistical different from that of histologically verified radiation necrosis.

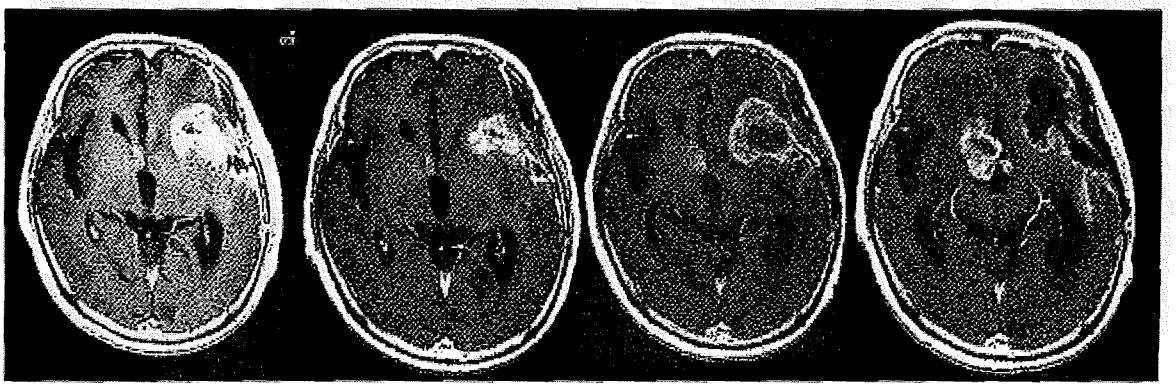


Fig.7 A typical case of pseudoprogression after BNCT

ないが，TPとの鑑別は困難であり，治療法の選択は難しい．われわれはBNCT後化学療法抜きでも高率にpsPDを経験し，これを報告した[9]，その典型例を**Fig.7**に示す．これは**Fig.1**でPETを紹介した症例と同一である．新規診断GBMに対してBNCTを行ったところ，1週間で急激な造影域の縮小を認めたが，2カ月後には浮腫とともに造影域の増大を認め，失語症の悪化を認めた．TPを疑い，開頭術を追加し，組織診断を行ったが，viableな腫瘍は存在せず，かつMIB-1 indexも著減していたため，部分摘出にとどめ経過を観察したところ，治療部位は縮小していったが，髄腔内播種のため死亡した．典型的なpsPDと考えており，詳細は拙著をご覧いただきたい[9]．初期の数例はTPを疑い，多くの症例に開頭術による生検術を追加したが，BNCT後4カ月以内の増大例は全例壊死主体であり，psPDと考えられた．また，症例を重ねるにつれ，psPDを疑った症例に2度目のPETを追加すると全例でBNCT前のL/N比より低下しており，PETでの鑑別が有用と思われた．

5…Tailor-made化学療法

Hegiらの解析[2]によればTMZが奏功するのは，MGMT promoterがmethylationされているpopulationとされており，われわれの観察でもまったく同じ感触を得ている．よって，われわれは初回手術時に摘出した腫瘍よりDNAを回収し，methylation-specific PCRにより，MGMT promoterのメチル化を検討している．TMZ使用にもかかわらず，造影域の増大をきたしたときにはPETによりTPもしくはRNの診断を行い，TPが疑われた折に，追加手術の適応がなければ化学療法のregimenの変更を行う．この際，MGMT promoterのメチル化が陽性ならPCVに，陰性ならprocarbazine（PCZ）併用TMZ投与，もしくはdose-dense TMZに切り替えている．**Fig.8**にメチル化陰性で，TMZ refractory GBMを紹介いただき，当院でPCZ-TMZ併用で著効を示した症例を提示する．PCZ-TMZは何例かに著効を経験しているが，やはり骨髄抑制は必発であり，大きな問題と考える．

おわりに

限られた紙面の都合もあり，考察は省かせていただく．現在われわれが有する知識と技術をつぎ込んで治療に当たっているが，それでも，髄腔内播種，放射線壊死の治療等，解決すべき問題はまだまだ存在する．

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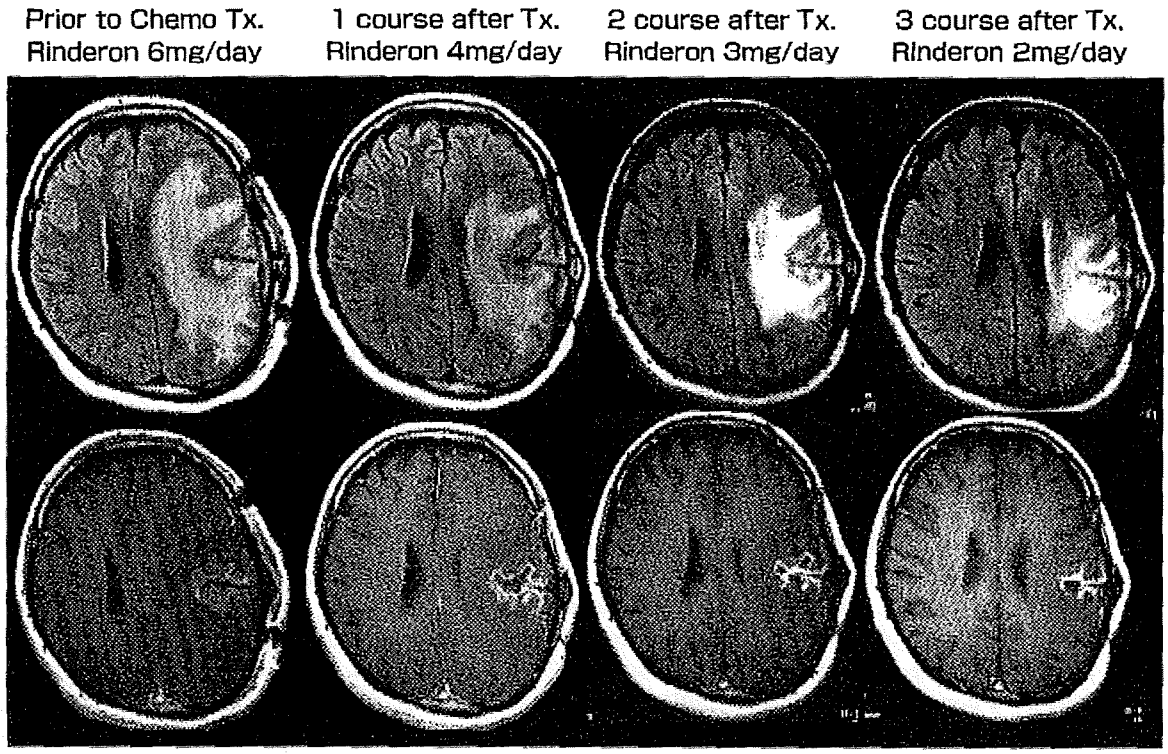

Fig.8 A typical case of tailor-made chemotherapy
The patient was refractory for TMZ chemotherapy with unmethylated promoter of MGMT. She was treated with PCZ and TMZ with good response.

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J. Radiat. Res., 50, 51–60 (2009)

Regular Paper

Boron Neutron Capture Therapy for Newly Diagnosed Glioblastoma

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Boron neutron capture therapy/Boronophenylalanine-PET/Glioblastoma/X-ray radiation therapy.

We evaluate the clinical results of a form of tumor selective particle radiation known as boron neutron capture therapy (BNCT) for newly-diagnosed glioblastoma (NDGB) patients, especially in combination with X-ray treatment (XRT). Between 2002 and 2006, we treated 21 patients of NDGB with BNCT utilizing sodium borocaptate and boronophenylalanine simultaneously. The first 10 were treated with only BNCT (protocol 1), and the last 11 were treated with BNCT followed by XRT of 20 to 30 Gy (protocol 2) to reduce the possibility of local tumor recurrence. No chemotherapy was applied until tumor progression was observed. The patients treated with BNCT (protocol 1 plus 2) showed a significant survival prolongation compared with the institutional historical controls. BNCT also showed favorable results in correspondence with the RTOG- and EORTC-RPA subclasses. The median survival time (MST) was 15.6 months for protocols 1 and 2 together. For protocol 2, the MST was 23.5 months. The main causes of death were cerebrospinal fluid dissemination as well as local recurrence. Our modified BNCT protocol showed favorable results of patients with NDGB not only for those with good prognoses but also for those with poor prognoses.

INTRODUCTION

Surgery followed by radiation therapy is still the standard treatment for glioblastoma (GB). The addition of temozolomide (TMZ) chemotherapy to the standard treatment has significantly increased the proportion of patients who survive longer than 2 years.[1)] However, additional progress is needed, as almost half of GB patients do not survive the first year after diagnosis.

Boron neutron capture therapy (BNCT) has been developed in the hope of achieving a breakthrough in GB treatment.[2,3)] BNCT, a form of tumor-selective particle radiation, comprises a binary approach. First, a boron-10 (^{10}B)-labeled compound delivers high concentrations of ^{10}B to the target tumor relative to the surrounding normal tissues. This is followed by thermal neutron irradiation. When neutrons collide with ^{10}B atoms, the ^{10}B (n, alpha) ^{7}Li neutron capture reaction releases alpha and ^{7}Li particles. These particles have the characteristics of high relative biological effectiveness and high linear energy transfer. In addition, the particles have extremely short tracks (5–9 micrometers), which results in relatively selective tumor cell kill without significant adjacent normal brain tissue damage. Therefore, if sufficient concentrations of boron compounds can be made to accumulate selectively in tumor tissues, BNCT would become an ideal radiotherapy.

Since the 1950s, BNCT has been used to treat high-grade gliomas, although the results have not been satisfactory.[4)] We modified the therapy in several ways to resolve problems previously existing, and applied this modified BNCT to malignant gliomas beginning in January, 2002[2,3)] by using Kyoto University Research Reactor (KUR).

First, we utilized an epithermal rather than a thermal beam to improve the distribution of thermal neutrons in deep sites.[5)] Second, we used both of the boron compounds that are currently available worldwide for BNCT: sodium borocaptate (BSH) and boronophenylalanine (BPA). These compounds reach different subpopulations of tumor cells and

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doi:10.1269/jrr.08043

accumulate in them in a different fashion.[6] BSH is not delivered into the normal brain through the blood-brain barrier, and the concentration of this compound in tumor tissue is related to both its vasculature and its concentration in the blood. BPA accumulates preferentially in the actively proliferating subpopulation. However, some of the compound inevitably accumulates in normal tissue. Therefore, the simultaneous use of both compounds cancels out the disadvantages of each.[7] Third, we used ^{18}F-BPA-positron emission tomography (PET) to estimate the BPA concentrations in the tissues.[8,9]

With these improvements, we were able to apply BNCT without craniotomy and with an accurate estimation of the absorbed dose. By implementing these modifications, we can rapidly shrink malignant gliomas on neuro-images, as reported elsewhere.[2,3]

Five years have passed since we first used this modified BNCT. Therefore, in the present manuscript, we can apply survival analysis to newly diagnosed glioblastoma (NDGB) patients who were treated with BNCT at our institute. To reduce the heterogeneous anti-tumor effects of BNCT and consequently improve patient survival, we combined BNCT with non-selective X-ray irradiation therapy (XRT) for the latter half of NDGB patients. We evaluated the survival results of BNCT, especially in combination with XRT.

METHODS

Patient enrollment

This study was approved by the ethics committee of Osaka Medical College, Takatsuki, Japan, and the Kyoto University Committee for Radiation Therapeutics, Kyoto, Japan. In addition, a written informed consent was obtained from each patient. From 2002 to 2006, we treated a total of 42 patients of malignant glioma using BNCT. Here, we report the results only for NDGB (WHO grade IV, n = 21) patients. Our eligibility criteria for this trial were as follows: 1) supratentorial NDGB (no history of radiation or chemotherapy); 2) no cerebrospinal fluid (CSF) dissemination upon diagnosis; 3) no tumor extension to the opposite hemisphere.

With protocol 1, we treated 10 patients from 2002 to 2004. With protocol 2, we treated 11 patients from 2004 to 2006. None of the patients underwent chemotherapy until tumor progression was confirmed histologically or by BPA-PET, as described below.

For a historical control, we used NDGB patients who were treated by surgical removal followed by XRT and chemotherapy (mainly ACNU, n = 27; 3 out of 27 were treated with TMZ) from 1990 to 2006 at Osaka Medical College, and in accordance with above criteria for BNCT. For the control group, all patients were operated on to achieve maximum tumor removal, as with the patients in the BNCT group, and patients with biopsy only were excluded from the group, as were the patients treated with BNCT at recurrence. From 2002 to 2006, we routinely recommended BNCT as the primary treatment for NDGB patients, however, approximately 4 months every year of the study, atomic reactors were not available for BNCT due to periodic maintenance. During these periods, all NDGB patients were enrolled in the control group.

Clinical regimen of BNCT

An approximate flowchart of our clinical BNCT regimen is depicted in Fig. 1. In both protocols 1 and 2, the patients received a BPA-PET to assess the distribution of BPA and to estimate the boron concentration in the tumors. The lesion/normal brain (L/N) ratio of BPA uptake can be estimated by using the data obtained from those assessments, and was the basis for dose planning as described previously.[2,3] Before BNCT we applied craniotomy to remove as much of the tumor as possible. Within a month after the craniotomy, BNCT was performed. In protocol 1, the patients were administered 100 mg/kg of BSH and 250 mg/kg of BPA for one hour intravenously 12 hours prior and just prior to neutron irradiation, respectively. Blood was sampled every 2 hours after BSH administration until neutron irradiation was completed, to monitor the boron concentration in the blood. The boron concentration from BSH in the blood during neutron irradiation was estimated from the measured ^{10}B concentration -time relationship. From the previous BNCT experience, which was performed with craniotomy, we hypothesized that the boron concentrations in tumor and blood contributed from BSH were equal just prior to neutron irradiation. The boron concentrations from BPA in the tumor

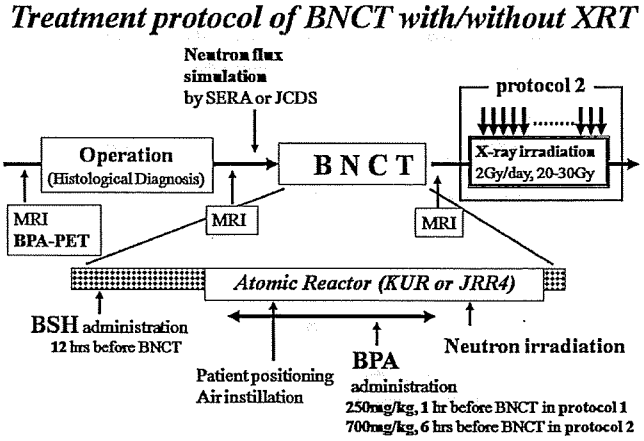

Fig. 1. A flow chart showing the treatment regimen of modified BNCT combined with external beam X-ray irradiation (protocol 2). BPA and BSH were simultaneously used in our BNCT, and the treatment was followed with conventional XRT 2 Gy daily fractionation. The total dose of XRT was determined based on the irradiated dose for the normal brain at the time of BNCT. In protocol 1, BPA was administered at 250mg/kg for one hour, and XRT was omitted. BNCT: boron neutron capture therapy, BPA: boronophenylalanine, BSH: sodium borocaptate, XRT: X-ray radiation therapy.

and normal brain were also estimated by the L/N ratio of BPA-PET. Judging from these boron concentrations contributed from each boron compound, neutron fluence rate simulated by dose-planning program (SERA or JCDS) and the factors of relative biological effectiveness of neutron beam and compound as shown in Table 1, total dose to tumor and normal brain could be estimated, as following formula.

Equivalent dose (Gy-Eq) = $D_B \times CBE_B + D_N \times RBE_N + D_\gamma \times$ hour

D_B: Boron dose (Gy) = $7.43 \times 10^{-14} \times$ boron concentration ($\mu g^{10}B/g$) $\times \Phi$ thermal neutron fluence

D_N: Nitrogen dose (Gy) = $6.78 \times 10^{-14} \times$ nitrogen concentration (weight %) $\times \Phi$ thermal neutron fluence

D_γ: Gamma-ray dose: = 0.83 Gy/hour

(These parameters are used in KUR)

Φ thermal neutron fluence = thermal neutron fluence rate ($n/cm^2/sec$) $\times$ radiation time

Here, Gy-Eq (Gy: Gray) corresponds to a biologically equivalent X-ray dose that can have equivalent effects on tumors and on the normal brain. To compare the effects of the $^{10}B(n, \alpha)^7Li$ reaction by different boron compounds relative to photons, the term compound biological effectiveness (CBE, below) has been defined as an alternative to the relative biological effectiveness (RBE).[11,12] The microdistribution of ^{10}B varies depending upon the pair of boron compounds and normal tissue. Therefore, this value is determined experimentally by using pure thermal neutron beam on each pair. The formula to calculate the value is,

CBE = {X-ray Dose – (Thermal Neutron Dose × RBE)} / $^{10}B(n, \alpha)^7Li$ Dose.

Table 1. RBE (relative biological effectiveness) and CBE (compound biological effectiveness) factor

Radiation			Tumor	Brain	Skin
Thermal Neutron		RBE	3.0	3.0	3.0
Epithermal Neutron		RBE	3.0	3.0	3.0
$^{10}B(n, \alpha)^7Li$	: BPA	CBE	3.8	1.35	2.5
	: BSH	CBE	2.5	0.37	0.8
γ-ray Dose		RBE	1.0	1.0	1.0

BPA: boronophenylalanine; BSH: sodium borocapate.

Dose response relationship of $^{10}B(n,alpha)^7Li$ reaction to tumor or normal tissue depends on the microdistribution of the ^{10}B which is different in each compound. But we can know only macroconcentration of ^{10}B (mg/g tissue) for dose calculation, and "RBE" is determined using this dose. Apparently this "RBE" is different from real RBE of alpha particle of $^{10}B(n,alpha)^7Li$ reaction and varies depending on compound and tissue. In BNCT, this "RBE" for each boron compound is termed as CBE (compound biological effectiveness) values. These RBE and CBE values are determined by human and experimental animal studies.

In protocol 2, 12 hours prior to neutron irradiation the patients were administered 100 mg/kg of BSH intravenously for one hour; later, for the 6 hours just prior to irradiation, they also received 700 mg/kg of BPA continuously. The neutron irradiation time was determined not to exceed 13 and 15 Gy-Eq to the normal brain in protocols 1 and 2, respectively. Within 2 weeks after neutron irradiation, a 2 Gy daily fraction of XRT was applied, for a total of 20 to 30 Gy, as shown in Fig. 1. The purpose of this boost XRT was to decrease the possibility of local recurrence, depending on 2 issues. One is to compensate possible heterogeneous distribution of boron compounds and the other is to compliment the lack of neutron fluence, especially in the deep part. The dose of XRT, therefore, total dose of XRT + BNCT was determined based on the BNCT dose for the normal brain, i.e., not exceeding biologically equivalent dose to 45Gy in the daily fractionation XRT. Radiation field of boost XRT was determined to cover the T2-high lesion in the MRI just before BNCT. The X-ray beam was delivered through anterior-posterior or bilateral opposing fields.

After treatment, all patients were carefully followed up with physical, neurological and neuroradiological examinations, and the toxicity and effectiveness of the treatment were evaluated at 1- to 3-month intervals. When MRI showed a new gadolinium (Gd)-enhanced lesion or increased perilesional brain edema, BPA-PET was again applied to assess the lesion for radiation necrosis or tumor progression.[10] If the lesion showed radiation necrosis, steroids, anticoagulants (chiefly warfarin) and vitamin E were administered. If the lesion indicated tumor progression, supplementary treatment such as chemotherapy or additional surgery was applied if possible. Actually, 11 cases were applied recraniotomy, as described in detail in the Results. Also 7 out of 21 BNCT cases were treated with TMZ, as mentioned in the Discussion. In the historical control group, additional treatments were also applied in case of tumor progression.

Survival analysis

The survival time from initial debulking surgery in BNCT patients was compared with that of the institutional historical controls that were treated with debulking surgery followed by XRT and chemotherapy, as described above. Estimates of the survival probability were calculated using the Kaplan-Meier method, and differences in survival curves were compared using the log-rank test. Data were analyzed using the JMP7 statistical software package (SAS Institute Inc., Cary, NC, USA). P values less than 0.05 were judged as statistical significant.

For the 21 patients who received BNCT, survival time was compared not only with that of the institutional historical controls but also with that of the corresponding recursive partitioning analysis (RPA) subclasses as defined by the Radiation Therapy Oncology Group (RTOG)[11] and the

European Organization of Research and Treatment of Cancer (EORTC)[12] as international historical controls. Based on this RTOG-RPA, GB was classified into 4 prognostic subgroups (classes III to VI), and the median survival time (MST) for Classes III, IV, V, and VI were 17.9, 11.1, 8.9, and 4.6 months, respectively.[11] Each patient treated with BNCT was stratified into his or her respective RPA class, and each patient's survival was introduced with special reference to this historical control. We could not apply any statistical analyses between our BNCT results and these international historical controls because raw data of the latter were not available.

We chose to use overall survival, not progression-free survival, as the primary endpoint. Our reasoning for this decision was as follows. Intensive treatments, such as chemoradiotherapy with TMZ, caused a high incidence of pseudoprogression (psPD) in the early phase of the treatments. It is impossible to distinguish between true tumor progression and pseudoprogression by Gd-MRI alone.[13,14] We experienced the same phenomenon, also with high frequency, in the patients treated with BNCT.[15] In addition, radiation necrosis is difficult to be distinguished from local tumor progression as stated above. Thus, progression-free survival was not suitable as the primary endpoint.

RESULTS

Patients' profiles and BNCT parameters

The patients' profiles and BNCT parameters are listed in Table 2. Cases 1 to 10 were treated using protocol 1 and cases 11 to 21 were treated using protocol 2. The L/N ratios of BPA uptake judged by BPA-PET ranged from 2.1 to 7.1. The minimal tumor doses for GTV in protocols 1 and 2 were 16.3 to 63.0 Gy-Eq and 26.9 to 65.4 Gy-Eq, respectively. In protocol 2, XRT of a total dose of 20–30Gy was started within 2 weeks after BNCT, as described above.

Survival

Patients treated with BNCT (n = 21) had a MST of 15.6 months (95% confidence interval (CI): 12.2–23.9) after diagnosis (Fig. 2A and Table 3). Here the date of diagnosis is the initial debulking surgery date, as descried above. This was significantly longer than the MST for the historical controls at our institute who were treated with surgical removal followed by XRT and chemotherapy (n = 27, MST was 10.3 months (95% CI: 7.4–13.2), log-rank test p = 0.0035). The RPA class distribution of 21 patients treated with BNCT at the initial diagnosis was as follows: Class III = 6 (29%); Class IV = 6 (29%); Class V = 8 (38%); Class VI = 1 (5%). The MSTs of the patients in classes III, IV, V, and VI were 23.5, 16.9, 13.2, and 9.8 months, respectively (Table 3). Of the 21 patients, 4 are still alive. In historical control, the RPA class distribution was as follows: Class III = 3 (11%); Class IV = 14 (52%); Class V = 8 (30%); Class VI = 2 (7%). The distributions of each RPA class in BNCT group and institutional historical control group are a little bit different. We compare the survival of both groups in low risk RPA (class III and IV) and in high risk RPA (class V and VI) separately. The MST of BNCT group in low risk group was 18.5 months (n = 12, 95% CI: 13.7–36.1) and that of historical control was 13.0 months (n = 17, 95% CI: 8.6–18.0). There is statistical significance in log-rank test (p = 0.028). The MST of BNCT group in high risk group was 12.2 months (n = 9, 95% CI: 9.8-undetermined) and that of historical control was 7.4 months (n = 10, 95% CI: 2.7–10.3). There is also statistical significance in log-rank test (p = 0.0083).

Table 2. Patient profile and parameters of BNCT in 21 cases with newlydiagnosed glioblastoma

Case	age, sex	BPA-PET	absorbed dose (Gy-Eq)[a] tumor[b]		XRT (dose(Gy))[c]	RTOG RPA class	Survival (months)
		(L/N)	max	min			
1	51, F	3.5*	50.6	23.8	–	5	9.9
2	73, M	7.1	57.3	27.0	–	5	10.4
3	56, F	3.5*	64.8	32.6	–	4	14.1
4	44, F	3.5*	47.5	46.2	–	3	62.2**
5	61, F	5.5	79.9	26.7	–	4	36.1
6	65, F	5.1	71.4	21.7	–	5	12.2
7	69, F	5.4	58.7	28.7	–	5	14.1***
8	57, F	3.2	37.7	16.3	–	4	13.7
9	61, M	3.7	57.6	23.2	–	5	15.6
10	49, F	3.2	37.7	16.3	–	4	18.5
11	62, F	4.8	115.0	63.0	30	6	9.8
12	16, F	6.6	149.0	39.7	24	3	17.4
13	69, F	3.5	89.5	65.4	20	4	23.9
14	36, F	4.3	96.4	42.3	30	3	23.5
15	63, F	3.5	60.6	26.9	20	5	41.5**
16	29, F	3.7	72.0	36.5	20	4	15.3
17	18, F	4.5	84.2	57.1	30	3	34.5**
18	59, M	3.5*	90.6	61.4	30	5	10.2***
19	15, M	3.3	122.0	43.1	30	3	11.0
20	69, F	2.1	52.0	26.9	30	5	22.2
21	46, F	2.1	64.0	44.6	20	3	17.7**

BPA: boronophenylalanine, PET: positron emission tomography, L/N: lesion to normal brain ratio, Gy-Eq: gray equivalent, XRT: X-ray radiation therapy, RN: radiation necrosis, rec.: recurrence

Cases 1 to 10 were treated by protocol 1 and cases 11 to 21 were treated by protocol 2.

[a]: Absorbed doses include contributions from gamma photons, ^{14}N (n, p) ^{14}C and ^{10}B(n, a) ^{7}Li reactions. A spot region of irradiated dose calculated by a SERA workstation is listed above.

[b]: Tumor was identified as a contrast-enhanced lesion by Gd on MRI.

[c]: XRT dose was identified as a total dose of 2 Gy daily fractionated external beam X-ray irradiation.

*: For these cases, BPA-PETs were not applicable and an L/N ratio of 3.5 was applied using the mean value from the literature (*Int J Rad Oncol Biol Phys* 40: 829–34, 1998).

**: alive

***: Cases 7 and 18 died from concomitant thyroid cancer and cerebrovascular disease, respectively.

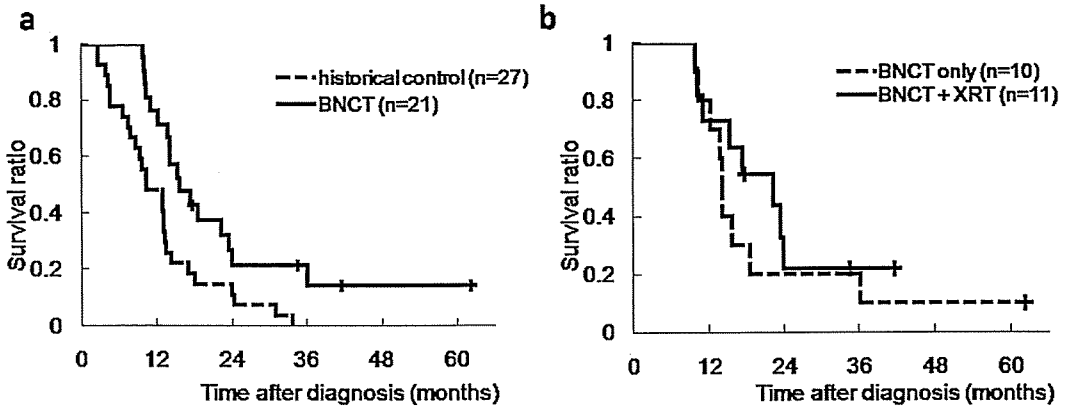

Fig. 2. Kaplan-Meier survival curves of newly diagnosed glioblastoma patients treated with BNCT. A: A continuous line represents the survival times of the patients treated with BNCT (protocols 1 plus 2, n = 21), and show an MST of 15.6 months. Four out of 21 cases are still alive. A broken line represents the survival times of our institutional historical controls (surgical removal, XRT and chemotherapy) (n = 27) and show an MST of 10.3 months (log-rank test, p = 0.0035). B: A continuous line represents the survival times of the patients treated with BNCT followed by XRT boost (protocol 2, n = 11), and show an MST of 23.5 months. Three out of 11 cases in protocol 2 are still alive. A broken line represents the survival times of the patients treated by protocol 1(n = 10), and show an MST of 14.1. There is no statistical significance in the difference in MSTs between these groups.

Table 3. Comparison of survival data among RPA class in the RTOG database[a], EORTC (RT/TMZ) trial[b] and in our cases treated with BNCT[c]

RTOG RPA class	RTOG original (1463 cases)[a]		EORTC (RT/TMZ)[b]		BNCT group (21 cases)[c]	
	case	Median (mo)	case	Median (mo)	Case[d]	Median (mo)
III	175	17.9	42	21.4	6	23.5
IV	457	11.1	152	16.3	6	16.9
V	395	8.9	93	10.3	8	13.2
VI	263	4.6	NR[e]		1	9.8

RPA: recursive partitioning analysis, RTOG: Radiation Therapy Oncology Group, EORTC: European Organization for Research and Treatment of Cancer, RT: radiation therapy, TMZ: Temozolomide, BNCT: boron neutron capture therapy

[a]: Curran W., *et al.* Recursive partitioning analysis of prognostic factors in three radiation therapy oncology group malignant glioma trials. *J Natl Cancer Inst* 1993;85:704–710.

[b]: Mirimanoff R.O., *et al.* Radiotherapy and temozolomide for newly diagnosed glioblastoma: Recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. *J Clin Oncol* 2006; 24: 2563–2569

[c]: BNCT group including 21 newly histologically confirmed glioblastoma patients treated with BNCTat Osaka Medical College between 2002 and 2006.

[d]: Three patients out of 8 in class III and 1 of 4 in class V were alive at the end point of this study. All of the patients in class IV had died.

[e]: not reported.

Therefore, it can be concluded that BNCT group shows the long survival in comparison with historical control not mainly by the difference of distribution of each RPA class in both groups. Our BNCT results for survival among the NDGB cases were favorable in comparison with those obtained from the corresponding RTOG- and EORTC- RPA subclasses (Table 3).

All patients receiving protocol 2 tolerated this treatment well. Of the 11 patients in protocol 2, 3 are still alive. The survival time from the date of diagnosis was calculated using the Kaplan-Meier method (Fig. 2B). The MST of the protocol 2 was 23.5 months (95% CI: 10. 2 – undetermined) after diagnosis (n = 11), and that of the protocol 1 patients (n = 10) was 14.1 months (95% CI: 9.9–18.5), although the difference was not statistically significant.

Reoperation after BNCT

Eleven cases were applied recraniotomy when the enhanced lesion on MRI increased in size, as stated above. Surgical specimen at recraniotomy in cases 3, 8, 13, 14 showed tumor progression. In these cases, only partial tumor removal could be done. Also surgical specimen in cases 1, 2, 4, 6, 14, 15, 20 showed mainly necrosis. Three (cases 1, 2, 6) out of these 7 treatment-related necrosis cases were considered as psPD because the lesions increased in size within 3 months after BNCT and the lesions were stable or decreased in size during the observation period after the recraniotomy.

Side effects of BNCT

All of the BNCT patients showed alopecia. Also, in the early period of this study, some patients showed transient

oliguria and fever during the first 24 hours after BNCT. We concluded these side effects were caused by recrystallization of BPA in urine. Thereafter, we overhydrated the remaining patients after BNCT, and no such side effects were observed again. Cases 11, 17, 18 other than above 7 histologically verified cases were considered as radiation necrosis judging from PET study. Four cases were symptomatic and other 6 cases were asymptomatic. We described radiation necrosis in the Discussion.

Representative case: Case 17

An 18-year-old female had a right parietal tumor partially removed in a hospital in May 2005 (Fig. 3, Column A). The histopathological diagnosis was GB. She was transferred to our hospital for BNCT for the remaining lesion. Prior to BNCT, we applied BPA-PET to confirm the BPA accumulation and simulation of the absorbed dose. The L/N ratio in the BPA-PET image was 4.5, as shown in Fig. 3, Row A. We performed re-craniotomy to remove the additional tumor (Fig. 3, Row B) and settled the Ommaya's reservoir to fill the cavity with air before the neutron irradiation, in order to increase the amount of neutrons reaching the bottom of the tumor. As a BNCT simulation, the minimum tumor dose and maximum normal brain dose were estimated to be 57.1 Gy-Eq (5.4 cm beneath the parietal scalp) and 10.8 Gy-Eq (2.5 cm beneath the scalp), respectively. An additional 30 Gy XRT (2 Gy × 15 Fr) was applied for the deep part of the tumor. The patient was followed-up with periodic MRI without any newly appearing lesions. Twenty-four months after BNCT, a small enhanced lesion was found. The patient returned to our clinic so that we could determine whether or not the lesion represented tumor progression. We applied BPA-PET again, and found no tracer uptake (Fig. 3, Row C). The lesion identified on MRI was considered to show radiation necrosis but not tumor progression. The MRI taken 26 months after BNCT is also shown in Fig. 3, Row C. A white arrow shows the absence of enlargement of the enhanced lesion on MRI. The patient was neurologically free and

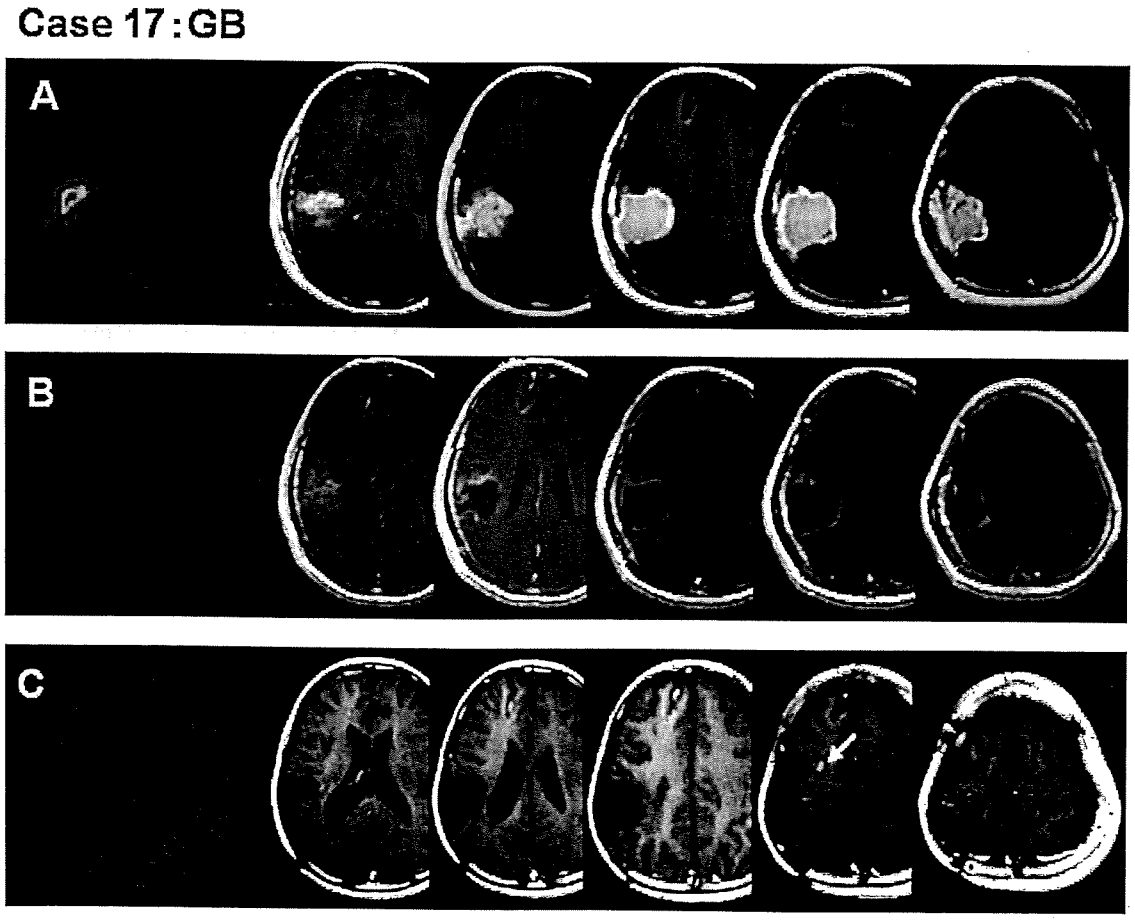

Fig. 3. Case 17: An 18-year-old female had a right parietal glioblastoma partially removed in a hospital in May 2005 (A). We performed re-craniotomy to remove the additional tumor and settled the Ommaya's reservoir to fill the cavity with air before neutron irradiation (B). MRI taken 26 months after BNCT is shown in C. The white arrow shows a newly appearing Gd-enhanced lesion, which was judged to be radiation necrosis. BPA-PETs taken prior to BNCT and 2 years after BNCT are listed in the left panel in rows A and C, respectively.

100% on KPS at the time this manuscript was prepared.

DISCUSSION

Comparisons of BNCT patients with institutional historical control and RTOG- and EORTC- RPA databases

BNCT has been applied to a limited extent for the treatment of malignant gliomas. So far, several clinical studies of BNCT have been reported.[16–19] In each of those studies, the MST was approximately 13 months. Although these survival times were similar to those obtained with surgery followed by XRT, no firm conclusions can be made as to whether the clinical results of BNCT are equivalent or superior to those of XRT. To improve the clinical effectiveness of BNCT for malignant gliomas, we have made several modifications, as described in the Introduction. With these modifications, it is likely that we can achieve more favorable results for BNCT on NDGB than were obtained in the previous trials. In our series (protocols 1 and 2, n = 21), the patients treated with BNCT had an MST of 15.6 months (95% CI: 12.2–23.9) after diagnosis. That of our institutional historical control (n = 27, MST: 10.3 months (95% CI: 7.4–13.2)) was significantly shorter (p = 0.0035, by log-rank test). However our historical control was obtained from 1990 to 2006. Since the BNCT series data were collected from 2002 to 2006, recent advancements in surgical procedure or chemotherapy may have influenced our BNCT series data. On the other hand, it is accepted that the extensive removal of NDGB showed a limited benefit for the survival of NDGB patients with large series study. Lacroix, *et al.*[20] reported that more than 98% removal of NDGB showed moderate benefit of the prolongation of MST such as 4 months or so, in comparison of less than 98% removal.[20] In BNCT group and institutional historical control group, 4 out of 21 patients and 5 out of 27 patients were received more than 98% removal of the tumor, respectively. Probably, advancement in chemotherapy, especially the advent of TMZ, may have improved the results of our BNCT cases in comparison with our historical control. Our discussion of the effects of TMZ in our BNCT series appears under the subheading *Further improvements* below.

Also, to apply a more objective comparison, we made reference to the RTOG- and EORTC-RPA databases. Previously, Hatanaka *et al.* reported good clinical results with BNCT.[21] However, Laramore *et al.*[22] analyzed the survival data of a subset of 12 patients who had been treated by Hatanaka between 1987 and 1994.[21] They concluded that there were no differences in their survival times compared with the RTOG-RPA classifications. Our patients in RTOG RPA classes III, IV, and V had MSTs of 23.5, 16.9, and 13.2 months compared with MSTs of 17.9, 11.1, and 8.9 months for these respective classes in the original RTOG trials[11] (Table 3). Of course, raw data from RTOG database is not yet available. It is impossible, and in any case would be meaningless to compare our BNCT data to RTOG-RPA data directly with statistics, as described above. Also, the RTOG-RPA database was published in 1993 and the data were collected in the late 1980s. So the same issue of possible data obsolescence arises, as it did with the institutional historical control, in light of recent advancements in surgical procedures and chemotherapy. To avoid the bias introduced by such advances, our results were also compared with the EORTC-RPA database.[12] An EORTC-RPA study was published recently, and all the patients in this study were treated with TMZ. At least, our study showed that the prognosis of BNCT patients was not bad in each RPA subclass of RTOG and EORTC. The response to BNCT was seemed to be favorable, especially in the poorer subclasses (RPA IV-VI).[11]

In our BNCT series, the MST of the patients treated with BNCT followed by XRT boost (protocol 2) was 23.5 months (95% CI: 10.2 – undetermined), while the MST of the patients treated with BNCT without XRT boost (protocol 1) was 14.1 months (95% CI: 9.9–18.5) (Fig. 2B), although the there was no statistical significance in survival between two protocols in log-rank test. We discuss the rationale for this modification in protocol 2 below.

Modifications in protocol 2

To the best of our knowledge, BNCT clinically has never been followed by a photon boost until the time of tumor progression. In the present study, we performed our new BNCT protocol combined with XRT for NDGB patients to diminish the possibility of tumor recurrence. This approach was based on experimental animal data showing that a significant therapeutic gain could be obtained when BNCT was combined with an X-ray boost.[23] Barth *et al.*[2] recently reported that an X-ray boost after BNCT could significantly enhance survival time in an experimental brain tumor model.

In our trial, we used BPA and BSH in combination. Here, the micro-distributions of BSH[24,25] and BPA[26–28] differed at the cellular level, and their simultaneous use could cover this heterogeneous distribution, especially on the tumor bulk.[7,29–31] To augment the absorbed dose of infiltrated tumor cells, where BPA should play an important role, we increased the amount of BPA from 250 mg/kg (protocol 1) to 700 mg/kg (protocol 2) and prolonged the infusion time from 1 hr (protocol 1) to 6 hrs (protocol 2). These changes were based on a BNCT study performed in Sweden[19,32] and on animal experimental data using secondary ion mass spectroscopy.[26,27] The Swedish group carried out a BPA-based trial using an epithermal neutron beam.[32] That study differed significantly from all previous clinical trials in that the total amount of BPA administered was 900 mg/kg, infused intravenously over 6 hours. The longer infusion time should theoretically give a more homogeneous distribution of boron compounds, even in the infiltrating lesion.[26,27,33,34] This approach by the Swedish group was well tolerated, and the MST for the 29 patients in their trial was 14.2 months after BNCT. In the present study, we modified this Swedish

method combining the BPA therapy with BSH. This was the rationale for our protocol 2.

Problems to be confronted

CSF dissemination, along with local progression, was a major cause of death after BNCT. This tendency was also confirmed in both protocols 1 and 2. Also, CSF dissemination was prominent even in the GB patients who had been treated with BNCT on recurrence.[35] In protocols 1 and 2 combined, we lost 7, 5, and 3 patients due to CSF dissemination, local tumor progression and both dissemination and local tumor progression, respectively (data not shown). CSF dissemination can be diagnosed by MRI or CSF cytology. About local tumor progression, we confirmed only 4 cases at recrainiotomy, as stated above. The rest cases were speculated as local tumor progression by follow-up MRI and responsiveness to steroids. It is generally accepted that more than 85% of tumor progression in GB patients arises within 2 cm of the original margin of the contrast-enhancing lesion by XRT.[36–38] These findings indicate that the local control of GB by BNCT is relatively good in comparison with XRT, but the problem of CSF dissemination remains. Some patients showed radiographical and neurological aggravation after BNCT with the XRT boost for NDGB; this tendency was more prominent in recurrent GB patients who had been treated with full-dose XRT and treated again with only BNCT upon recurrence. The lesions were occasionally removed when we could not control them with medication. Histological examination often showed radiation necrosis with no evidence of tumor residues, and these patients were well controlled after surgery. Even some NDGB patients, such as case 17 (protocol 2) showed radiation necrosis. This is probably caused by the elevated absorbed dose for the normal brain with the combination of additional XRT in protocol 2. Management of these pathologies with the correct diagnosis by BPA-PET is also important for patients who receive high-dose irradiation, as case 17 shows.[10] This radiation necrosis in protocol 2 may be diminished by additional XRT with gradation of the absorbed dose, more in the deeper and less in the shallower lesions, using multi-leaf collimators.

Further improvements

Recently, Stupp *et al.*[1] reported that an oral alkylating agent, TMZ, given concomitantly with XRT followed by six 28–day cycles of TMZ alone, significantly extended survival in NDGB. As a result, concurrent XRT and TMZ, followed by 6 monthly cycles of adjuvant TMZ, became the new standard of care for patients with NDGB. It should be pointed out that, in our BNCT patients, no chemotherapy was applied to patients in either protocol until tumor progression was confirmed. In protocols 1 and 2, 2 and 5 patients, respectively, were treated with TMZ when they showed enlargement in Gd-enhanced MRI. In 3 of those cases, BPA-PET and histology proved that there was no tumor progression. In the EORTC study group (XRT plus concomitant TMZ chemotherapy followed by subsequent periodic use of TMZ as chemotherapy), Mirimanoff *et al.*[12] reported an excellent result with RPA sub-classifications for NDGB, as shown in Table 3. Our BNCT group (n = 21) showed almost equal MST in RPA classes III and IV and slightly better MST in RPA class V in comparison with this EORTC study (Table 3), irrespective of the fact that limited numbers of patients were given TMZ only when they were diagnosed with a recurrence, as described above. In addition, TMZ shows a limited benefit when administered for a GB relapse. Brada, *et al.*[39] reported that TMZ showed a modest survival benefit for recurrent GB, with a 5.4 month median prolongation after TMZ administration. Taken together, the results indicate that in our BNCT series, TMZ might show a limited contribution to the prolongation of survival.

In any case, BNCT has not been clinically evaluated when given sequentially or concomitantly with cancer chemotherapy. BNCT is likely to benefit from being combined with chemotherapeutic agents such as TMZ, and such combinations should be further researched. Further study is now under way for this protocol; modified BNCT with XRT boost, followed by chemotherapy. To obtain definitive results of the survival benefit of BNCT for NDGB, a strictly designed phase 3 study is necessary.

CONCLUSIONS

In conclusion, we can achieve favorable results from BNCT in NDGB patients. We applied two major modifications to the current BNCT protocol (protocol 2) in addition to our former protocol (protocol 1). The first modification is a longer-term and larger BPA infusion, and the second is the additional application of XRT.

ACKNOWLEDGEMENTS

This work was supported in part by Grants-in-Aid for Scientific Research (B) (16390422 and 19390385) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (MEXT) to S-I. M.; by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare of Japan to S-I. M. (P. I., Hideki Matsui); by the Regional Science Promotion Program of the Japan Science and Technology Corporation; and by the "Second-term Comprehensive 10-Year Strategy for Cancer Control" of the Ministry of Health, Labor, and Welfare of Japan to S-I. M. This work was also supported in part by the Takeda Science Foundation for Osaka Medical College; by a Grant-in-Aid for Cancer Research from MEXT (12217065) to K. O.; and by a Grant-in-Aid for Scientific Research for Young Researchers (B) (18791030) from MEXT to S. K.

S. K., S-I. M., and K. O. contributed equally to this work

as primary investigators.

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