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CASE REPORT

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A rare case of extraventricular neurocytoma

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Abstract We report an unusual case of extraventricular (cerebral) neurocytoma with a long, 25-year history, and which appeared to transform to neuroblastoma. In 1978, an 18-year-old woman was treated for right frontal oligodendroglioma. Eighteen years later (in 1996), recurrence of tumor in the fourth ventricle was noted and was treated with gamma-knife radiotherapy. The tumor shrunk transiently, but 7 years later (in 2004), MRI study demonstrated a second recurrence and ventricular dissemination. Partial removal was performed, and histological examination revealed that tumor cells had round or oval nuclei with halos. Frequent mitoses and vascular proliferation were observed. The MIB-1 LI was 80%. Despite postoperative whole-brain radiotherapy to a total dose of 30 Gy, the tumor progressed, and she died at 4 months after the second surgery.

Key words Extraventricular · Neurocytoma · Neuroblastoma

Introduction

Central neurocytoma (CN) is a definite clinicopathological entity that has been well known to neurosurgeons since 1993.[1] It commonly occurs as an intraventricular mass, and is often calcified, but may also occur as a periventricular parenchymal mass or even in locations remote from the ventricles, as so-called extraventricular (cerebral) neruocytoma.[2–4] Neurocytomas frequently contain cells with small round nuclei and a surrounding halo of empty-appearing cytoplasm that closely resemble the cells of oligodendroglioma. We report an unusual case of extraventricular neurocytoma, initially treated as an oligodendroglioma in 1978. The patient developed two recurrences 18 and 25 years after initial surgery, and the tumor appeared to transform to neuroblastoma.

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Case presentation

In 1978, an 18-year-old woman presented with progressive headache and nausea. Cranial computed tomography (CT) revealed a right frontal lobe tumor with calcification. The tumor was partially removed, followed by local irradiation. Unfortunately, her clinical records had been discarded, and her precise history of treatment was therefore unknown. The pathological diagnosis was oligodendroglioma. A few years later, she discontinued visiting the hospital.

In 1996, she complained of diplopia and vertigo and consulted her previous physician. CT examination revealed a fourth ventricular tumor with calcification (Fig. 1). She rejected radical resection and underwent gamma-knife radiotherapy. The tumor decreased in size (Fig. 2), and her symptoms were improved.

In 2004, the patient presented with disturbance of consciousness and gait disturbance and was admitted to our hospital. Magnetic resonance imaging (MRI) revealed lateral ventricular and fourth ventricular tumor as well as hydrocephalus (Fig. 3). In February 2004, the tumor was partially removed. The pathological diagnosis was central neuroblastoma. Although postoperative whole-brain radiotherapy was performed to a total dose of 30 Gy, the tumor progressed (Fig. 4), and she died 4 months after the second surgery.

Pathological findings

We reexamined the first specimens. The tumor cells had round or oval nuclei with a finely speckled chromatin

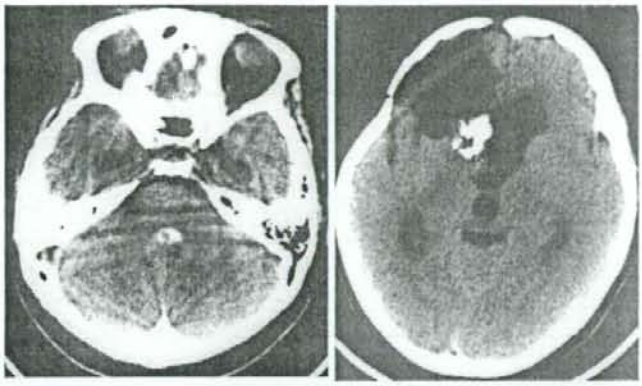

Fig. 1. Computed tomography (CT) examination in 1996 revealed a fourth ventricular and right lateral ventricular calcified tumor

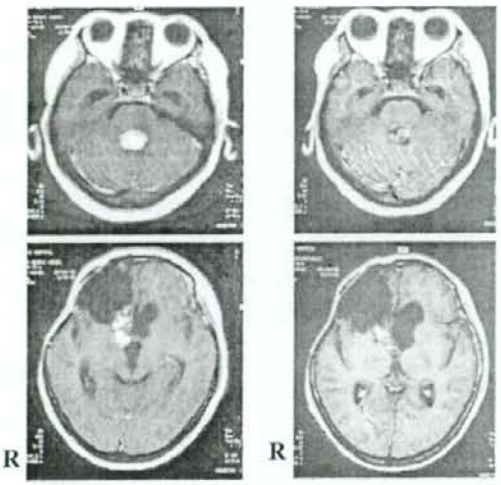

Fig. 2. Magnetic resonance imaging (MRI) at the time of first recurrence, showing that the volume of the fourth ventricular tumor had decreased after gamma-knife surgery. *Right column*, pregamma-knife status; *left column*, postgamma-knife status

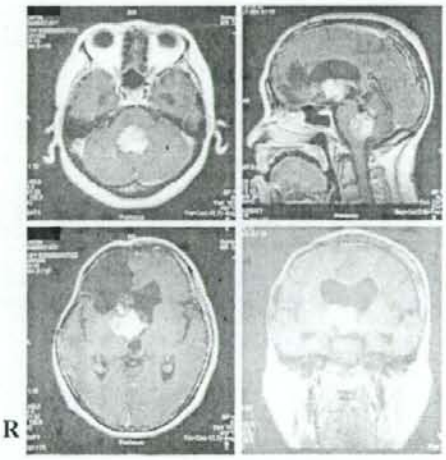

Fig. 3. MRI at the time of second recurrence shows enlargement of the fourth ventricular and lateral ventricular tumors as well as hydrocephalus

pattern with halos. In 1978 ("central neurocytoma" had not been described), the pathological diagnosis was oligodendroglioma. The general architecture was monotonous. Immunohistological study revealed strong expression of synaptophysin and neuron-specific enolase (NSE). Staining for glial fibrillary protein (GFAP) was negative. The MIB-1 labeling index (LI) was very low. Thus, we diagnosed the tumor as neurocytoma (Fig. 5).

The specimens from the second operation displayed nuclear atypia, frequent mitoses, and microvascular proliferation. Immunohistological study revealed expression of synaptophisin and NeuN. Staining for GFAP was partly positive. The MIB-1 LI was extremely high, at 80%. The pathological diagnosis was neuroblastoma (Fig. 6).

Discussion

Central neurocytoma (CN), named by Hassoun et al.[5] in 1982, is thought to be a benign neoplasm exhibiting neuronal differentiation, typically located in the lateral ventricles, and corresponding to WHO grade II.[1] CN may also occur as a periventricular parenchymal mass, or even in locations remote from the ventricles, as so-called extraventricular neurocytoma.[2-4] Neurocytomas frequently contain cells with small round nuclei and a surrounding halo of empty-appearing cytoplasm that closely resemble the cells of oligodendroglioma. Before Hassoun's work, almost all central neurocytomas may have been diagnosed as intraventricular oligodendrogliomas. In the present case, the

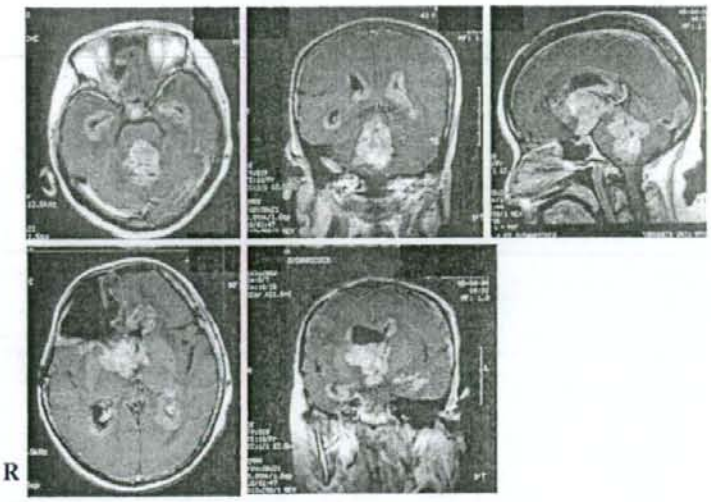

Fig. 4. MRI after whole-brain radiotherapy shows tumor progression as well as dissemination throughout the ventricular system

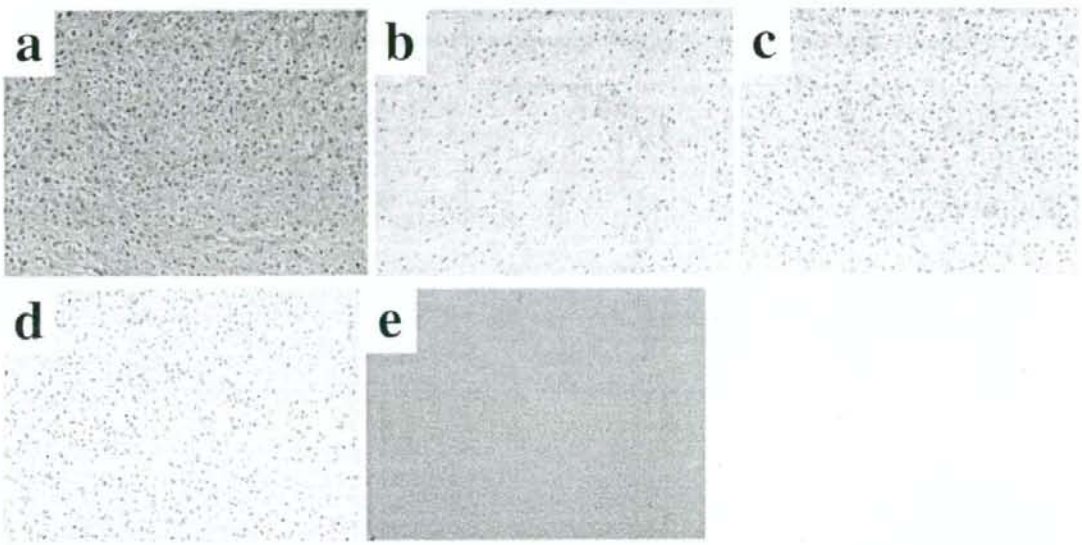

Fig. 5. The tumor cells had round or oval nuclei with finely speckled chromatin pattern with halos (**a**: H&E). Immunoreactivity for synaptophysin (**b**) and NSE (**c**) was positive; immunoreactivity for GFAP was negative (**d**); and MIB-1 LI was very low (**e**). **a–e** ×200

tumor was diagnosed as an oligodendroglioma at initial treatment.

We reexamined the tumor pathologically and diagnosed extraventricular neurocytoma based on the pathological findings and the patient's medical history (right frontal tumor). Some cases have been reported of neurocytoma with a poor clinical outcome, and anaplastic histological features, including apparent mitotic activity, microvascular proliferation, and necrosis, have sometimes been observed.[6-10] The MIB-1 LI varies from low to high. Soylemezoglu et al. reported that tumors with MIB-1 LI greater than 2% had a relapse rate of 63%, compared with a rate of 22% for tumors with a MIB-1 LI less than 2%.[11] They proposed considering CNs with a MIB-LI greater than 2% and/or vascular proliferation as "atypical central neurocytoma."[11]

In the present case, the MIB-1 LI was extremely high (80%), and mitoses and vascular proliferation were prominent; we therefore diagnosed neuroblastoma. Horten and Rubinstein collected and reviewed 35 cases with cerebral neuroblastoma. They proposed the criteria for the histological pattern of neuroblastoma.[12] According to the histologi-

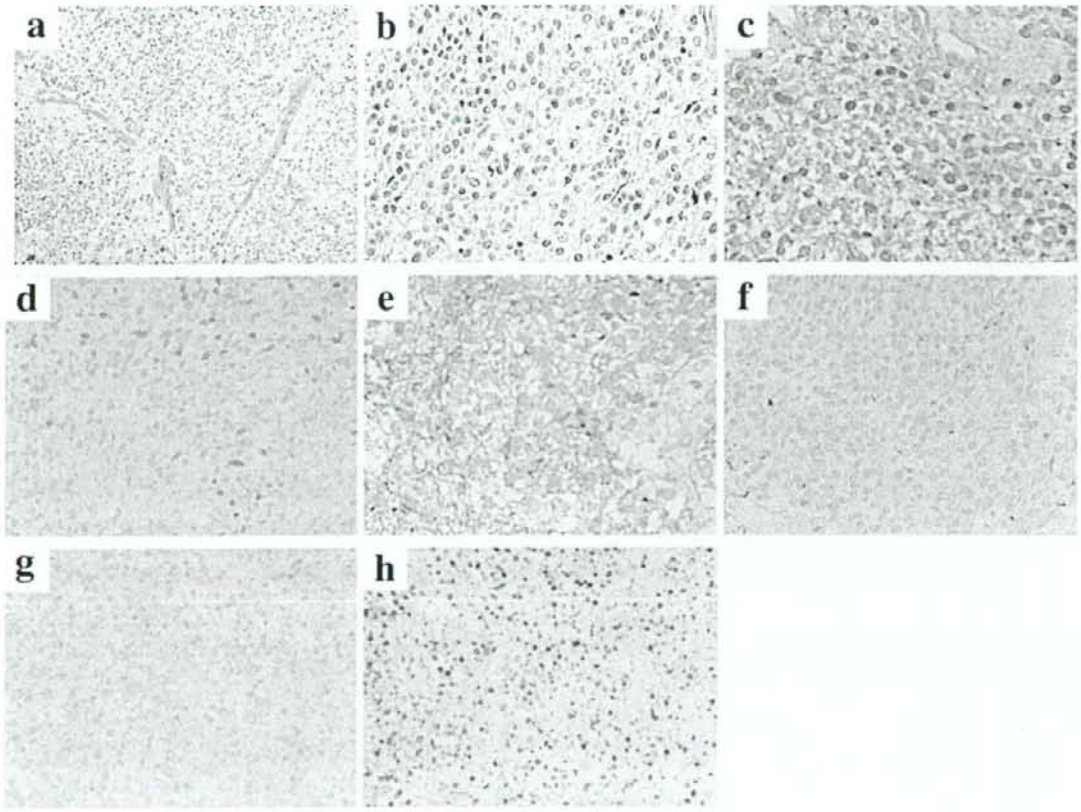

Fig. 6. Tumor cells display nuclear atypia, frequent mitoses, and microvascular proliferation (**a**, **b**: H&E). Immunoreactivity for synaptophysin (**c**), TUJ1 (**d**), and NFP (**e**) was positive; immunoreactivity for GFAP was partially positive (**f**); and immunoreactivity for OLG2 (**g**) was negative. MIB-1 LI was 80% (**h**). **a** ×40; **b**, **c**, **e** ×400; **d**, **f**–**h** ×200

cal classification of tumors of the central nervous system proposed by WHO in 2000, neuroblas toma is classified in the supratentorial primitive neuroectodermal tumor group with ganglioneuroblastoma.[13] Tong et al. reported that central neurocytomas are genetically distinct from neuroblastoma.[14]

For patients with typical neurocytomas, complete resection is considered the best treatment, and after incomplete resection patients benefit from radiotherapy. In our case, radiotherapy and gamma-knife radiosurgery were effective, although not curative. To our knowledge, there has been no previous report of transformation of neurocytoma to neuroblastoma. It is possible that the neuroblastoma originated from a different site or was a radiation-induced secondary tumor. However this may be, our case is of considerable interest from both pathological and clinical perspectives.

The most appropriate treatment for CN has yet to be clearly established. It has been suggested that radiation therapy is advisable in patients with recurring tumors and histological evidence of malignancy. The role of chemotherapy is less clear, and few reports have addressed this issue.[15–17] von Koch et al. reported that chemotherapy including procarbazine, CCNU, and vincristine was effective for recurrent central neurocytoma.[18] However, because our patient's general condition was already very poor at her final admission, we could not treat her with chemotherapy.

Long-term controlled studies are needed for evaluation of the efficacy of postoperative radiation therapy, and close, long-term follow-up is necessary for patients with extraventricular neurocytoma.

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症例報告

Isomorphic astrocytomaの1手術例

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An Operative Case of Isomorphic Astrocytoma

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Abstract

Diffuse astrocytomas are classified as WHO Grade II tumors. Recently, a subtype presenting with better prognosis has been proposed, and it is known as "isomorphic astrocytoma." A clinical case that we encountered was believed to be categorized as this subtype; it has been presented in this report.

The patient was a 20-year-old male with a chief complaint of intractable epileptic seizures. He experienced his first attack at 16 years of age in July 2001, and it was a generalized seizure. Anticonvulsants prescribed by a previous doctor had no effect on controlling the seizures. MRI performed in March 2004 showed a lesion approximately 2.0 cm in diameter in the left temporal lobe. The patient was referred to our institution for further investigation of the lesion and therapy.

Electroencephalography and magnetoencephalography were used to assess the lesion at seizure focus. The tumor was resected under awake surgery. The pathological diagnosis was diffuse astrocytoma, but this tumor was considered to be the isomorphic subtype. Some parts of the tumor showed a relatively high MIB-1 labeling index (LI) of 9.2%, and additional 50-Gy radiotherapy was performed. The postoperative course was uneventful and despite decreasing the anticonvulsant dosage, he has remained seizure free.

Isomorphic astrocytoma is characterized by prolonged epileptic seizures, a low MIB-1 LI, and better prognosis. In our case, since the MIB-1 LI was higher in some parts of the tumor, the appropriate therapy for WHO Grade II tumors was performed. However, this case was considered representative of isomorphic astrocytoma. No reports of this tumor subtype have been previously described in Japan. Therefore, this report is the first case of isomorphic astrocytoma reported to Japanese literature.

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Key words : diffuse astrocytoma, isomorphic astrocytoma, epilepsy

はじめに

Diffuse astrocytomaは星細胞腫群(astrocytic tumor)のうち，浸潤性に脳実質を侵す分化型グリオーマである。WHO分類(2000)では，限局した発育を示すpilocytic astrocytomaとの対比の意味で"diffuse"がつけられている。わが国では原発性脳腫瘍の7.7%を占め，

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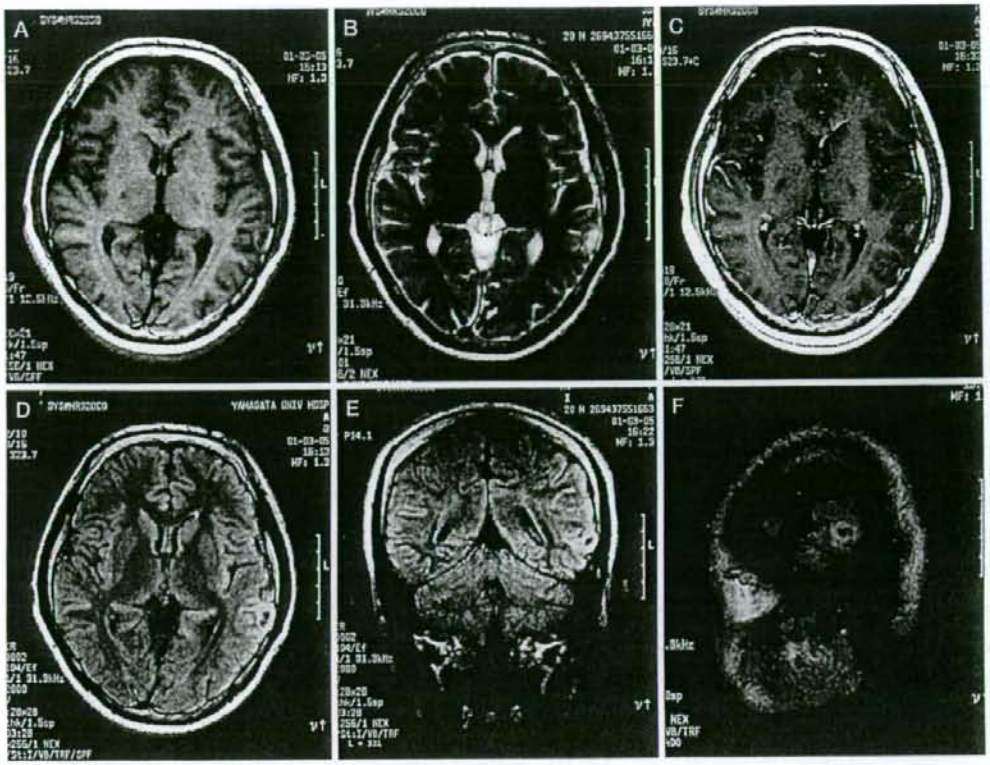

Fig. 1 Preoperative MRI

T_1-weighted (A), T_2-weighted (B), Gd-enhanced-(C), and FLAIR-(D, E, F) MRI showing a mass lesion in the left temporal lobe.

25～59歳に好発する。大脳半球，特に前頭葉に多く，次いで側頭葉，頭頂葉に発生する。臨床悪性度はWHO Grade IIに属する。組織学的には3つの亜型に分類され，①線維性(fibrillary)，②原形質性(protoplasmic)，および③肥胖性(gemistocytic)星細胞腫がある。症状としては，脳実質へのびまん性の浸潤によりさまざまな局所症状が出やすく，頭蓋内圧亢進による症状は初期には出にくい。てんかん発作を起こすことが多いことも特徴の1つである[1]。

これまでdiffuse astrocytomaと診断されていた星細胞腫のなかに，長期のてんかん歴を持ち，diffuse astrocytomaに比べて良好な予後をたどる一群，すなわちisomorphic astrocytomaが存在するとの報告がなされるようになった[2-4]。今回われわれは，このisomorphic astrocytomaと考えられた1例を経験したので報告する。

I．症　　例

患　者　20歳，男性

主　訴　難治性てんかん

既往歴，家族歴　特記事項なし

現病歴　2001年7月，全身性強直間代発作があり近医を受診した。MRIを施行されたが異常所見を指摘されず，バルプロ酸の内服を開始した。その後も数カ月に1回の頻度で複雑部分発作が出現し，ゾニサミドの併用を開始した。しかし発作のコントロールは不良で，月に3，4回の頻度に増加した。その後のMRIで異常を指摘され2005年1月，当科を紹介され受診した。

初診時神経学的所見　発作間欠期の状態では神経学的異常は認められなかった。複雑部分発作を月に3，4回の頻度で認めていた。当科外来受診後，カルバマゼピン投与も試みたが効果は認められなかった。

神経放射線学的所見　2001年に前医でMRIを施行さ

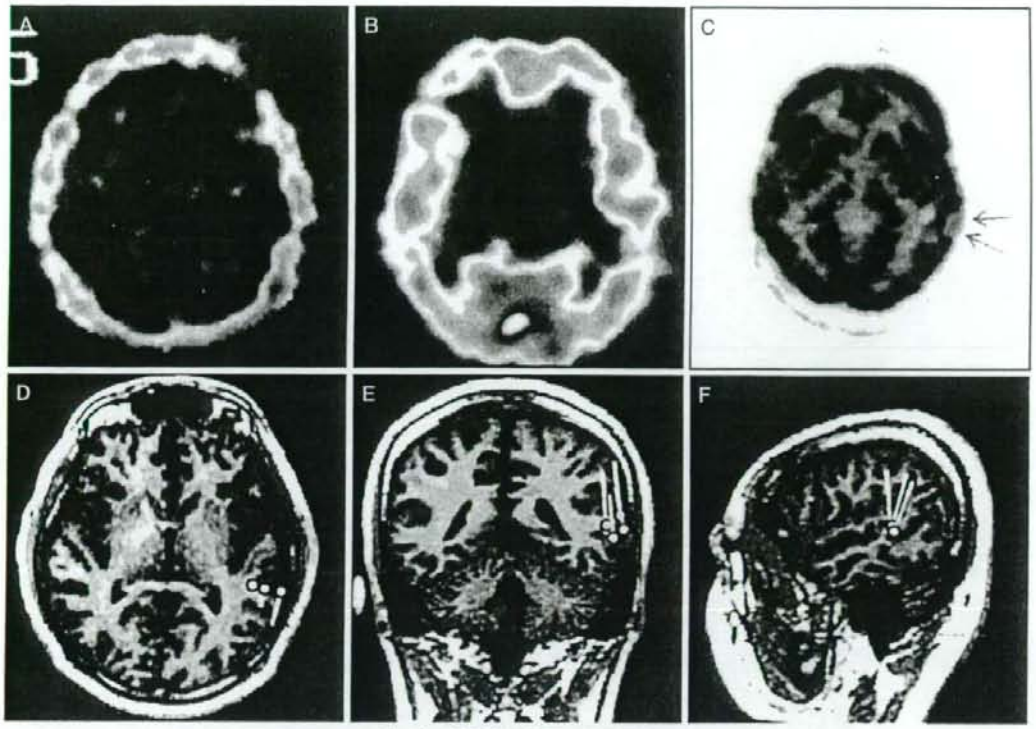

Fig. 2 SPECT, PET and MEG
Thallium-SPECT (A), Iomazenil-SPECT (B) and FDG-PET-(C) show a low-uptake lesion in the left temporal lobe. MEG results revealed that epileptic activity was detected in the left temporal lesion (D, E, F).

れているが，病変は指摘されなかった。われわれが retrospective に見直してみると T_2 強調画像で左側頭葉に内部が高信号，周辺部が等～高信号の病変が認められた。

当科初診時の CT では，単純，造影とも明らかな異常は指摘できなかった。

MRI では左側頭葉に皮質の腫大がみられ，内部は T_1 強調画像で低信号，T_2 強調画像では高信号である。周辺部は等～高信号である。造影効果は認められない（Fig. 1）。FLAIR の冠状断，矢状断をみると，病変は中側頭回後方に位置する。MRS（magnetic resonance spectroscopy）では NAA（N-acetyl aspartate）ピークの低下が認められる。明らかな choline ピークの上昇はみられず，low grade glioma のパターンと考えられた。

SPECT では，タリウムで病変部は低集積，イオマゼニルでも病変部位に一致した低集積が認められた。FDG（fluorodeoxyglucose）PET でも病変部位は低集積を示した。MEG によるてんかん焦点の検索を行うと，てんかん焦点は病変部に一致して推定された（Fig. 2）。

脳血管造影では明らかな腫瘍濃染などの異常所見はみられなかった。

手術所見　以上の結果から，術前診断として左側頭葉の low grade glioma，あるいは皮質形成異常による症候性てんかんと診断し，摘出術を施行することとした。

症例は生来右利きであり，言語有意半球の病変と考えられたため，言語野を同定しつつ摘出術を施行する目的で覚醒下手術を施行した。術中所見では病変部はやや赤色で腫大しており，皮質脳波では病変後方に spike の出現を認めた。Language mapping を行ったところ，病変の後下方の電気刺激によって picture naming の障害が出現した（Fig. 3）。

病変周囲の脳溝を鋭的に分け，病変切除を施行した。摘出後の皮質脳波で spike の消失を確認し手術を終了した。

術後経過　術後 MRI では，病変は全摘出されている（Fig. 4）。

術直後一過性の軽度の物品呼称の障害が出現したが，約 1 週間で完全に症状は消失した。WAIS-R では，術前は VIQ59，PIQ59，IQ52 であったものが，術後 1 カ月の

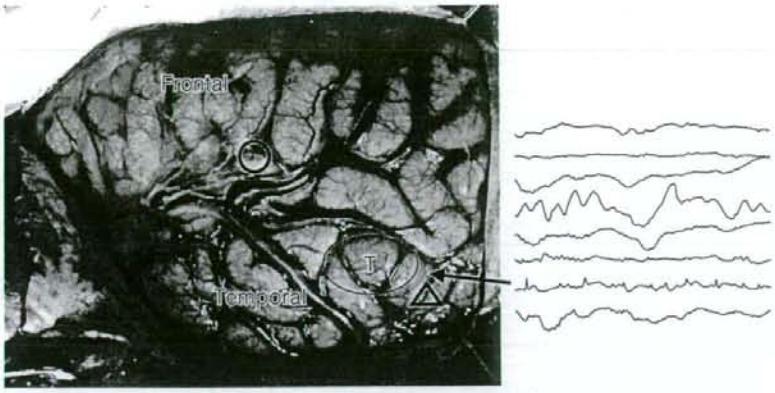

Fig. 3 Intraoperative photograph
The lesion showed swelling and was reddish. On the EEG rerealed spike activity in the posterior part of the lesion. T: tumor, ○: dysarthria, △: anomia.

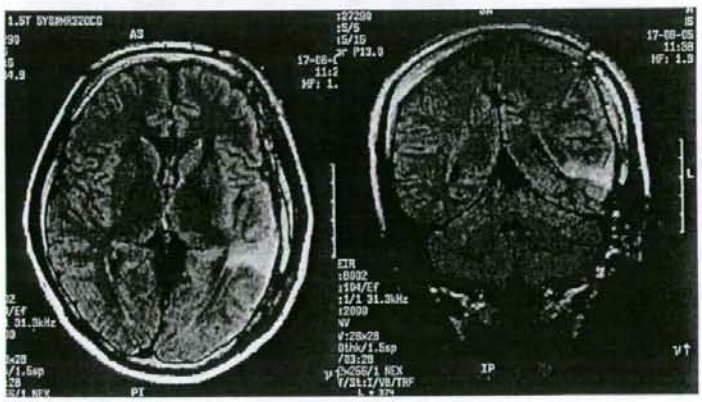

Fig. 4 Postoperative MRI
The lesion was removed completely.

時点ではVIQ72, PIQ70, IQ67と著明に改善した。てんかん発作に関しても術前はゾニサミド200 mg, バルプロ酸1,000 mg, カルバマゼピン400 mgでコントロールしていたが, 術後10カ月経過した現在, カルバマゼピン400 mgの内服のみで完全に消失している。

病理学的所見(Fig. 5) 病変部では腫瘤の形成はなく, 正常のグリア細胞とほぼ同大の核を持つ腫瘍細胞がびまん性に増生しており, 細胞密度が軽度に増加している。核の異型は乏しく核分裂像は認められない。核異型が乏しいため, 腫瘍細胞と背景のグリア細胞の鑑別はかなり困難である。既存の脳組織の破壊はなく, 反応性星細胞もみられない。免疫組織化学的には, GFAP(+/−), CD34, NeuN, NFPは陰性であった。MIB-1 LIは大部分で3%以下であったが, 最も高い部位で9.2%と軽度高値であった。

以上の病理学的所見より, 本例はdiffuse astrocytoma, WHO Grade IIと診断したが, 核異型の乏しさ, 免疫染色の結果, および病歴よりisomorphic astrocytomaと考えられた。しかし, 部分的にMIB-1 LIが軽度高値を示したため, 後療法としてWHO分類Grade IIの治療に準じて50 Gyの放射線照射を追加した。

II. 考　　察

Isomorphic astrocytomaは, 2004年にSchrammらによってdiffuse astrocytomaのうち長期のてんかん歴を持ち, 組織学的にMIB-1 LIが低く, 臨床的にはdif-

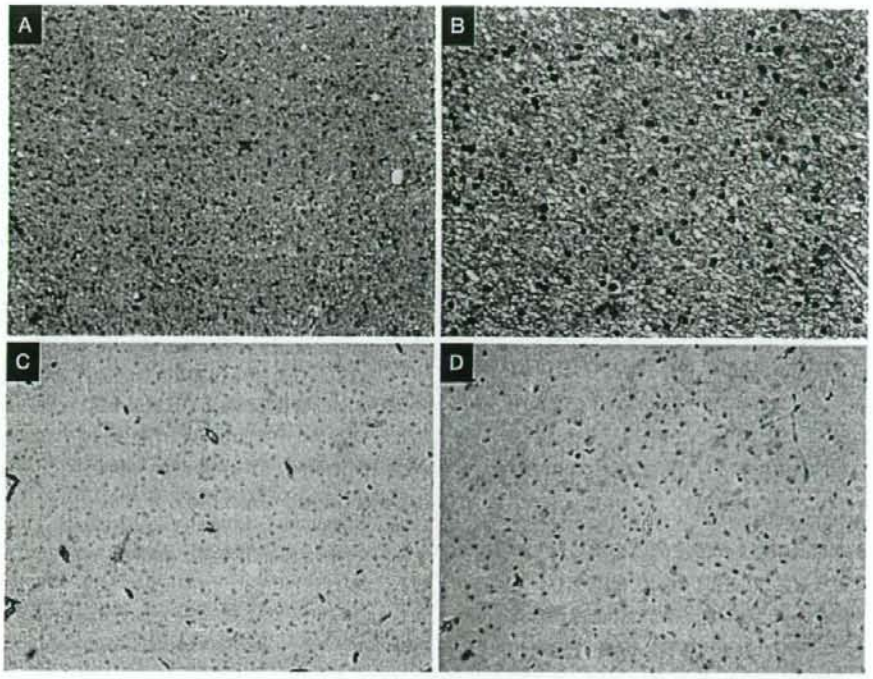

Fig. 5 Pathological findings
Diffuse proliferation of the glial cells was noted and cellularity is slightly elevated (A: H&E×100, B: H&E×400). Immunoreactivity for CD34 was negative (C×100) and the MIB-1 LI was low (D×200).

Table The differential diagnosis of the brain tumor and associated long-term epilepsy

Tumor	WHO Grade	Nuclear atypia	MAP2	CD34
isomorphic astrocytoma	I	No	−	−
astrocytoma	II	Yes	+	−
oligodendroglioma	II	Yes	+	−
ganglioglioma	I	Var.	+	+

The features of the isomorphic astrocytoma were as follows: no nuclear dysmorphism and negative results on immunohistochemical staining for MAP2 and CD34. Var: variable.

fuse astrocytoma よりも予後良好という特徴を示す一群が存在するとして提唱された新しい概念である[2,3]。彼らは，diffuse astrocytoma の中で，長期てんかん歴を持つ一群の予後が，そうでない群と比べ良好であることを見出し，clinical entity として LEAT (long-term epilepsy associated tumors) という概念を打ち出した。その後 LEAT の病理像を詳細に検討し，isomorphic astrocytoma という新しい腫瘍を提唱した[4]。予後良好なことから，isomorphic astrocytoma は WHO 分類では Grade I に相当すると述べられている。

長期のてんかん発作を特徴とする脳腫瘍としては，astrocytoma のほかに oligodendroglioma，ganglioglioma といったものがある[5,6]。その中で，isomorphic astrocytoma の病理学的特徴として，異型が少なく細胞密度の比較的低い組織像，核異型や核分裂像が少ないこと，および増殖能が低い（MIB-1 LI＜1％）ことが挙げられる。また，免疫組織化学的に MAP2 や CD34 に不染性であることにより oligodendroglioma や ganglioglioma との鑑別が可能であるとされている（Table）[3]。

本症例も，isomorphic astrocytoma の病理学的特徴を

核の異型性，細胞密度で示し，isomorphic astrocytoma と病理診断された。ただし，MIB-1 LI が大部分で 3％程度であったが，一部では 9.2％と高値であり，既報告の isomorphic astrocytoma とは異なる点である。このような腫瘍の一部には，MIB-1 LI が局所的に高い例もあるのではないかと考えた。画像診断的な特徴は報告されていないが，本症例では，石灰化，嚢胞形成などは認められなかった。臨床像では難治性てんかんを示し，さらに年単位の経過で画像上腫瘍の増大がみられないことが特徴であるが，本例も同様の臨床経過および画像所見を認めた。

以上の病理学的所見，画像所見，および臨床像から isomorphic astrocytoma の subtype と判定した。Subtype と判定した理由は MIB-1 LI が一部で高値であったことによる。治療としては，WHO Grade I 相当である isomorphic astrocytoma subtype ではあるが，腫瘍の一部分の MIB-1 LI が軽度高値を示したため摘出術の後，後療法として放射線局所照射を追加した。

これまでの報告の通り，本症例においても術後の epilepsy のコントロールは良好で術後 10 カ月経過した現在も発作は消失し，Engel's Class I となっている。

近年，low grade glioma の治療においては，本邦でも生存率の改善が認められるものの[7]，基本的に浸潤性性格を持つ glioma の治療を考えると摘出術のみで治癒が得られる症例は少なく，当科では WHO Grade II の症例に対しては術後の放射線療法を施行することをプロトコールとしている[8]。その一方で，長期生存例の放射線療法による晩期の高次脳機能への影響など，生存率だけでなく患者一人ひとりの ADL を保った治療が重要であり，これまで diffuse astrocytoma として一律に扱われてきた腫瘍群の中から予後良好群を分別することで，より適切な治療が可能になると考えられる。今後は，当科でも病理学的に isomorphic astrocytoma であり手術的に全摘出できたと考えられる症例には，放射線療法を施行しないプロトコールを考えている。このことからも，subtype としての isomorphic astrocytoma を認識することは大変重要と考えられる。

しかし，isomorphic astrocytoma は非常に新しい概念であり，今後の症例の見直しや積み重ね，長期フォローが必要と考えられる。

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原著

補足運動野症候群を呈した神経膠腫手術例の検討

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Supplementary Motor Area Syndrome with Frontal Glioma

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Shinya Sato*, Takamasa Kayama*

Abstract

The supplementary motor area (SMA) is a region located within each cerebral hemisphere at the posterior medial border of the frontal lobe. It is considered to play an important role in planning, initiating and maintaining sequential motor actions. In this report, we aimed to confirm or invalidate the somatotopic organization of the SMA, correlates the pattern of clinical symptoms observed after SMA removal with the extent of resection. Althogh there was no apparent change shown in the monitoring of intraoperative motor evoked potential (MEP), four patients displayed postoperative SMA syndrome on the side of the body contralateral to the SMA resection. All patients developed postoperative severe hemiplegia. One dominant frontal glioma patient was followed by transient mutism and motor aphasia. In this study, there is no correlation between extent of SMA resection and postoperative clinical pattern of deficits.

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Key words : supplementary motor area syndrome, glioma, MEP

はじめに

補足運動野 (supplementary motor area: SMA) は，前頭葉内側部に存在し，前交連 (anterior commissure: AC) を通り，前交連 (AC) ―後交連 (posterior commissure: PC) line に垂直な VCA (vertical commissure anterior) line により，前方の pre SMA と後方の SMA proper とに区別される[1]。SMA proper は運動の企画，開始，維持を担っているとされ，前方から顔面，上肢，下肢という体部位局在がある[2]との報告もなされている。

SMA の障害により，対側の麻痺と，優位半球病変では mutism が生じるが，それらは比較的急速に回復することが特徴とされ，補足運動野症候群 (SMA 症候群) と呼ばれている。今回われわれは，当科で開頭術を行った前頭葉 glioma のうち SMA 症候群を呈した 4 例において，SMA の摘出範囲と従来報告されている SMA の体部位局在との関係についての検証をしたので報告する。

I．対象と方法

当科にて，1994 年から現在まで開頭術を行った 44 例の前頭葉 glioma のうち，術後 SMA 症候群を呈した症例は 4 例 (9.1%) であった (Table 1)。この 4 症例において，①術前後の MRI における SMA 領域と腫瘍局在，切除範囲の関係を比較し，②運動障害および言語障害の経過と，SMA の切除範囲との関係について検討した。

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Table 1 Summary of the 4 patients.

Case	Age/Sex	Lesion side	Dominant or non-dominant	Pathology	MIB-1 L.I.	MEP D-wave
1	49/F	left	dominant	oligodendroglioma	5.0	no change
2	35/M	right	non-dominant	oligodendroglioma	10.6	no change
3	51/F	right	non-dominant	oligodendroglioma	3.2	no change
4	18/M	right	non-dominant	oligoastrocytoma	7.4	no change

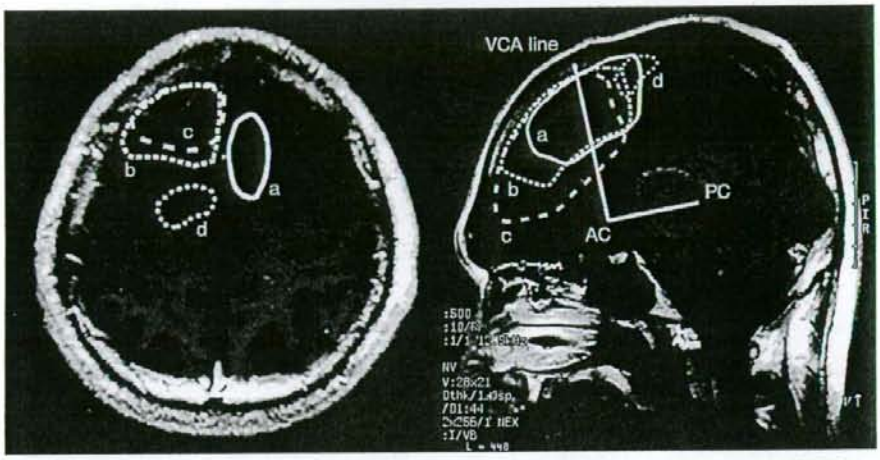

Fig. 1 Resection area in the 4 patiens on T_1 weighted magnetic resonance image (MRI)

年齢は18～51歳(平均38.2歳)，男性2例，女性2例であった。病変は左側が1例，右側が3例であり，左側の1例のみが優位側病変で，他3例は非優位側病変であった。病理診断は，oligodendroglioma 3例，oligoastrocytoma 1例である。MIB-1 labeling index は3.2～10.6%(平均6.6%)であった。全例で術中MEPモニタリングを施行したが，D-waveのamplitudeの低下や消失は認めなかった。

II. 代表症例

〈症例1〉 49歳 女性

診 断 左前頭葉 oligodendroglioma

経 過 全身痙攣にて発症した。頭部MRIでは左前頭葉に径4 cmのT_1 low，T_2 high，Gdにて淡く造影される病変を認めた。Low grade gliomaの診断で，開頭腫瘍摘出術を施行した。術中MEPモニタリングでD-responseの変化なく腫瘍を全摘出した。術直後よりDejong 1の右片麻痺とmuteを認めた。術後1日目より麻痺は徐々に改善，術後1週間では上下肢ともにDejong 3まで回復した。また，術後2週間より発語も可能となり，その後，術後1カ月ほどで運動障害，言語障害ともほぼ完全に消失した。病理診断はoligodendrogliomaであった。放射線化学療法を施行し，術後2年が経過した現在も再発はみられていない。

III. 結 果

1. 術前後のMRIの比較 (Fig. 2)

症例1は優位半球，症例2，3，4は非優位半球に腫瘍が存在していた。全例で切除範囲がVCA line後方に及んでおり，SMA properの領域が部分摘出されていた。症例1，2，3はSMA proper前方が主体であり，症例4ではSMA proper後方に限局していた。全例で切除範囲は，一次運動野や言語野には及んでいなかった。

2. 臨床症状の経過 (Fig. 3)

優位半球病変を切除した症例1においてのみ一過性のmutismを認め，非優位半球病変の症例2，3，4では言語障害は認めなかった。また，全例で反対側に上下肢の運動麻痺を認め，上肢のみあるいは下肢のみに限局した運動麻痺は認めなかった。また，症状の経過としては，

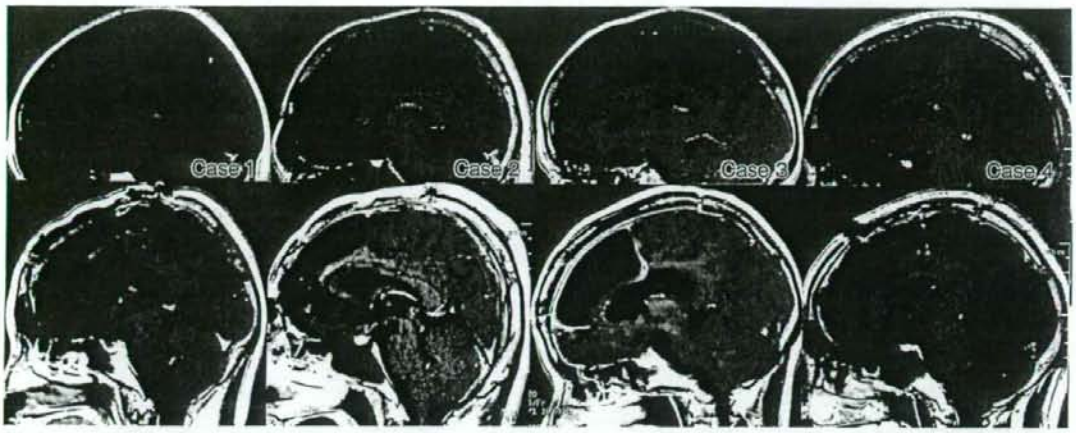

Fig. 2 Pre(upper line)-and post(lower line)-oparative Gd-enhanced,T1 weighted magnetic resonance images obtained on the 4 patients

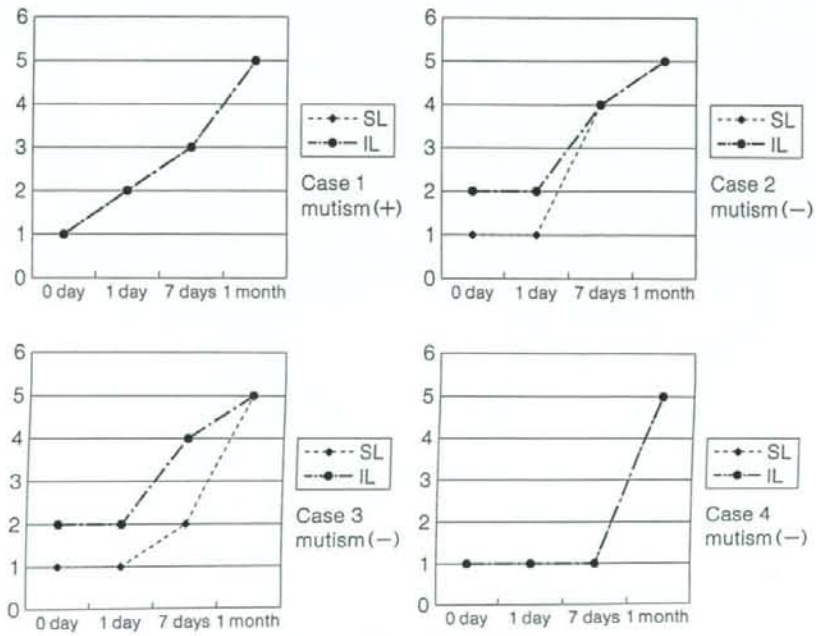

Fig. 3 Postoperative course
Y-axis: Dejong grade, X-axis: Time course, SL: Superior Limb, IL: Inferior Limb

Rostomily らの報告[3]と同様に術後数日から回復し始め，術後1カ月後にはほぼ完全に回復した。

IV. 考　　察

SMA は，解剖学的には一次運動野の前方内側に存在し，前交連から AC-PC line に垂直に引いた VCA line によって前方の preSMA と後方の SMA proper とに組織学的に区別される[1]。従来より報告されている SMA の高次の運動調節に関する働きは，SMA proper が担っていると考えられている。

今回の検討では，術中の MEP モニタリングでも異常

を認めず，術後MRIで一次運動野の障害が認められないにもかかわらず，術後一過性の対側の運動障害や優位半球病変ではmutismを認めた。これまでの報告[4,5]でも，MEPモニタリングでは術後のSMA症候群を予測できないと報告しており，当科の症例もこれに矛盾しないものであった。これはMEPモニタリングの限界と考えられるが，逆にMEPモニタリングが保たれた例ではSMA切除後麻痺が生じたとしても一過性である可能性が高いことから，モニタリングとして大変有用であると考えられる。KrainikらはfMRIを用いて術前にSMAを同定し，fMRIで同定されるSMAの摘出範囲と術後の症状の重症度，回復までの期間に相関がある[1]と報告しているが，fMRIで同定されたSMAが前下方のみ摘出された場合でも術後にmutismと片麻痺を呈した[6]と報告しており，今回のわれわれの検討と同様に，SMAの体部位局在には一致していなかった。症状の回復過程はこれまでRostomilyら[3]が報告しているのと同様であり，今回の4症例では，摘出された解剖学的SMAの容量と症状の程度，回復までの時間に相関はないと思われた。SMAの体部位局在については，FotaineらがSMAにも一次運動野同様に体部位局在があり，SMAの切除部位によって出現する症状が異なる[2]と報告しているが，症例3のようにSMA前方のみが摘出された場合でも，症例4のようにSMA後方のみの摘出の場合でも上下肢ともに麻痺が出現し，従来報告されているようなSMAの体部位局在に相応する症例は認められなかった。SMAの体部位局在については，Zentnerらのように疑問視する報告[5]もあり，今後fiber trackingやawake surgry等の知見から，SMAの機能が解明されてくることが期待される。SMA症候群は，low grade gliomaで発生しやすいといわれている[7,8]が，われわれの症例も4例全例oligo系のlow grade gliomaであった。これは腫瘍がゆっくりと成長し，正常神経細胞と共存していられるためと考えられる。摘出後比較的速やかに症状が回復する機序としては，Krainikらのように対側のSMAが賦活化され機能が補われる[9]との報告がみられ，今後検討が必要と考えている。

今回のわれわれの検討では明らかなSMAの体部位局在を見出すことができなかったが，それはこれまで報告されているような体部位局在がそもそも存在しないのか，一次運動野や運動のプログラミングに関わるその他の部位との皮質下の線維連絡の離断範囲の違いによるものなのか，その他の原因によるものなのか，今後症例を重ねて検討していく必要があると考えられる。

V. 結　　語

SMA症候群を呈した前頭葉gliomaの開頭術後症例の検討を行った。SMAの切除範囲にかかわらず，全症例において一過性の上下肢の運動障害を認め，一次運動野のような体部位局在は明らかではなかった。SMAそのものの機能，またSMAとその他部位との線維連絡など，今後の検討が必要であると考えている。

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Enhanced antitumor effect of combined-modality treatment using convection-enhanced delivery of hydrophilic nitrosourea with irradiation or systemic administration of temozolomide in intracranial brain tumor xenografts

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Objective: *Convection-enhanced delivery (CED) is a local infusion technique that delivers chemotherapeutic agents directly to the central nervous system, circumventing the blood–brain barrier and reducing systemic side effects. We previously reported the safety and efficacy of CED of ACNU (nimustine hydrochloride: 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride), a hydrophilic nitrosourea, in rat brain tumor models. This study evaluated the efficacy of combined-modality treatments using CED of ACNU with irradiation or systemic administration of temozolomide.*

Methods: *Antitumor efficacy and toxicity of the treatment were evaluated using rat 9L intracranial brain tumor models.*

Results: *Combined treatment using CED of ACNU with irradiation produced significantly longer survival time than each treatment alone (versus CED: $p<0.001$, versus irradiation: $p<0.05$, log-rank test) or systemic administration of ACNU with irradiation ($p<0.001$). Long-term survival (120 days) and eradication of tumor occurred only in this combined-treatment group. We also showed that CED of ACNU plus systemic administration of temozolomide significantly enhanced survival rate compared with each treatment alone (versus CED: $p<0.001$, versus systemic temozolomide: $p<0.05$).*

Discussion: *Multimodality treatment using CED of ACNU, radiotherapy and systemic chemotherapy with temozolomide is a promising strategy for treatment of brain tumors.* [Neurol Res 2008; **000**: 000–000]

Keywords: Brain tumor; convection-enhanced delivery; nimustine hydrochloride; radiotherapy; temozolomide

INTRODUCTION

Despite aggressive multimodal therapy, the prognosis of patients with high-grade glioma remains dismal. Though radiotherapy plus concomitant and adjuvant temozolomide, a novel oral alkylating agent, improves the survival of patients with high-grade glioma, median survival is still less than 15 months[1], and further exploration is needed in order to increase the efficacy of temozolomide-based regimens in the treatment of high-grade glioma.

In the application of systemic chemotherapy to intracranial malignancies, poor penetration of most anticancer drugs across the blood–brain barrier (BBB) into the central nervous system remains a major obstacle[2,3]. Even with use of agents that penetrate the BBB, tumoricidal drug concentrations are difficult to obtain in tumor tissue without systemic side effects. On the other hand, local methods of delivery that bypass the BBB, including direct injection or biodegradable polymer or wafer implantation, yield only limited distribution of drug within the target site[4].

In an effort to improve drug distribution within brain tissue, several recent studies have demonstrated the possibility of use of convection-enhanced delivery (CED)[5,6]. With the use of a pressure-driven bulk flow process, CED distributes agents to clinically relevant volumes of solid tissues[5]. Therapeutic drugs delivered via CED circumvent the BBB, and high drug concentrations reach the site of injection and are widely

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distributed within the target site. Compared with routes of administration dependent on diffusion from injection or implantation sites, CED yields a greater volume of distribution and is designed to direct a drug to specific target volumes[7–10]. In addition, CED minimizes systemic exposure, resulting in fewer systemic side effects.

Many investigators are now applying this technique to brain tumors. A large variety of antineoplastic drugs, such as immunotoxins[7,11–14], boronated drugs[15,16], liposomal drugs[17–19] and free antineoplastic agents[8,20,21], have been administered using this technique, all with promising outcomes. In particular, 1,3-bis-chlorethyl-1-nitrosourea (BCNU), which had one of the most proven efficacies in systemic chemotherapy for high-grade glioma before the advent of temozolomide, has been considered as a promising candidate for CED, and it has been reported that BCNU locally delivered via CED provided favorable therapeutic outcomes in rat models of glioma[21]. The efficacy of TMZ delivered via CED has also been reported in rat models of glioma[22], and together with the findings noted above, suggests that CED of alkylating agents is a promising strategy for treating intracranial malignancies.

We previously demonstrated that a hydrophilic nitrosourea, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU), could be safely and effectively administered via CED in an *in vivo* rat brain tumor model[23]. However, our findings then were still unsatisfactory, and some factors limiting antitumor efficacy were noted: (1) the limitation of the therapeutic window of ACNU delivered via CED due to the local CNS toxicity ascribed to the non-specific cytotoxicity of ACNU; (2) the short tissue retention time of ACNU infused once via CED in brain; (3) the heterogeneous distribution of ACNU administered via CED within tumors, which resulted in partial response and local recurrence of brain neoplasms.

To improve the variability in efficacy within tumors, this study examined the efficacy of combined treatment using CED of ACNU with irradiation or systemic concomitant administration of temozolomide in rat 9L brain tumor models.

The findings obtained in this study advocate the new concept of a multimodal approach using CED of ACNU with radiotherapy or systemic chemotherapy of temozolomide for the treatment of malignant glioma.

MATERIALS AND METHODS

ACNU and temozolomide

ACNU was provided by Sankyo Co. Ltd (Tokyo, Japan). Infusion solutions of ACNU were prepared by diluting ACNU in saline to a concentration of 0.5 mg/ml. TMZ was provided by Schering-Plow K.K. (Osaka, Japan) and was dissolved in a solution of 10% dimethyl sulfoxide (Sigma, St Louis, MO, USA) in 0.9% saline.

Tumor cell line

9L gliosarcoma cells (American Type Culture Collection, Rockville, MD, USA) were maintained as monolayers in complete medium consisting of Eagle's minimal essential medium supplemented with 10% fetal calf serum, non-essential amino acids and 100 U/ml penicillin G. Cells were cultured at 37°C in a humidified atmosphere consisting of 95% air and 5% CO_2.

Animals and intracranial xenograft technique

All protocols used in the animal studies were approved by the Institute for Animal Experimentation of the Tohoku University Graduate School of Medicine.

Male Fisher 344 rats weighing ~200 g were purchased from Japan SLC Inc. (Japan SLC Inc., Shizuoka, Japan). To produce the intracranial xenograft tumor model, 9L gliosarcoma cells were harvested by trypsinization, washed once with Hanks balanced salt solution (HBSS) without Ca^{++} and Mg^{++}, and resuspended in HBSS for implantation. Cells (5×10^5) in 10 µl HBSS were implanted in the striatal region of Fisher 344 rat brains as follows: under deep isoflurane anesthesia, rats were placed in a small-animal stereotactic frame (David Kopf Instruments, Tujunga, CA, USA). A sagittal incision was made to expose the cranium, followed by a burr hole in the skull at 0.5 mm anterior and 3 mm lateral to bregma using a small dental drill. Cell suspension (5 µl) was injected over 2 minutes at a depth of 4.5 mm from the brain surface; after a 2 minute wait, another 5 µl was injected over 2 minutes at a depth of 4.0 mm, and after a final 2 minute wait, the needle was removed and the wound was sutured.

Convection-enhanced delivery

CED of ACNU or saline was performed using a volume of 20 µl as described previously[24]. Briefly, the infusion system consisted of a reflux-free, step-design infusion cannula[25] connected to a loading line (containing ACNU or saline) and an olive oil infusion line. A 1 ml syringe (filled with oil) mounted onto a micro-infusion pump (BeeHive; Bioanalytical Systems, West Lafayette, IN, USA) regulated the flow of fluid through the system. Based on chosen coordinates, the infusion cannula was mounted onto stereotactic holders and guided to the target region of the brain through burr holes made in the skull. The following ascending infusion rates were applied to achieve the 20 µl total infusion volume: 0.2 µl/min (15 minutes) + 0.5 µl/min (10 minutes) + 0.8 µl/min (15 minutes).

Whole-brain irradiation in a single fraction

Irradiation was conducted with X-rays generated by a Shimadzu HF-320 apparatus (Shimadzu Mectem, Shiga, Japan) operated at 200 kV and 10 mA with 0.5 mm Cu and 1.0 mm Al filters. The dose rate to the brain was 0.72 Gy/min and total dose of 8.64 Gy was given in 12 minutes. The animals were anesthetized before irradiation with intraperitoneal sodium pentobarbital 30 mg/kg body weight. The whole body of each animal, excluding the head, was covered with lead sheets, and four rats were irradiated at a time.

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Toxicity of combined treatment with CED of ACNU plus irradiation in normal rodent CNS

Nine male Sprague–Dawley rats weighing ~200 g (Japan SLC Inc.) were assigned to three groups: (1) a radiation group, receiving whole-brain irradiation to a total dose of 8.64 Gy (n=3); (2) a CED group, receiving a single 20 µl CED infusion of ACNU (n=3); (3) a CED plus radiation group, receiving CED of ACNU and irradiation 3 days after CED (n=3). ACNU was used at half the maximum tolerated dose (MTD), 0.01 mg/rat, as determined in a previous study[23]. Rats were monitored daily for survival and weekly for weight and general health. All rats were euthanized on the thirtieth day after conclusion of treatment, and their brains were removed, fixed, subjected to paraffin sectioning (5 µm) and stained with hematoxylin and eosin (H&E).

Antitumor effect of combined treatment using CED of ACNU with irradiation in a rat 9L brain tumor model

Forty rats with 9L tumor cells were randomized into five experimental groups with eight animals each. The groups were as follows: (1) a control group, receiving CED of saline; (2) a CED group, receiving CED of ACNU at a dose of 0.01 mg/rat; (3) a radiotherapy group, receiving whole-brain irradiation to a total dose of 8.64 Gy; (4) a systemic chemotherapy plus radiotherapy group, receiving intravenous (i.v.) injection of ACNU at a dose of 0.4 mg/rat (2 mg/kg: clinically tolerable dose for i.v. administration[26]) followed by whole-brain radiation to a total dose of 8.64 Gy; (5) a CED plus radiotherapy group, receiving CED of ACNU at a dose of 0.01 mg/rat, followed by whole-brain radiation to a total dose of 8.64 Gy. Seven days after tumor cell implantation, a single CED infusion (20 µl; saline) was performed for the control and radiotherapy groups, while the CED group and the CED plus radiotherapy group were given a single 20 µl CED infusion of 0.5 mg/ml ACNU. The systemic chemotherapy plus radiotherapy group received a bolus IV injection via a tail vein (0.4 ml; 0.1 mg/ml ACNU). Ten days after tumor implantation, whole-brain irradiation in a single fraction to a total dose of 8.64 Gy was given to the radiotherapy group, the systemic chemotherapy plus radiotherapy group and the CED plus radiotherapy group. Rats were monitored daily for survival and general health. Animal weights were reported weekly. The study was terminated 120 days after tumor implantation, when the surviving animals were euthanized and their brains stained with H&E. Results for the survival studies are indicated using Kaplan–Meier curves. Survival rates of the treatment groups were compared with the log-rank test.

Toxicity of combined treatment with CED of ACNU plus systemic administration of temozolomide in normal rodent CNS

Nine male Sprague–Dawley rats weighing ~200 g (Japan SLC Inc.) were assigned to three groups: (1) a CED group, receiving a single 20 µl CED infusion of ACNU at doses of 0.01 mg/rat (n=3); (2) a temozolomide group, receiving intraperitoneal injection of temozolomide for 5 days (n=3); (3) a CED plus temozolomide group, receiving both CED of ACNU and systemic administration of temozolomide (n=3). Temozolomide was given systemically (intraperitoneal administration) at a dose of 350 mg/m^2/day daily for 5 days[27]. CED of ACNU was performed on the third day after starting temozolomide administration. Rats were monitored daily for survival and weekly for weight and general health. All rats were euthanized on the thirtieth day after the conclusion of treatment, and their brains were removed, fixed, subjected to paraffin sectioning (5 µm) and stained with H&E.

Antitumor effect of combined treatment using CED of ACNU with systemic administration of temozolomide in a rat 9L brain tumor model

Thirty-eight rats that received 9L tumor cell implants were randomly divided into four groups: a control group (n=8), a CED group (n=10), a temozolomide group (n=10) and a combination group (n=10). Seven days after tumor implantation, CED infusions of 20 µl ACNU at a dose of 0.01 mg/rat were performed in the CED and combination groups. In the control and temozolomide groups, CED infusion of 20 µl 0.9% saline was performed as a control. In the temozolomide and combination groups, temozolomide (350 mg/m^2/day) was given systemically (intraperitoneal administration) daily for 5 days, starting on day 5 after tumor implantation. Survival rates in the treatment groups were compared using the log-rank test. Survival was estimated using Kaplan–Meier curves.

RESULTS

Toxicity of combined treatment using CED of ACNU with irradiation

Rats receiving whole-brain irradiation to a total dose of 8.64 Gy exhibited no radiation-induced changes in the CNS (*Figure 1a*). In animals receiving CED of ACNU at 0.01 mg/rat, brain tissues exhibited only evidence of minor trauma at the site of the infusion cannula in the striatum (*Figure 1b*). Animals that received CED of ACNU at 0.01 mg/rat, followed by whole-brain irradiation to a total dose of 8.64 Gy, also had minor trauma at the site of infusion, but no increase in tissue damage was found (*Figure 1c*).

No systemic toxicity was observed following any of these treatments. All rats survived without neurological or behavioral signs.

Synergistic antitumor effect of combined treatment using CED of ACNU with irradiation in a 9L rat brain tumor model

As shown in *Figure 2*, all animals in the control group died due to tumor progression by day 21 and mean survival was only 17.8 days (median: 18.5 days). CED of ACNU at half MTD (0.01 mg/rat) yielded no improvement in survival. All animals died by day 36 and mean survival was 21.0 days (median: 17.0 days).

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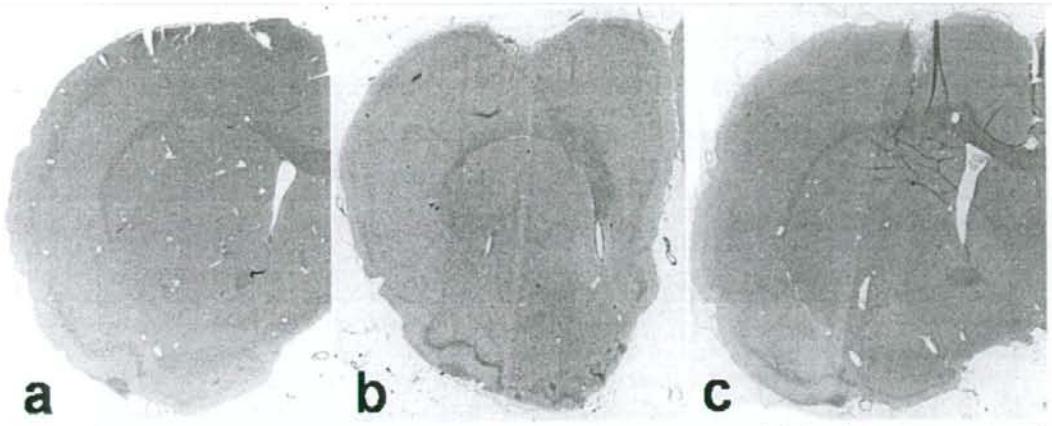

Figure 1: Evaluation of toxicity of combined treatment with CED of ACNU and irradiation in normal adult rat brains. Representative H&E sections 30 days after conclusion of treatment. Rats receiving whole-brain irradiation to a total dose of 8.64 Gy exhibited no radiation-induced changes (**a**). Rats treated with CED of ACNU at half MTD (0.01 mg/rat) had evidence only of minor trauma at the site of the infusion cannula in the striatum (**b**). Rats that received CED of ACNU at 0.01 mg/rat, followed by whole-brain irradiation to a total dose of 8.64 Gy, had minor trauma at the site of the infusion, but no other apparent local damage (**c**)

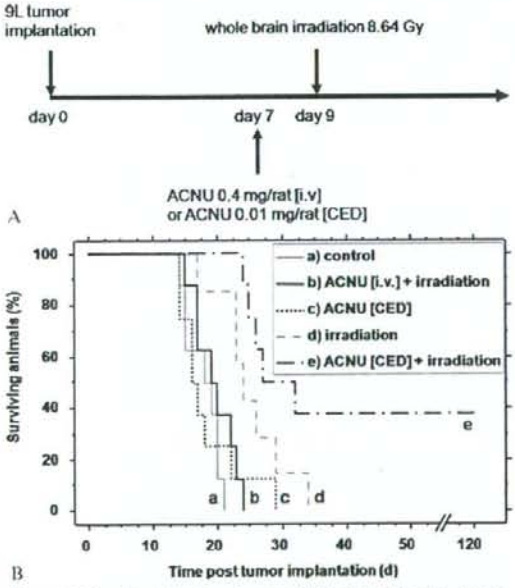

Figure 2: Combined treatment using CED of ACNU plus irradiation. (**A**) Experimental design of the survival study. CED infusion of saline, systemic administration of ACNU (0.4 mg/rat) and CED of ACNU at 0.01 mg/rat were performed 7 days after tumor implantation. Whole-brain irradiation was performed 9 days after tumor implantation. (**B**) Survival of treated animals was observed for 120 days and is indicated using Kaplan–Meier curves. Animals were treated with CED of saline (**a**), i.v. administration of ACNU plus irradiation (**b**), CED of ACNU alone (**c**), irradiation alone (**d**) or CED of ACNU plus irradiation (**e**)

Animals receiving whole-brain irradiation died by day 34, with a mean survival of 25.1 days (median: 24.0 days). This group had a significantly improved survival rate compared with the control group ($p<0.01$, log-rank test). Animals treated with systemic administration of ACNU plus irradiation died by day 24, with a mean survival of 19.6 days (median: 19.5 days). No survival advantage was observed compared with the control group. Animals treated with using CED of ACNU plus irradiation exhibited significantly improved survival rate compared with controls ($p<0.001$), animals receiving CED alone ($p<0.001$), those with systemic ACNU plus radiation ($p<0.001$) and those with radiation alone ($p<0.05$); as a result of this combined treatment, three of eight animals (37.5%) survived beyond day 120 (median: 29.5 days).

Histopathologic evaluation of brain tissue was performed in all animals at death or after sacrifice. Only three animals, in the group receiving CED of ACNU plus irradiation, survived to the end of the study at day 120 and exhibited complete pathologic response (*Figure 3a*). Tumor progression was observed in the brains of all rats that died (*Figure 3b*).

Toxicity of combined treatment using CED of ACNU with systemic temozolomide

In animals receiving CED of ACNU at 0.01 mg/rat, no increase in tissue damage was observed compared with the previous study (*Figures 1b* and *4a*). Animals receiving intraperitoneal injection of temozolomide (350 mg/m^2/day) for 5 days exhibited no change in the CNS (data not shown). Animals that received combined treatment with CED of ACNU plus systemic administration of temozolomide had minor trauma at the site of infusion, but no clear increase in CNS toxicity was found in them (*Figure 4b*).

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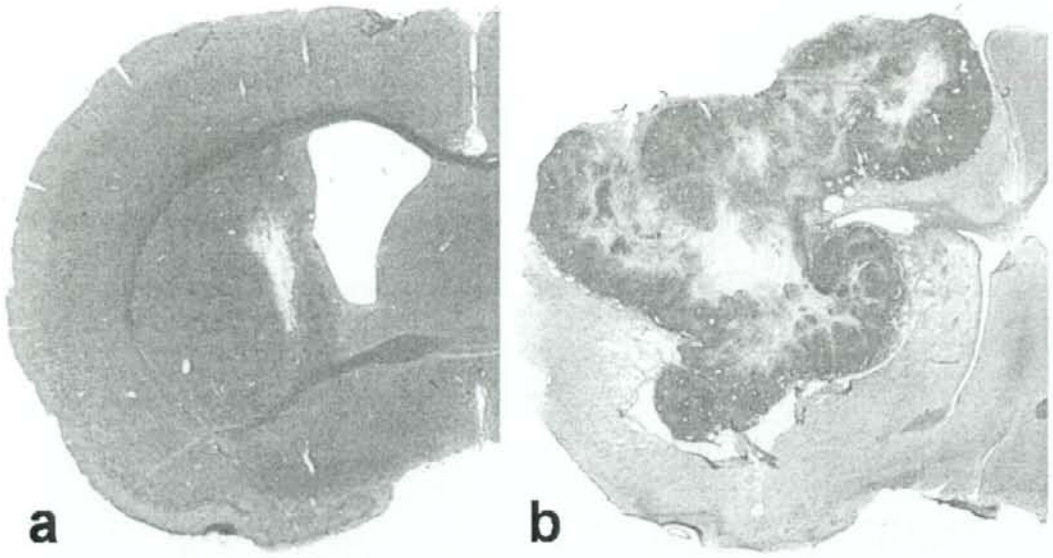

Figure 3: Representative brain sections from surviving and non-surviving animals. (a) Brain section obtained from one of the survivors treated by CED of ACNU at 0.02 mg/rat. None of the survivors had residual tumor. (b) Brain section from a rat of the control group showing typical tumor found in all non-surviving animals, in which tumor progression led to death

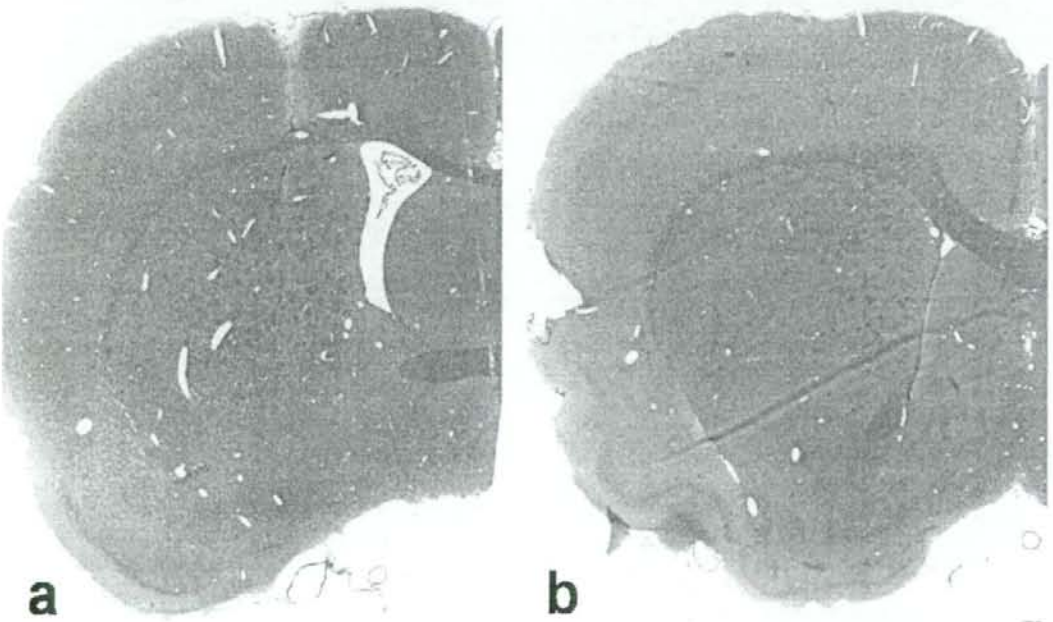

Figure 4: Evaluation of toxicity of combined treatment using CED of ACNU and systemic administration of temozolomide in normal adult rat brains. Representative H&E sections 30 days after the conclusion of treatment. Rats receiving CED of ACNU at half MTD (0.01 mg/rat) had evidence only of minor trauma at the site of the infusion cannula in the striatum (a). Rats that received CED of ACNU plus systemic temozolomide had minor trauma at the site of the infusion, but no other drug-induced damage (b)

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