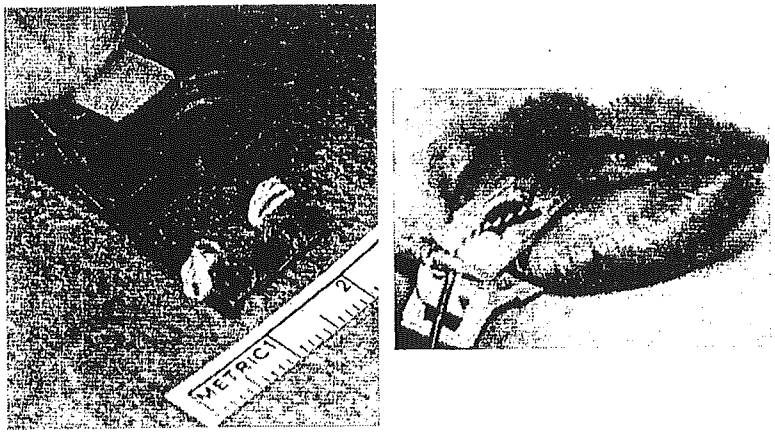

Fig. 1 Stimulation electrode for the lower lip.

The SSEPs were recorded at least twice to assess reproducibility. Recordings from bipolar chains of electrodes were used for the lower lip SSEPs. The reference electrode was placed at the nasion for the median nerve SSEPs.

RESULTS

The first cortical component for contralateral lower lip stimulus (N15), with a peak latency around 15 ms,[6] was obtained in seven of the nine patients. A mass lesion involving the hand and/or face area of the somatosensory cortex was found to abolish the median nerve and/or lower lip SSEPs. Neither lower lip nor median nerve SSEPs could identify the central sulcus in case 9, where identification of the motor cortex by direct stimulation was used with the aid of the neuronavigation system. The lesion in case 7 was also located in the hand-digit-face sensory area, but polarity inversion of the lower lip SSEPs was obtained because the inferior extension of the tumour was more limited than in case 9, preserving the lower lip somatosensory area. The area of the hand representation was not exposed by the craniotomy in case 4.

Representative case report

Case 1: A 38-year old male presented with a protoplasmic astrocytoma manifesting as left facial seizures in August 2000. He underwent partial tumour resection and five courses of three-drug

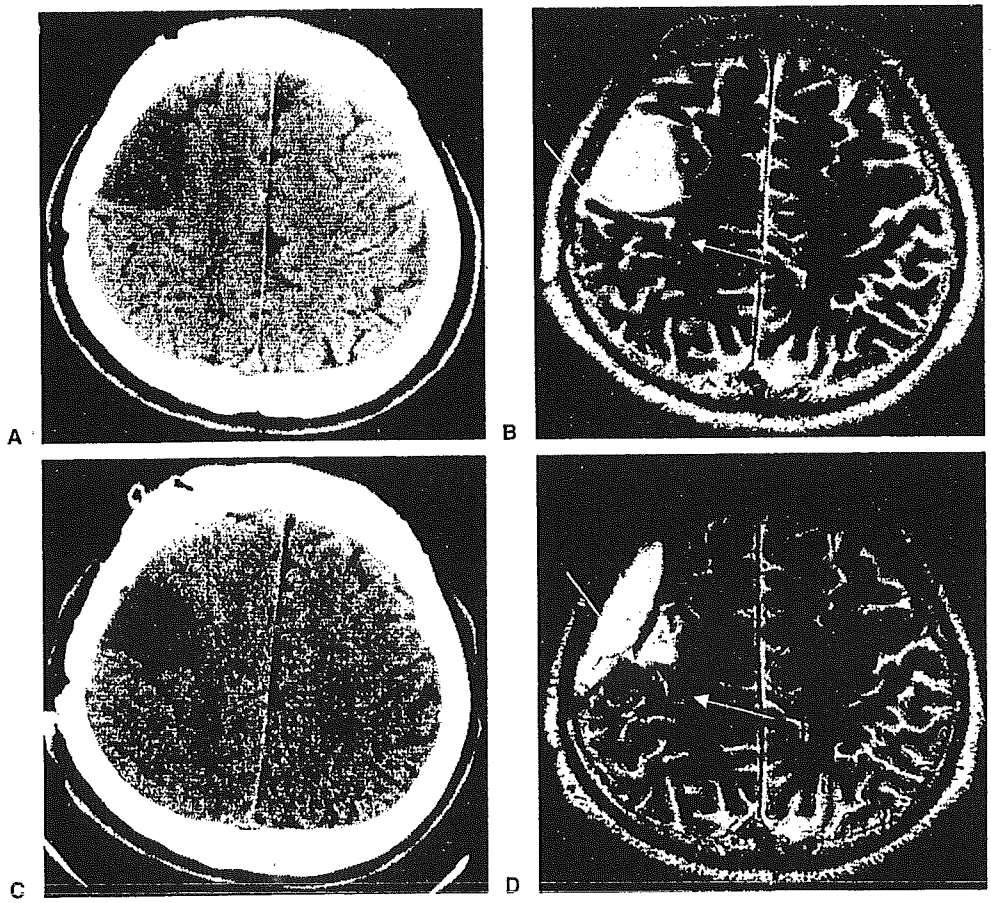

Fig. 2 *Case 1.* Arrows indicate the central sulcus: (A) Preoperative unenhanced computed tomography (CT) scan showing a hypodense glioma close to the central sulcus; (B) Preoperative axial T2-weighted magnetic resonance (MR) image showing the hyperintense glioma; (C) Postoperative CT scan; (D) Follow-up axial T2-weighted MR image.

chemotherapy. However, seizure activity increased in May 2002 and he was admitted to our hospital in July 2002. Left facial seizures occurred approximately 10 times per day. T2-weighted magnetic resonance (MR) imaging demonstrated a hyperintense lesion in the right precentral gyrus. The lesion was hypodense on CT and did not enhance with contrast (Fig. 2(A) and (B)). The tumour was located in the face motor area based on anatomical landmarks, including the inverted omega-shaped central sulcus on axial and functional MR imaging. Neurological and neuropsychological examinations were normal.

A frontoparietal craniotomy was performed under general anaesthesia. After incision of the dura, a silicone sheet containing eight electrodes was placed on the brain perpendicular to the central sulcus to record cortical SSEPs (Fig. 3(A) and (B)). Phase reversal of the lower lip SSEPs at about 15 ms was observed between electrodes 1–2 and 2–3 (Fig. 3(D)). Thus, the central sulcus was identified to be under electrode 2. Polarity inversions of the median nerve SSEPs across the central sulcus at about 20 ms were observed between electrodes 2 and 3 (Fig. 3(E)). The distance between these two points along the central sulcus was 35 mm. The tumour was totally removed up to the hand-digit motor area, using direct cortical and subcortical stimulation techniques and a neuronavigation system (Fig. 2(C), (D), and 3(D)).

Postoperatively, no neurological or neuropsychological deficits were observed. Left facial seizure activity disappeared. Histopathological examination revealed that the tumour had transformed to an anaplastic astrocytoma. As adjuvant therapy, the patient received 72 Gy of hyperfractionated radiation to an extended local field and chemotherapy using nimustine hydrochloride (ACNU). Careful, repeated observation for 46 months has not indicated any recurrence. Karnofsky score was 100% at last follow up.

DISCUSSION

The method presented for recording SSEPs using lower lip stimulation is straightforward with low risk. Seizures do not occur during evoked potential recording and anaesthetic variability is less prominent than during cortical stimulation. Evoked potential localisation can also be used in young children, in whom the exposed cortex is typically electrically poorly excitable.[4]

Recording of SSEPs has several limitations. Loss of N20 or P20 occurs in 9% of patients, thus phase reversal is not identified, presumably because electrodes are not exactly aligned over the sensory and motor hand areas.[1] Such "off axis" alignment of electrodes may also provide misleading results during localisation of the central sulcus.[5] SSEP phase reversal of N20–P20 was successful in 92% of 230 patients with tumours of the sensorimotor region, but recording of a typical N20–P20 phase reversal may be difficult in cases of large central and postcentral lesions.[3] In these situations, combined cortical SSEP recording for median nerve and trigeminal nerve lower lip stimulation can localise the central sulcus, and also trace its course between two points.

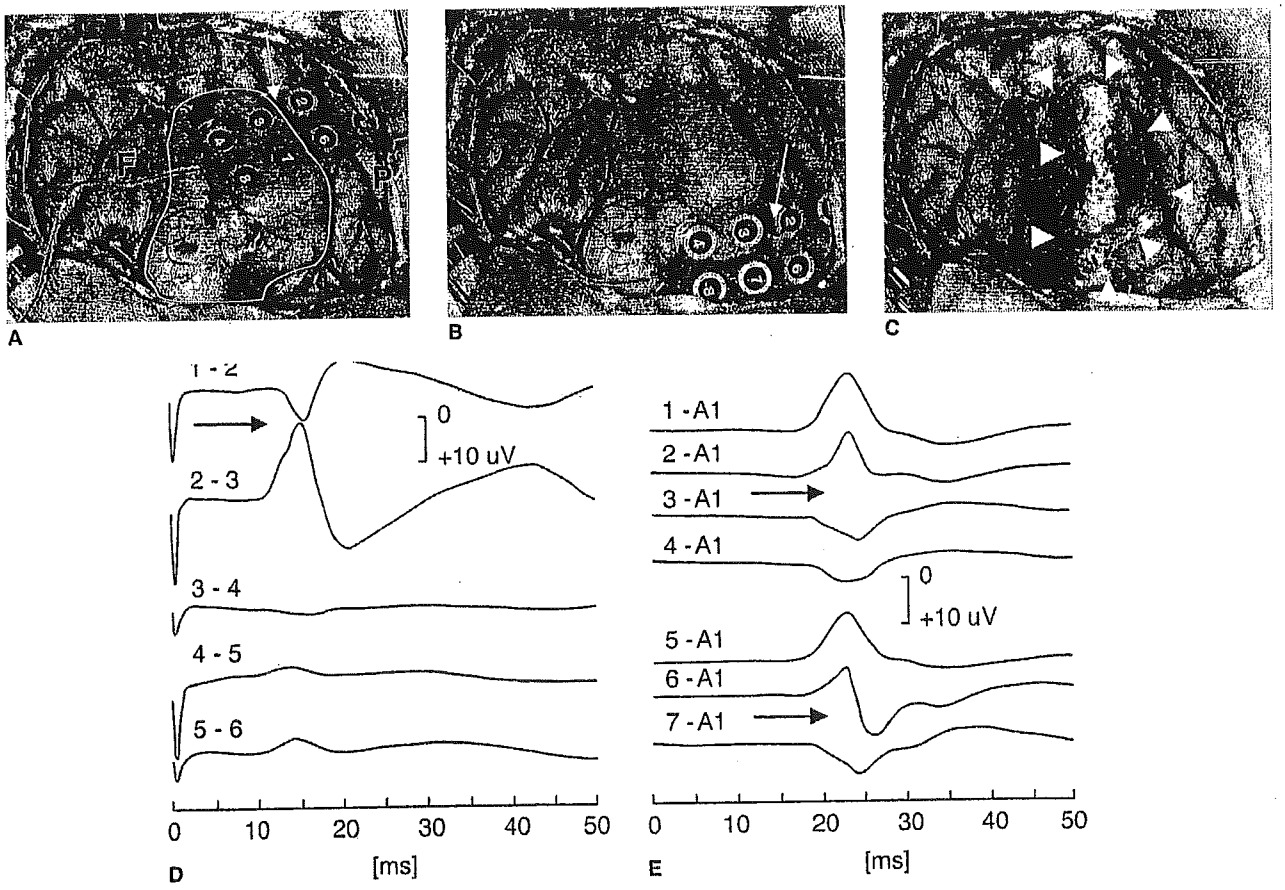

Fig. 3 *Case 1.* (A) and (D) Direct cortical somatosensory evoked potential recordings from stimulation of the lower lip. Electrodes 1, 2, 5, and 6 are posterior (parietal) and electrodes 3, 4, 7, and 8 are anterior (frontal). Recordings from bipolar chains of electrodes were used. Phase reversal is seen between electrode 1–2 and 2–3, marking the position of the central sulcus (black arrow, D). The continuous white line (A) indicates the location of the tumour. The white arrow indicates the central sulcus. F = frontal lobe; P = parietal lobe. (B) and (E) Direct cortical somatosensory evoked potential recordings from stimulation of the media nerve. Electrodes 1, 2, 5, and 6 are posterior (parietal) and electrodes 3, 4, 7, and 8 are anterior (frontal). Phase reversal is seen between electrodes 2–3 and 6–7 marking the position of the central sulcus (black arrows, E). The white arrow indicates the central sulcus. (C) Intraoperative photograph after gross total resection of the tumour. Arrowheads indicate the resection cavity.

Previously reported methods of SSEP recording[2] using stimulation of the chin, lips, tongue and palate have localised the face area of the human sensorimotor cortex. The upper and lower lip representations overlap adjacent to the hand area, and have been reported to provide little additional localising information if the hand area has been identified. The representation of the lower lip is slightly more lateral than the upper lip, but separate stimulation of the upper and lower lip provides no practical advantage. Polarity inversion of potentials across the sulcus has been reported to be a less reliable criterion for trigeminal SSEPs, partly because most recordings are made with 10 mm inter-electrode spacing, and inconsistent recording of polarity inversions may simply be due to lack of optimal recording sites, or because the dipole generators may have a more radial orientation, with little or no polarity inversion across the cortical surface.[2]

In contrast, our study indicates that lower lip SSEPs can localise the face representation areas of the somatosensory and motor cortex under general anaesthesia using polarity inversion. Our stimulating apparatus was tightly attached to the lower lip, resulting in constant stimulation and we used higher intensity and lower frequency stimuli than the 0.4 and 4 Hz frequency, and intensities of two or three times the sensory threshold used in previous studies of trigeminal SSEPs.[8–12] The most suitable intensity of nine times the sensory threshold was indicated by a previous magnetoencephalography (MEG) study and was well tolerated, even when the patient was awake. Our experience is that the stable pressure of the clip electrode is particularly useful to maintain constant resistance and electrode contact and to achieve a constant stimulus intensity, which helps to minimise subject apprehension. Longer inter-stimulus intervals evoked a larger amplitude of N15m. The present study used biphasic waveforms for electrical stimulus and recordings from bipolar chains of electrodes, which reduces artefacts.

We could not establish the normal distance between the phase reversal points of median nerve and lower lip SSEPs, because pin-point definition of the phase reversal was difficult in cortical SSEPs using strip electrodes, and because some patients had no clear-cut phase reversal of median nerve SSEPs. Our MEG study of normal subjects found that the distance between the N15m source of the lower lip somatosensory evoked fields (SEFs) and the N20m source of the median nerve SEFs is 23.0 ± 7.2 mm (mean ± sd) in the left hemisphere and 18.6 ± 2.1 mm in the right hemisphere.[1] This distance may increase and/or the SSEP sources may shift upward in patients with gliomas in and around the orofacial central region. Combination stimulus of the median nerve and the lip would be particularly useful in such cases.

CONCLUSION

Recording of SSEPs using median nerve and lower lip stimulation as described above can be used to localise the central sulcus and the hand and orofacial area laterally and adjacent to the Sylvian fissure.

REFERENCES

1. Cedzich C, Taniguchi M, Schafer S, Schramm J. Somatosensory evoked potential phase reversal and direct motor cortex stimulation during surgery in and around the central region. Neurosurgery 1996;38:962–970.
2. McCarthy G, Allison T, Spencer DD. Localization of the face area of human sensorimotor cortex by intracranial recording of somatosensory evoked potentials. J Neurosurg 1993;79:874–884.
3. Romstock J, Fahlbusch R, Ganslandt O, Nimsky C, Strauss C. Localisation of the sensorimotor cortex during surgery for brain tumours: feasibility and waveform patterns of somatosensory evoked potentials. J Neurol Neurosurg Psychiat 2002;72:221–229.
4. Sartorius CJ, Wright G. Intraoperative brain mapping in a community setting. Technical considerations. Surg Neurol 1997;47:380–388.
5. Wood CC, Spencer DD, Allison T, McCarthy G, Williamson PD, Goff WR. Localization of human sensorimotor cortex during surgery by cortical surface recording of somatosensory evoked potentials. J Neurosurg 1988;68:99–111.
6. Nagamatsu K, Nakasato N, Hatanaka K, Kanno A, Iwasaki M, Yoshimoto T. Neuromagnetic localization of N15, the initial cortical response to lip stimulus. Neuroreport 2001;12:1–5.
7. Berger MS, Ojemann GA, Lettich E. Neurophysiological monitoring during astrocytoma surgery. Neurosurg Clin N Am 1990;1:65–80.
8. Bennett MH, Jannetta PJ. Trigeminal evoked potentials in humans. Electroencephalogr Clin Neurophysiol 1980;48:517–526.
9. Findler G, Feinsod M. Sensory evoked response to electrical stimulation of the trigeminal nerve in humans. J Neurosurg 1982;56:545–549.
10. Larsson LE, Prevec TS. Somato-sensory response to mechanical stimulation as recorded in the human EEG. Electroencephalogr Clin Neurophysiol 1970;28:162–172.
11. Leandri M, Parodi CI, Zattoni J, Favale E. Subcortical and cortical responses following infraorbital nerve stimulation in man. Electroencephalogr Clin Neurophysiol 1987;66:253–262.
12. Seyal M, Browne JK. Short latency somatosensory evoked potentials following mechanical taps to the face. Scalp recordings with a non-cephalic reference. Electroencephalogr Clin Neurophysiol 1989;74:271–276.

原　著

悪性星細胞腫の治療成績の変遷と手術療法の意義

隈部俊宏　高井良尋　嘉山孝正　冨永悌二

原著

悪性星細胞腫の治療成績の変遷と手術療法の意義

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Surgical Aspects for Improving the Prognosis of Malignant Astrocytic Tumors

by

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This study retrospectively examined the median survival times in 370 patients with malignant astrocytic tumors treated in our institute between 1982 and 2003; follow-up review of these patients continued through the end of April, 2004. The study period was divided into three treatment eras as follows: 1982 to 1988, when patients underwent no preoperative magnetic resonance (MR) imaging evaluation (pre MR era); 1989 to 1996, when patients underwent preoperative MR imaging (post MR era); and after 1997, when patients underwent preoperative MR imaging with functional brain mapping and intraoperative neuronavigation system monitoring (post MAP era). Patients with glioblastoma (GB) treated after 2000 were separately classified as the 21st century era because of the large number of patients compared with other eras, and changes to radiation treatment protocols. One hundred and eighty-one patients had anaplastic astrocytoma (AA) and 189 had GB. The patients were also divided into younger (under 60 years) and elderly (60 years or over) groups, and total resection and partial resection or biopsy groups. Survival rates were determined using the Kaplan-Meier method. The statistical significance of differences between life table curves was determined using the logrank test. The median survival time of 62 patients with GB in the 21st century era was 23.1 months. The median survival time in the elderly group was significantly shorter than in the younger group in both patients with AA ($p=0.0004$) and GB ($p<0.0001$). The median survival time of patients with AA or GB in both younger and elderly groups tended to improve according to treatment advances. The total resection group had significantly longer median survival time than the partial resection or biopsy group, in both patients with AA ($p<0.0001$) and GB ($p=0.0002$) and in the younger (AA: $p=0.0001$, GB: $p=0.0040$) and elderly (AA: $p=0.0230$, GB: $p=0.0265$) groups. Therefore, the outcomes for patients with malignant astrocytic tumors improved according to treatment advances, especially following total resection, despite inhomogeneity in diagnostic criteria, determination of the extent of tumor removal, and treatment protocols.

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Key words: anaplastic astrocytoma, decade of diagnosis, extent of tumor resection, glioblastoma, survival

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はじめに

髄芽腫や胚細胞腫などの，化学療法への感受性が高い疾患に関しては，洗練された治療プロトコールにより，近年，治療成績の改善が得られている．一方，悪性神経膠腫に対してはメタアナリシスの結果にて，化学療法の

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治療効果は1年生存率で6%，生存中央値（median survival time；MST）で2カ月の延長にとどまると報告されている[3]．現況での日本における最善の治療方法は，MRI画像上で確認される領域の腫瘍を残存なく摘出し，綿密な放射線治療と，公的に認められているニトロソウレア剤による化学療法を追加することである．

われわれ脳神経外科医の使命は，手術療法をいかに確実に遂行できるかにある．手術摘出度が治療成績に重大な影響を及ぼすことは，すでに多くの報告がなされている[4]．そして，残存腫瘍が少ないほど化学療法の治療効果が高いことも報告されている[5]．

1980年代後期に導入されたMRI，1990年代半ばから盛んに行われるようになった脳機能マッピングとニューロナビゲーションシステムの導入は，悪性神経膠腫に対する手術療法をより論理的に行ううえで，大きな意義があったと考えられる．これら画像（解剖）情報と機能情報が術者にフィードバックされることにより，悪性神経膠腫手術のポイントが少しずつ明らかになってきている．

しかし，このような手術療法の進歩に伴った治療成績の向上は，いまだ結果として報告されていない．全米の26%の人口を包括するSurveillance Epidemiology and End Results Program (SEER Program) による検討[1]では，過去30年間の悪性脳腫瘍の治療成績は改善傾向にあるも，glioblastoma（GB）に関してはまったく改善されていなかったと報告している．日本脳腫瘍統計[6]によると，GBの2年生存率と3年生存率は，1985～1990年では20.5%，12.9%，1991～1996年では19.6%，11.4%とやはり改善されていない．

東北大学脳神経外科（以下，当科）にて治療した悪性星細胞腫の治療成績の変遷を検討し，治療成績が向上していることと，画像上，全摘出された症例の予後は良好なことを報告したい．

方　法

1982年以降，2003年12月までに当科にて治療した181例のanaplastic astrocytoma（AA）と189例のGBの治療成績の変遷を検討した．視床，基底核部，脳幹部を含めた後頭蓋窩病変も治療中に組織が得られ，AAもしくはGBと診断されたものはすべて本統計に含まれている．AAの平均年齢は44.5±17.5歳，GBの平均年齢は53.1±15.4歳であった．手術療法を行ううえで，最も重要な画像情報であるMRIが東北大学にて稼働したのが1989年であり，脳機能マッピングとニューロナビゲーションシステムの導入が1997年であることから，この22年間を3つに分けて，1982～1988年を「MR導入前期」，1989～1996年を「MR導入期」，1997年以降を「脳機能マッピング期」と定義した．なお，GBの症例数は当科では近年増加傾向にあり，脳機能マッピング期は100例と多くなっていることと放射線治療方法を変更したことから，2000年以降を「21世紀」として統計した．腫瘍悪性度，年齢，手術摘出度は比重の大きい予後決定因子であることから[1)2)4)~6)]，各時期ごと，手術摘出度，年齢による治療成績をAAとGB別々に検討した．年齢は60歳以上を「高齢者群」，60歳未満を「若年者群」と定義[2)]し，両群別々に治療成績の推移，手術摘出度による治療成績を検討した．2004年4月30日の段階で統計し，生存曲線はKaplan-Meier法にて算出し，logrank法にて有意差を検定した．

本統計は後ろ向き検討であるため，手術摘出度の評価および治療内容が画一ではない点が大きな問題となる．

手術摘出度は術後なるべく早期の造影画像によって評価した．MRI導入前のCTによって評価された全摘出と，術後遅くとも72時間以内に造影MRIと拡散強調画像を撮影して，厳密に摘出度を判定したうえでの全摘出とでは判断される意味合いが異なるのは明らかである．今回の統計では，全体が造影される病変はその領域を，部分的に造影される，もしくは造影されない病変はT1強調画像で低信号を示す領域を腫瘍と判断して，術後残存していないものを全摘出，それ以外のものを部分摘出，もしくは生検術として分類した．散在性に造影され，T1強調画像で塊として低信号を示す領域が認められないような症例においては，摘出度の判定はいまだ難しく統一された見解はない．

放射線療法はGBに対しては，1985年までは1回2 Gyの均等分割照射での全脳30 Gyと拡大局所30 Gy，1988年までは不均等分割照射で全脳27 Gyと拡大局所27 Gy，1995年までは不均等分割照射で拡大局所へ63 Gy，1999年までは1回1.5 Gyを1日2回照射する加速過分割照射で拡大局所へ60 Gy，2000年以降は1回2 Gyの均等分割照射での全脳30 Gyを術後5日目以内に開始し，続いて加速過分割照射で拡大局所へ30 Gy，さらに残存造影領域があった場合，6 Gyを隔日で4回造影結節に絞って照射する方法をとった．AAに対しては，1985年までは1回2 Gyの均等分割照射での全脳30 Gyと拡大局所30 Gy，1986年までは不均等分割照射で全脳27 Gyと拡大局所27 Gy，1995年までは不均等分割照射で拡大局所へ63 Gy，1996年以降は1回1.2 Gyを1日2回照射する過分割照射で拡大局所へ72 Gyの照射を行っ

Table 1 Survival in patients undergoing surgery for glioblastoma

Category	No. of patients	Median survival time (months)	Survival (%) 2-year	3-year	5-year	p Value*
Era						
pre MR (1982～1988)	47	10.6	10.6	0.0	0.0	0.0002**
post MR (1989～1996)	42	17.6	27.0	24.3	7.1	
post MAP (1997～1999)	38	16.0	26.3	15.8	5.3	0.0401***
21st (2000～2003)	62	23.1	41.7	24.7		
Extent of tumor resection						
Total	102	17.9	33.4	24.5	7.8	0.0002
Partial or biopsy	87	11.9	19.2	6.6	2.2	
Age						
Under 60 years	111	18.2	36.3	22.8	8.7	<0.0001
60 years or over	78	11.7	11.9	5.1	0.0	

*The statistical significance of differences was determined using the logrank test.
**Between pre MR era and post MR era
***Between post MAP era and the 21st century era
MAP: brain mapping

Table 2 Survival in younger patients (under 60 years) undergoing surgery for glioblastoma

Category	No. of patients	Median survival time (months)	Survival (%) 2-year	3-year	5-year	p Value*
Era						
pre MR (1982～1988)	30	12.0	50.0	0.0	0.0	0.0034**
post MR (1989～1996)	25	20.0	37.0	32.4	11.1	
post MAP (1997～1999)	21	17.4	33.3	23.8	9.5	0.0226***
21st (2000～2003)	35	26.4	57.6	39.2		
Extent of tumor resection						
Total	65	19.8	43.9	33.9	13.2	0.0040
Partial or biopsy	46	16.7	26.5	9.1	3.0	

*The statistical significance of differences was determined using the logrank test.
**Between pre MR era and post MR era
***Between post MAP era and the 21st century era

た．化学療法は nimustine hydrochloride (ACNU) を基本薬剤として使用した．顆粒球増多因子が使用できるようになってから，100 mg/m^{2}を照射期間中に 2 回静注し，その後外来にて 2 カ月に 1 度の維持療法を 2 年間行っている．

結　果

1 GB

「MR 導入前期」47 例の MST は 10.6 カ月であったが，「MR 導入期」42 例の MST は 17.6 カ月と危険率 0.0002 で向上していた．「脳機能マッピング期」38 例の MST は 16 カ月で「MR 導入期」と差が認められなかったが，「21 世紀」の 62 例の MST は 23.1 カ月であり，「脳機能マッピング期」と危険率 0.0401 をもって治療成績が向上していた(**Table 1**)．なお MIB1 陽性率は，「MR 導入期」30.6±14.4% (16 例)，「脳機能マッピング期」30.9±11.6% (34 例)，「21 世紀」37.2±14.9% (62 例) と，いずれも高い増殖能を示しており，GB の治療成績の向上は，少なくとも組織学的により良性のものが多くなったからではない．全摘出が行われた 102 例の MST は 17.9 カ月で，部分摘出もしくは生検術に終わった 87 例の 11.9 カ月に比較すると，危険率 0.0002 をもって良好であった (**Table 1**)．「若年者群」111 例の MST は 18.2 カ月で，「高齢者群」78 例の MST は 11.7 カ月であり，危険率 0.0001 未満をもって「高齢者群」の予後は不良であった (**Table 1**)．

次に年齢別に治療成績の推移を検討した．「若年者群」では，「MR 導入前期」30 例の MST は 12 カ月であったが，「MR 導入期」25 例では 20 カ月と危険率 0.0034 で有意に治療成績が向上していた．「脳機能マッピング期」21 例の MST は 17.4 カ月で「MR 導入期」との差が認められなかったが，「21 世紀」35 例の MST は 26.4 カ月で

Table 3 Survival in elderly patients (60 years or over) undergoing surgery for glioblastoma

Category	No. of patients	Median survival time (months)	Survival (%) 2-year	3-year	5-year	*p* Value*
Era						
pre MR (1982～1988)	17	6.0	0.0	0.0	0.0	0.0014**
post MR (1989～1996)	17	12.7	12.5	12.5	0.0	
post MAP (1997～1999)	17	14.1	17.6	5.9	0.0	ns***
21st (2000～2003)	27	20.3	15.5	0.0	0.0	
Extent of tumor resection						
Total	37	16.2	13.8	6.9	0.0	0.0265
Partial or biopsy	41	9.9	10.2	3.4	0.0	

*The statistical significance of differences was determined using the logrank test.
**Between pre MR era and post MR era
***ns: not significant (between post MAP era and the 21st century era)

Table 4 Survival in patients undergoing surgery for anaplastic astrocytoma

Category	No. of patients	Median survival time (months)	Survival (%) 2-year	3-year	5-year	10-year	*p* Value*
Era							
pre MR (1982～1988)	60	15.0	36.7	26.7	15.0	6.7	0.0014**
post MR (1989～1996)	58	27.5	54.7	44.1	30.6	26.5	
post MAP (1997～2003)	63		73.9	67.7	51.3		0.0152***
Extent of tumor resection							
Total	64	62.2	74.2	69.1	52.1	33.8	<0.0001
Partial or biopsy	117	22.6	44.4	32.6	19.1	15.2	
Age							
Under 60 years	139	33.8	58.9	50.0	35.4	27.6	0.0004
60 years or over	42	17.2	42.1	31.8	18.2	0.0	

*The statistical significance of differences was determined using the logrank test.
**Between pre MR era and post MR era
***Between post MR era and post MAP era

あり，「脳機能マッピング期」と危険率 0.0226 をもって治療成績が向上していた（**Table 2**）．全摘出された 65 例の MST は 19.8 カ月で，それ以外の 46 例の 16.7 カ月に比較すると危険率 0.0040 をもって良好であった（**Table 2**）．

「高齢者群」でも，「MR 導入前期」17 例の MST 6 カ月に比較して，「MR 導入期」17 例の MST 12.7 カ月は危険率 0.0014 をもって有意に改善していた．「脳機能マッピング期」17 例の MST は 14.1 カ月，「21 世紀」27 例の MST は 20.3 カ月で有意差を認めなかった（**Table 3**）．全摘出された 37 例の MST は 16.2 カ月で，それ以外の 41 例の 9.9 カ月に比較すると危険率 0.0265 をもって良好であった（**Table 3**）．

2 AA

「MR 導入前期」60 例の MST は 15 カ月であったが，「MR 導入期」58 例の MST は 27.5 カ月と危険率 0.0014 で向上し，「脳機能マッピング期」63 例では危険率 0.0152 でさらに成績の向上が確認された（**Table 4**）．全摘出が行われた 64 例の MST は 62.2 カ月で，部分摘出もしくは生検術に終わった 117 例の 22.6 カ月に比較すると 0.0001 未満の危険率をもって良好であった（**Table 4**）．GB に比較すると AA では「高齢者群」の比率がかなり少なくなるが，年齢はやはり大きな予後決定因子であり，「若年者群」139 例の MST は 33.8 カ月であったのに比較して，「高齢者群」42 例の MST は 17.2 カ月であり，危険率 0.0004 をもって「高齢者群」の予後は不良であった（**Table 4**）．

次に年齢別に治療成績の推移を検討した．「若年者群」では，「MR 導入前期」40 例の MST は 15 カ月であったが，「MR 導入期」47 例では 36.7 カ月と危険率 0.0012 で有意に治療成績が向上していた．「脳機能マッピング期」52 例では，治療成績は改善傾向にあるも有意差は認められなかった（**Table 5**）．全摘出された 55 例の MST は

Table 5 Survival in younger patients (under 60 years) undergoing surgery for anaplastic astrocytoma

Category	No. of patients	Median survival time (months)	Survival (%) 2-year	3-year	5-year	10-year	p Value*	
Era								
pre MR (1982～1988)	40	15.0	35.0	27.5	17.5	10.0	0.0012**	
post MR (1989～1996)	47	36.7	63.3	50.2	35.7	33.2		ns***
post MAP (1997～2003)	52		74.4	69.6	52.4			
Extent of tumor resection								
Total	55	81.3	73.6	69.5	53.3	37.8	0.0001	
Partial or biopsy	84	23.5	49.2	36.9	22.8	20.5		

*The statistical significance of differences was determined using the logrank test.
**Between pre MR era and post MR era
***ns: not significant (between post MR era and post MAP era)

Table 6 Survival in elderly patients (60 years or over) undergoing surgery for anaplastic astrocytoma

Category	No. of patients	Median survival time (months)	Survival (%) 2-year	3-year	5-year	10-year	p Value*	
Era								
pre MR (1982～1988)	20	10.3	40.0	25.0	10.0	0.0	ns**	
post MR (1989～1996)	11	13.8	18.2	18.2	9.1	0.0		0.0094***
post MAP (1997～2003)	11	36.0	71.6	59.7	47.7			
Extent of tumor resection								
Total	9	39.4	77.8	66.7	44.4	22.2	0.0230	
Partial or biopsy	33	10.3	32.2	21.7	9.7	0.0		

*The statistical significance of differences was determined using the logrank test.
**ns: not significant (between pre MR era and post MR era)
***Between post MR era and post MAP era

81.3 カ月で，それ以外の 84 例の 23.5 カ月に比較すると危険率 0.0001 をもって良好であった（Table 5）．

「高齢者群」では，「MR 導入前期」20 例の MST 10.3 カ月に比較して，「MR 導入期」11 例の MST 13.8 カ月は有意差がないが，「脳機能マッピング期」11 例の MST は 36 カ月で危険率 0.0094 をもって有意に改善していた（Table 6）．全摘出された 9 例の MST は 39.4 カ月で，それ以外の 33 例の 10.3 カ月に比較すると危険率 0.0230 をもって良好であった（Table 6）．

考　察

今回の統計は，数多く挙げられる予後決定因子がまったく統一されていない対象に対して，同一の治療を行っていない後ろ向きの検討である．したがって，結論として述べられる内容は限られる．今回の統計から得られる結論は，AA，GB ともにその治療成績は時代変遷に伴って改善傾向にあり，全摘出した症例の予後は有意に良好であったということである．「若年者群」と「高齢者群」別に，時代変遷と手術摘出度による治療成績を検討したが，どちらの年齢群においても AA，GB ともに時代変遷に伴って治療成績は改善傾向にあり，全摘出した症例の予後は有意に良好であった．

1 つの問題点として，診断基準が変更されたために，より良性の病変を GB として分類してしまっているのではないかという懸念が挙げられる．すべての症例を同一もしくは複数の神経病理医が，一時期に臨床経過を知らされない状態で診断することが望ましいが，今回の統計では行うことができなかった．GB に関して MIB1 陽性率を検討した結果，少なくとも近年の GB の治療成績向上は，増殖能の低い症例が集まった結果ではないことは断言できるのではないかと思われる．

治療成績の改善がなぜ得られたのか，今後細かく検討していく必要があるが，次のような事項が関与していると予想される．①状態を悪化させることなく可及的腫瘍摘出を行うようになったこと，②CT simulator を用いた正確な放射線治療や，残存腫瘍に対する定位的放射線治療の追加がより局所制御において有効になっている可能性，③化学療法を行うにあたり，顆粒球増多因子および制吐剤が開発され，合併症をきたすことなく初期治療を全うできるようになったこと，④再発の早期発見に努めていること，⑤再発時に局所再発であれば状況を判断し

積極的に再摘出術を行っていること，⑥手術適応にならない再発病変に対するガンマナイフ治療，⑦び漫性再発に対する ifosfamide, cisplatin, etoposide による化学療法の追加（校費負担），⑧播種病変に対する methotrexate 髄腔内投与もしくは ACNU 還流療法[7]，⑨状態が悪化し外来通院が不可能になった後の関連病院もしくは在宅介護を受け持ってくれる訪問医療との連携など，まだまだ多くの細かい事項が関与していると思われる．1例たりとも同じ経過を辿らないのが悪性神経膠腫であり，関与する因子をすべて統計して治療成績を検討するのは難しい．

本邦においても開始されつつある悪性神経膠腫の治療方法確立に向けての前向き試験が，さらに信頼に足るデータを与えてくれるものと期待したい．

文　献

1) Barnholtz-Sloan JS, Sloan AE, Schwartz AG: Relative survival rates and patterns of diagnosis analyzed by time period for individuals with primary malignant brain tumor, 1973-1997. *J Neurosurg* **99**: 458-466, 2003.
2) Fujimura M, Kumabe T, Tominaga T, Jokura H, Shirane R, Yoshimoto T: Routine clinical adoption of magnetic resonance imaging was associated with better outcome after surgery in elderly patients with a malignant astrocytic tumour: A retrospective review. *Acta Neurochir (Wien)* **146**: 251-255, 2004.
3) Jeremic B, Milicic B, Grujicic D, Dagovic A, Aleksandrovic J: Multivariate analysis of clinical prognostic factors in patients with glioblastoma multiforme treated with a combined modality approach. *J Cancer Res Clin Oncol* **129**: 477-481, 2003.
4) Keles GE, Anderson B, Berger MS: The effect of extent of resection on time to tumor progression and survival in patients with glioblastoma multiforme of the cerebral hemisphere. *Surg Neurol* **52**: 371-379, 1999.
5) Keles GE, Lamborn KR, Chang SM, Prados MD, Berger MS: Volume of residual disease as a predictor of outcome in adult patients with recurrent supratentorial glioblastomas multiforme who are undergoing chemotherapy. *J Neurosurg* **100**: 41-46, 2004.
6) The Committee of Brain Tumor Registry of Japan: Report of Brain Tumor Registry of Japan (1969-1996). *Neurol Med Chir (Tokyo)* **43 (Suppl)**: 36-43, 2003.
7) Ushio Y, Kochi M, Kitamura I, Kuratsu J: Ventriculolumber perfusion of 3-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]-1-(2-chloroethyl) -1-nitrosourea hydrochloride for subarachnoid dissemination of gliomas. *J Neurooncol* **38**: 207-212, 1998.

要　旨

悪性星細胞腫の治療成績の変遷と手術療法の意義

隈部　俊宏　　高井　良尋　　嘉山　孝正　　冨永　悌二

181 例の anaplastic astrocytoma（AA）と 189 例の glioblastoma（GB）に関して，1982～1988 年までを「MR 導入前期」，1989～1996 年を「MR 導入期」，1997 年以降を「脳機能マッピング期」，GB に関してはさらに 2000 年以降を別にして 4 期に分類し，治療成績の変遷を検討した．さらに AA と GB 別々に 60 歳以上の「高齢者群」と 60 歳未満の「若年者群」に分類して，それぞれの群での治療時期，手術摘出度による生存中央値（median survival time; MST）を検討した．

この結果，「高齢者群」の予後は「若年者群」と比較して有意に不良であったが，どちらの年齢群においても AA，GB ともに時代変遷に伴って治療成績は改善しており，全摘出した症例の予後は有意に良好であった．

本統計は後ろ向き検討であるため，診断基準，手術摘出度の評価および治療内容が画一ではないが，AA，GB ともに時代変遷に伴って治療成績は改善傾向にあり，全摘出した症例の予後は有意に良好であったといえる．

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Clinical Study

C-kit expression in germinoma: an immunohistochemistry-based study

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Key words: c-kit, germinoma, immunohistochemistry, PLAP, tumor marker

Summary

In our immunohistochemical study of 25 human primary intracranial germinomas and germinomas with syncytiotrophoblastic giant cells (STGC), we stained the same sections for c-kit and placental alkaline phosphatase (PLAP). Immunohistochemical expression was graded using a semi-quantitative scoring system where 3+ = 51–75%, and 4+ = 76–100%. Of the 25 cases, 7 (28%) were graded 3+ and 18 (72%) 4+ for c-kit; 8 (32%) were 3+ or 4+ for PLAP. All 3 cases negative for PLAP-staining were strongly positive and all embryonal carcinomas, immature teratomas, and yolk sac tumors were negative for c-kit staining. The soluble isoform of c-kit (s-kit) is reportedly detectable in cerebral spinal fluid of patients with germinomas and germinomas with STGC. C-kit and s-kit may be powerful tumor markers for germinomas with or without STGC.

Introduction

In Japan, central nervous system (CNS) germ cell tumors (GCTs) account for 1.8–3% of all primary brain tumors and for 9–15% of pediatric brain tumors [1]. GCTs are divided into pure germinomas, non-germinomatous GCTs (NGGCTs), and mature teratomas. The prognosis of patients with NGGCTs remains dismal; their median survival is less than 2 years [2]. On the other hand, germinomas, the most frequent type of intracranial germ-cell tumor, can be cured by chemotherapy and radiotherapy and mature teratomas are curable by surgery. As the early diagnosis of GCTs has important therapeutic implications, reliable tumor makers are needed for GCTs. High levels of α-fetoptotein (AFP), human chorionic gonadotropin (HCG),or β-subunit HCG (β-HCG) in serum or cerebrospinal fluid (CSF) alert to the possible presence of cells comprising NGGCTs. These tumors need not be histologically confirmed before surgery, in fact, surgical intervention before the administration of adjuvant therapy may increase the risk of tumor cell dissemination. We obtained good results by subjecting NGGCT patients to neo-adjuvant therapy (NAT) [3]. On the other hand, in the absence of reliable tumor markers, germinomas are confirmed histologically after biopsy or other surgical procedures. While placental alkaline phosphatase (PLAP) is considered a good tumor-marker candidate for germinomas, many patients with these tumors are serum-negative for PLAP. Shinoda et al. [4] suggested that the immunohistochemical PLAP-negativity of some germinoma cells may be due to changes in the antigenicity of PLAP in the course of tumor-cell differentiation.

The proto-oncogene c-kit encodes a transmembrane tyrosine kinase receptor (KIT) for stem cell factor (SCF) [5]. KIT is expressed in a number of cell types such as germ cells, melanocytes, mast cells, interstitial cells of Cajal, and in a subset of malignant neoplasms. SCF/KIT-mediated signaling plays an important role in normal spermatogenesis and primordial germ cell migration and survival [6–8]. Moreover, c-kit mutations identified in several types of tumor cells are thought to play an important role in tumor formation [9–12] and they may be an additional parameter for predicting the metastatic risk of gastrointestinal stromal tumors (GISTs) [13]. The soluble form of the c-kit receptor (s-kit), a product of c-kit cleavage, was detected in CSF of patients with intracranial germinomas [14]. The CSF concentration of s-kit may be a useful marker to diagnose germinomas and predict recurrence or dissemination in patients with germinomas or germinomas with STGCs [15]. We compared the reactivity for c-kit and PLAP of immunohistochemically stained adjacent tissue sections from patients with germ cell tumors.

Materials and methods

Specimens from 25 patients operated at the Department of Neurosurgery of Kumoamoto and Kagoshima University Medical School for pure germ cell tumors were used. All patients had undergone surgical tumor removal or biopsy during the years from 1992 to 2004. The surgical specimens were of adequate size, however, some of the biopsy specimens were too small for evaluation by semi-quantitative staining analysis and could not be used. Samples were fixed in 10% formalin, embedded in paraffin, 4–5 μm sections were cut, deparaffinized with xylene, rehydrated in a graded series of ethanol, and immersed in 0.3% methanolic hydrogen peroxide to

eliminate endogenous peroxidase activity. The sections were then incubated overnight with 1:200 diluted polyclonal antibody against human c-kit (DAKO JAPAN CO. Ltd, Kyoto) or human PLAP (Biomedia Corp., Foster City, CA). Identical sections were stained for both c-kit and PLAP. We used the avidin–biotin complex method; for detection we used 0.05 % diaminobenzine tetrahydrochloride. To provide a negative control, tumor sections were incubated with normal rabbit serum instead of primary antibody. Slides were counterstained with hematoxylin. Tissue sections from testicular seminomas were used as a positive control.

Immunohistochemical expression was graded using a semi-quantitative scoring system where – = 0%, 1+ = 1–25%, 2+ = 26–50%, 3+ = 51–75%, and 4+ = 76–100%. For the semi-quantitative analysis of c-kit we used at least 10 high-power fields. Usually the samples were small, however, all included samples were large enough to allow the inspection of 10 different fields.

Results

A summary of the 25 patients is presented in Table 1. None had received radio- or chemotherapy before surgery. The same sections from surgical specimens were stained for both c-kit and PLAP. As a positive control for PLAP staining we used full-term placental tissue. We confirmed a clear reaction on the outer and occasional inner surfaces of syncytiotrophoblasts (data not shown) to ascertain that the results were not attributable to the antibody or staining techniques we used. All 25 specimens exhibited a strong immunoperoxidase reaction for c-kit (3+, n = 7; 4+, n = 18). On the other hand, only 8 of the 25 cases (32%) were graded as 3+ or 4+ for PLAP staining and 3 cases were PLAP-negative (Table 2). In case 1, the surface membrane of all tumor cells was strongly stained for c-kit, however, these cells were PLAP-negative (Figure 1). Case 2 was unusual in that there was strong positivity for both c-kit and PLAP (Figure 2). Among the 25 cases, only 2 (8%) were graded as strongly positive (4+) for PLAP although all 25 exhibited strong (3+ or 4+) positivity for c-kit. As these findings were not attributable to either the quality of the antibody used or to our staining technique, we postulate that they are due to differences in antigenic expression by the germinoma cells. Moreover, we found that among the NGGCTs we examined (2 immature teratomas, 2 yolk sac tumors, and 1 embryonal carcinoma), none contained cells positive for c-kit (Figure 3). To exclude the possibility that their location in pineal or suprasellar regions contributed to their c-kit positivity, we subjected other pineal or suprasellar region tumors

Table 1. Characteristics of the patients with germinoma and germinoma with STGC

Age	4–32 (Median = 18)
Gender	Male: 18 Female: 7
Diagnosis	Germinoma: 18 Germinoma with STGC: 7
Location of tumor	Pineal: 12 Suprasellar: 7 Pineal and suprasellar: 3 Basal ganglia: 3

Table 2. The semi-quantitative scoring of the immunohistochemical staining of c-kit or PLAP

	–	1+	2+	3+	4+	Ave.	Total
C-kit	0	0	0	7	18	3.7*	25
PLAP	3	5	11	6	2	1.9*	25

PLAP: Placental alkaline phosphatase, Ave.: Average, *P < 0.0001 Wilcoxon signed-rank test.

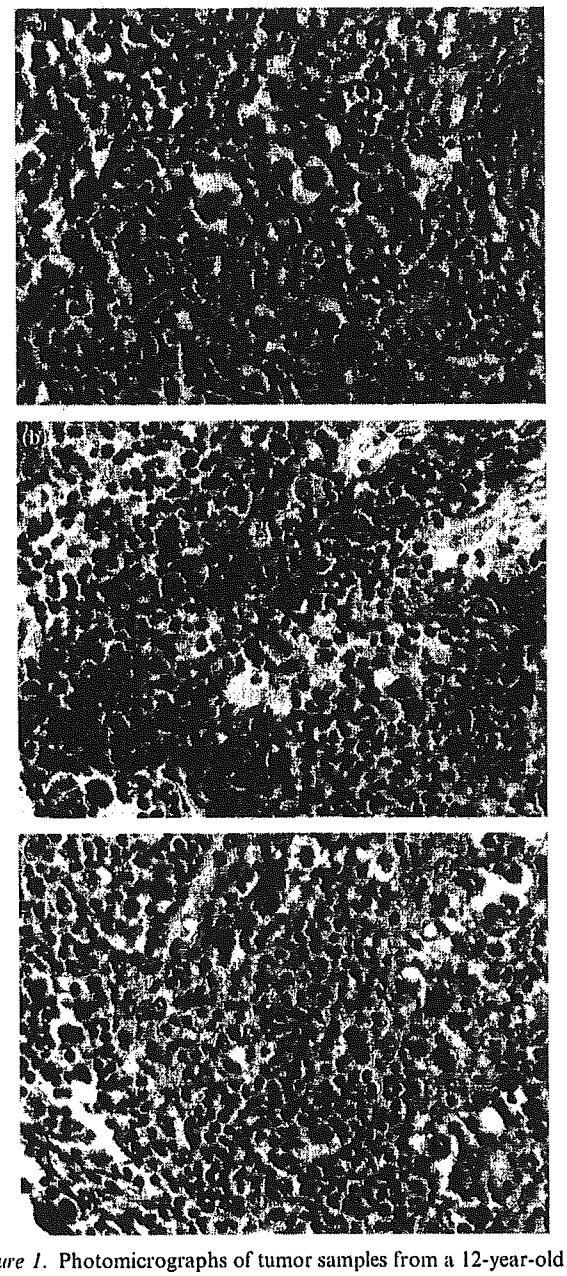

Figure 1. Photomicrographs of tumor samples from a 12-year-old boy with germinoma. (a) H&E staining demonstrates the typical two-cell pattern of germinoma. (b) The tumor cells are strongly positive for c-kit. (c) Compared to c-kit, staining for PLAP is weak.

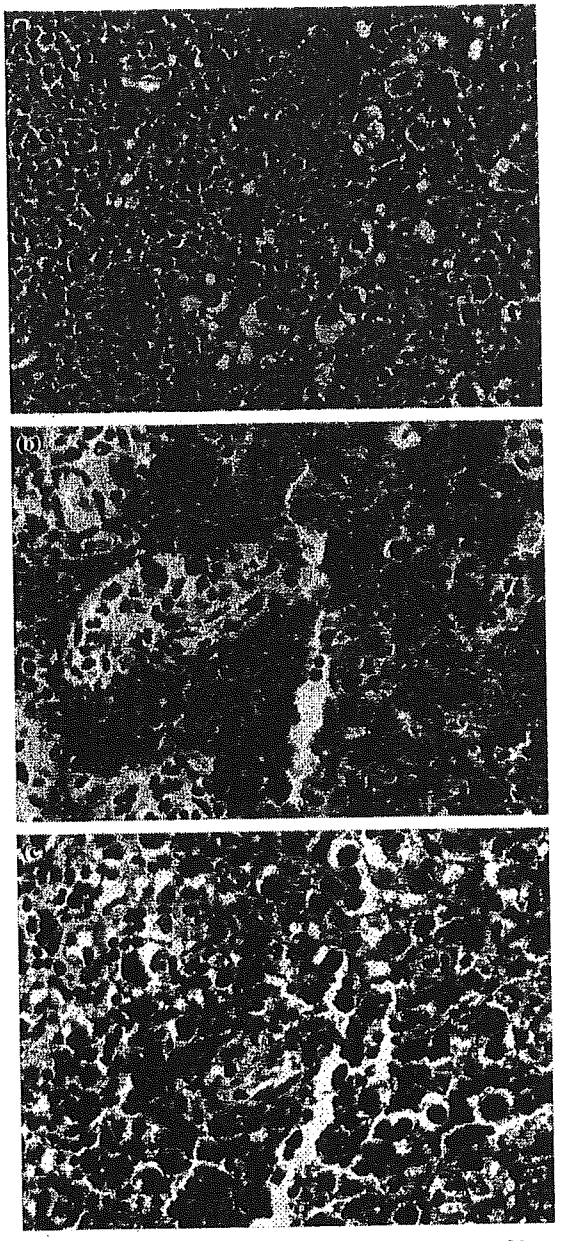

Figure 2. Photomicrographs of tumor samples from a 21-year-old male with germinoma. (a) H&E staining demonstrates the typical two-cell pattern of germinoma. (b) The tumor cells are strongly positive for c-kit. (c) Staining for PLAP is also positive.

(one each pineocytoma, pineoblastoma, pituitary adenoma and suprasellar meningioma) to c-kit staining; none were positive (Figure 4). As NGGCTs and other pineal or suprasellar tumors were negative for PLAP staining, germinomas did not exhibit different specificity for c-kit and PLAP. However, the sensitivity of germinomas for c-kit and PLAP staining was significantly different (Table 2), indicating that c-kit is more reliable than PLAP as a tumor marker for primary intracranial germinomas and germinomas with STGC.

Discussion

C-kit and its ligand SCF, a 21-kDa glycoprotein, are especially important for the proliferation of stem cells, mast cells, melanocytes, and germ cells. The binding of SCF to c-kit results in the dimerization of receptor molecules and activation of receptor kinase activity. The phosphorylated intracellular domain of c-kit recruits several signal molecules and mediates the interaction with the SH2 domain of the translocated protein. Several binding proteins have been described, e.g. p85 of PI-3-kinase, SHP-1, and SH-2 [16–18]. Active mutations of c-kit have been reported in testicular germ-cell tumors, GISTs, and intracranial germinomas. Our immunohistochemical study showed that all intracranial germinomas examined were strongly positive for c-kit and that all NGCCTs were c-kit-negative. While c-kit is thought to be crucial in the development and proliferation of intracranial germinomas, the underlying mechanism(s) remains unclear. Some molecules downstream of the c-kit signal are important for cell proliferation, e.g. PI-3 kinase, AKT, and MAP-kinase; they may be related to tumorigenesis or proliferation in intracranial germinomas [19–21]. At present it is not known why c-kit is highly expressed in germinomas but not in NGGCTs although in testicular seminomas, c-kit activity is thought to be important for maintaining the germ-cell character. As SCF is expressed ubiquitously in human tissues, it is readily available for use by tumor cells. Germinomas are often found in young individuals and they are rare in adults older than 30 years, moreover, their incidence is higher in boys than girls. The c-kit/SCF system may play a yet not well-understood role in these features.

Our immunohistochemical study revealed that while all specimens from 25 patients operated for primary intracranial germinomas or germinomas with STGC were strongly positive for c-kit, the same sections stained only weakly or not at all for PLAP. We offer the following considerations for this observation. While c-kit is a cell-membrane protein, the PLAP protein is located mainly in the cytoplasm and PLAP antigenicity may be delicate and difficult to preserve for immunohistochemical staining. Also, intracranial germinoma cells may be at different stages of differentiation and during some of these stages, the cells may not manifest PLAP antigenicity. Miyanohara et al [14]. reported that s-kit is expressed at significant levels in CSF from patients with germinomas and germinomas with STGC. They also performed immunohistochemical staining studies on germ cell tumors and their results coincided with ours. However, while they demonstrated positive staining in some well-differentiated teratomatous structures in immature teratomas, we found that these tumors did not stain. In conclusion, we suggest that c-kit may be a useful a clinical marker for germinomas and germinomas with STGC and that the staining sensitivity is higher for c-kit than PLAP.

Acknowledgements

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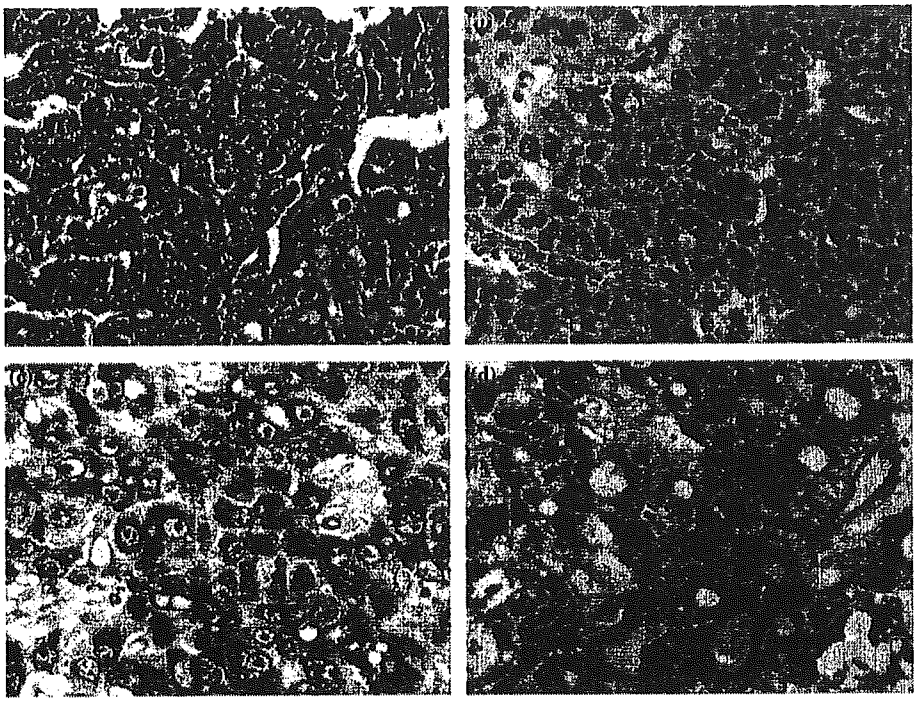

Figure 3. Photomicrographs of tumor samples from non-germinomatous germ cell tumors. (a) H&E staining of embryonal carcinoma. Dense and clear tumor cells demonstrate the papillary pattern. An atypical mitosis is seen in the center of this photograph. (b) The section shown in (a) is negative for c-kit staining. Several mitotic cells are present. (c) α-Fetoprotein (AFP) staining of yolk sac tumor. The cytoplasm of tumor cells is positive for AFP staining. (d) The section shown in (c) is negative for c-kit staining.

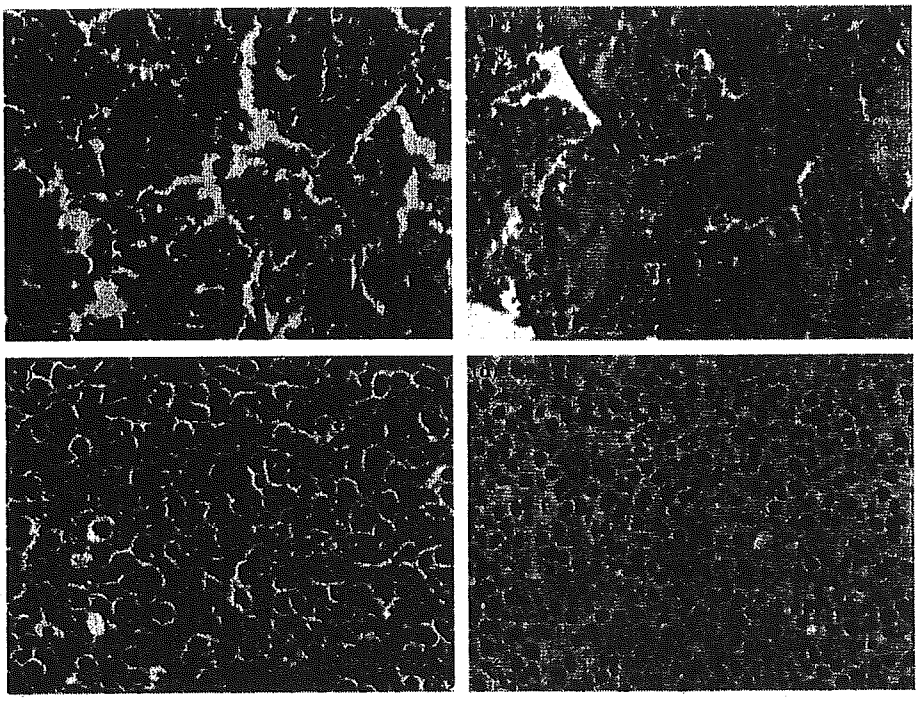

Figure 4. Photomicrographs of samples from tumors in the pineal or suprasellar region. (a) H&E staining of pineocytoma. Some of the tumor cells are aligned in a rosette pattern. (b) The section shown in (a) is negative for c-kit staining. (c) H&E staining of pituitary adenoma. Round and homogeneous tumor cells are present. There are no findings of malignancy such as mitoses or pleomorphism. (d) The section shown in (c) is negative for c-kit staining.

References

1. Kuratsu J, Ushio Y: Epidemiological study of primary intracranial tumors in childhood. A population-based survey in Kumamoto Prefecture, Japan. Pediatr Neurosurg 25: 240–246, 1996
2. Matsutani M: Combined chemotherapy and radiation therapy for CNS germ cell tumors – the Japanese experience. J Neurooncol 54: 311–316, 2001
3. Kochi M, Itoyama Y, Shiraishi S et al.: Successful treatment of intracranial nongerminomatous malignant germ cell tumors by

administering neoadjuvant chemotherapy and radiotherapy before excision of residual tumors. J Neurosurg 99: 106–114, 2003
4. Shinoda J, Miwa Y, Sakai N et al.: Immunohistochemical study of placental alkaline phosphatase in primary intracranial germ-cell tumors. J Neurosurg 63: 733–739, 1985
5. Yarden Y, Kuang WJ, Yang-Feng T et al.: Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 6: 3341–3351, 1987
6. Matsui Y, Toksoz D, Nishikawa S et al.: Effect of steal factor and leukaemia inhibitory factor on murine primordial germ cells in culture. Nature 353: 750–752, 1991
7. Packer Al, Besmer P, Bachvarova RF: Kit ligand mediates survival of type A spermatogonia and dividing spermatocytes in postnatal mouse testes. Mol Reprod Dev 42: 303–310, 1995
8. Pesce M, Farrace MG, Piacentini M et al.: Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). Development 118: 1089–1094, 1993
9. Tian Q, Frierson HF Jr., Krystal GW et al.: Activating c-kit gene mutations in human germ cell tumors. Am J Pathol 154: 1643–1647, 1999
10. Kemmer K, Corless CL, Fletcher JA et al.: KIT mutations are common in testicular seminomas. Am J Pathol 164: 305–313, 2004
11. Beghini A, Tibiletti MG, Roversi G et al.: Germline mutation in the juxtamembrane domain of the kit gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. Cancer 92: 657–662, 2001
12. Looijenga LH, Leeuw Hde, Oorschot Mvan et al.: Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors. Cancer Res 63: 7674–7678, 2003
13. Wardelmann E, Losen I, Hans V et al.: Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. Int J Cancer 106: 887–895, 2003
14. Miyanohara O, Takeshima H, Kaji M et al.: Diagnostic significance of soluble c-kit in the cerebrospinal fluid of patients with germ cell tumors. J Neurosurg 97: 177–83, 2002
15. Takeshima H, Kuratsu J: A review of soluble c-kit (s-kit) as a novel tumor marker and possible molecular target for the treatment of CNS germinoma. Surg Neurol 60: 321–324, 2003
16. Serve H, Hsu YC, Besmer P: Tyrosine residue 719 of the c-kit receptor is essential for binding of the P85 subunit of phosphatidylinositol (PI) 3-kinase and for c-kit-associated PI 3-kinase activity in COS-1 cells. J Biol Chem 269: 6026–6030, 1994
17. Yi T, Ihle JN: Association of hematopoietic cell phosphatase with c-kit after stimulation with c-kit ligand. Mol Cell Biol 13: 3350–3358, 1993
18. Tauchi T, Feng GS, Marshall MS et al.: The ubiquitously expressed Syp phosphatase interacts with c-kit and Grb2 in hematopoietic cells. J Biol Chem 269: 25206–11, 1994
19. Tsai M, Chen RH, Tam SY et al.: Activation of MAP kinases, pp90rsk and pp70-S6 kinases in mouse mast cells by signaling through the c-kit receptor tyrosine kinase or Fc epsilon RI: rapamycin inhibits activation of pp70-S6 kinase and proliferation in mouse mast cells. Eur J Immunol 23: 3286–3291, 1993
20. Shearman MS, Herbst R, Schlessinger J et al.: Phosphatidylinositol 3′-kinase associates with p145c-kit as part of a cell type characteristic multimeric signalling complex. EMBO J 12: 3817–3826, 1993
21. Wandzioch E, Edling CE, Palmer RH et al.: Activation of the MAP kinase pathway by c-kit is PI-3 kinase dependent in hematopoietic progenitor/stem cell lines. Blood 104: 51–57, 2004

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Comparative Clinical Study of the Anti-Emetic Effects of Oral Ramosetron and Injected Granisetron in Patients With Malignant Glioma Undergoing ACNU Chemotherapy

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Abstract

The effectiveness of ramosetron tablets and granisetron injection was compared for reducing the frequency of nausea, vomiting, and anorexia in patients with malignant glioma undergoing ACNU chemotherapy. Patients with malignant glioma to be treated with ACNU chemotherapy were randomly assigned to receive oral ramosetron (20 patients) or intravenous granisetron (19 patients) prior to ACNU injection. Gastrointestinal toxicity within 48 hours of ACNU injection was compared to that in patients who had received ACNU chemotherapy with dopamine D2 receptor-blocker as a historical control group. Within 24 hours of the administration of ACNU, 15 of the 20 patients treated with ramosetron and 16 of the 19 treated with granisetron were nausea-free, and 14 of the former and 14 of the latter regained their normal appetite. There was no significant difference in the anti-emetic effects. Ten of the 17 controls experienced no vomiting within 6 hours of the injection of ACNU, five were nausea-free within 24 hours, and two retained their normal appetite within 24 hours. Oral ramosetron has the same anti-anorectic and anti-emetic effects as intravenous granisetron. Ramosetron tablets are less expensive and are easy to take, so should be on the list of first-choice anti-emetic drugs for patients treated with ACNU chemotherapy.

Key words: glioma, chemotherapy, anti-emesis

Introduction

Many patients receiving anticancer drugs experience severe gastroenterologic symptoms such as nausea and vomiting that reduce their quality of life. The currently used chemotherapeutic agents, nimustine hydrochloride (ACNU), lomustine (CCNU), and carmustine (BCNU), are highly active emetic agents. More than 70% of patients suffer nausea and vomiting during the first 24 hours after ACNU infusion despite the administration of high-dose dopamine D2-receptor antagonists.[4] One mechanism by which anticancer drugs induce nausea and vomiting involves peripheral serotonin (5-HT3) receptor of the afferent vagus nerve.[1] Specific 5-HT3 receptor antagonists significantly reduce such emetic side effects. Ramosetron hydrochloride reduces cisplatin-induced emesis and can be administered as orally disintegrating tablets, which is less expensive than administration by injection.[8]

The present study investigated the effect of ramosetron tablets on the frequency of nausea, vomiting, and anorexia in patients with malignant glioma undergoing ACNU chemotherapy and compared the results to those obtained in patients receiving granisetron infusions.

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Patients and Methods

I. Patients

Patients with malignant glioma (anaplastic astrocytoma [n = 23] or glioblastoma [n = 16]) aged

Table 1 Characteristics of patients receiving ramosetron or granisetron before the first ACNU administration

	Ramosetron	Granisetron	p Value
No. of patients	20	19	
Median (range) age, years	55 (21-72)	48 (20-75)	
Average ACNU dose, mg/m^2	78.5	78.4	0.921
Sex			
male	11	14	0.320
female	9	5	
Tumor grade			
anaplastic astrocytoma	11	12	0.519
glioblastoma	9	7	
Tumor site			
frontal	8	10	0.981
temporal	5	4	
parietal	2	1	
occipital	1	1	
basal ganglia	2	2	
insula	1	1	
suboccipital	1	0	
Performance status			
0	13	11	0.865
1	7	7	
2	0	1	
Irradiation on the same day			
+	16	13	0.480
−	4	6	

20 to 75 years were scheduled to receive their first course of chemotherapy consisting of only ACNU (80 mg/m^2) or ACNU (70 mg/m^2) in combination with other agents with or without radiotherapy. Patients receiving simultaneous administration of cisplatin were excluded. During the study period, the patients received only the ACNU regimen and no other drugs that could influence the study outcome such as other anti-emetic, antipsychotic, or sedative drugs. Informed consent was obtained from all patients. The study protocol was approved by the Human Subjects Committee of our institute.

II. Study design

Patients were randomly assigned to receive either ramosetron or granisetron before the first ACNU injection. Patients in the ramosetron group (n = 20) took one ramosetron tablet (0.1 mg) 1 hour before the intravenous (i.v.) administration of ACNU. Patients in the granisetron group (n = 19) received i.v. drip infusion of granisetron (3.0 mg) diluted with saline to a total volume of 100 ml starting 1 hour before ACNU injection. Radiation therapy was administered on the same day to 16 patients in the ramosetron group and 13 in the granisetron group. Their baseline characteristics were not statistically different (Table 1). No patients in this study needed additional anti-emetic drugs following ACNU injection for at least 48 hours.

The degree of gastrointestinal toxicity was estimated according to the National Cancer Institute Common Toxicity Criteria.[9] Vomiting was recorded as grade 0 (no vomiting), grade 1 (one episode in the course of 24 hours), grade 2 (2-5 episodes within 24 hours), grade 3 (≥6 episodes within 24 hours), and grade 4 (≥6 episodes with the patient requiring intensive care). The severity of nausea was recorded as grade 0 (no nausea), grade 1 (mild nausea with tolerance for solid foods and fluids), grade 2 (moderate nausea with tolerance for fluids but not solids), and grade 3 (severe nausea with tolerance for neither solids nor fluids). Anorexia was recorded as grade 0 (normal appetite), grade 1 (ability to eat a third of a meal), grade 2 (ability to eat half a meal), grade 3 (inability to eat and requiring i.v. fluids), and grade 4 (requiring i.v. hyperalimentation).

The optimal dosage was also assessed in the ramosetron group. Our results were compared with the clinical records of 17 patients with malignant glioma treated with ACNU who had received no anti-emetic drugs except for dopamine D2 receptor blockers during the period from 1991 to 1993.

III. Statistical analysis

Student's t-test, the chi-square test, or the Wilcoxon test was used to analyze the efficacy of ramosetron and granisetron depending on data characteristics. The significance level was $p < 0.05$.

Results

No vomiting occurred during the first 24 hours of ACNU administration in 19 of the 20 patients in the ramosetron group and 17 of the 19 in the granisetron group (Table 2). There was no significant difference in the prevention of vomiting.

Nausea was prevented within 6 hours of ACNU administration in 16 of the 20 patients in the ramosetron group and 17 of the 19 in the granisetron group, and within 24 hours in 15 of the 20 patients in the ramosetron group and 16 of the 19 in the granisetron group (Table 3). There was no significant difference in the prevention of nausea.

Anorexia was blocked within 6 hours of ACNU administration in 17 of the 20 patients in the ramosetron group and 15 of the 19 in the granisetron group, and within 48 hours in 16 of the 20 patients in the ramosetron group and 14 of the 19 patients in the granisetron group (Table 4). There was no significant difference in the prevention of anorexia.

Five of six patients asked to assess the ease of taking the ramosetron tablets were able to take them easily and without water, the other took the tablet

Table 2 Effectiveness of ramosetron and granisetron for the prevention of vomiting within 48 hours of ACNU administration

Time after ACNU (hr)	Drug	No. of patients	No. (%) of patients by NCI grades*				
			0	1	2	3	4
0–6	Ramosetron	20	20 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Granisetron	19	18 (94.7)	1 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
6–24	Ramosetron	20	19 (95.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Granisetron	19	18 (94.7)	1 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
24–48	Ramosetron	20	19 (95.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Granisetron	19	19 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

*According to the National Cancer Institute (NCI) Common Toxicity Criteria[9]: grade 0, no vomiting; grade 1, one episode within 24 hours; grade 2, 2–5 episodes within 24 hours; grade 3, ≥6 episodes within 24 hours; grade 4, ≥6 episodes with the patient requiring intensive care.

Table 3 Effectiveness of ramosetron and granisetron for the prevention of nausea within 48 hours of ACNU administration

Time after ACNU (hr)	Drug	No. of patients	No. (%) of patients by NCI grades*			
			0	1	2	3
0–6	Ramosetron	20	16 (80.0)	2 (10.0)	0 (0.0)	2 (10.0)
	Granisetron	19	17 (89.5)	1 (5.3)	1 (5.3)	0 (0.0)
0–24	Ramosetron	20	15 (75.0)	2 (10.0)	1 (5.0)	2 (10.0)
	Granisetron	19	16 (84.2)	1 (5.3)	1 (5.3)	1 (5.3)
24–48	Ramosetron	20	16 (80.0)	1 (5.0)	1 (5.0)	2 (10.0)
	Granisetron	19	16 (84.2)	1 (5.3)	0 (0.0)	2 (10.5)

*According to the National Cancer Institute (NCI) Common Toxicity Criteria[9]: grade 0, no nausea; grade 1, mild nausea with tolerance for solids and fluids; grade 2, moderate nausea with tolerance for fluids only; grade 3, severe nausea with intolerance for solids and fluids.

Table 4 Effectiveness of ramosetron and granisetron for the prevention of anorexia within 48 hours of ACNU administration

Time after ACNU (hr)	Drug	No. of patients	No. (%) of patients by NCI grades*				
			0	1	2	3	4
0–6	Ramosetron	20	17 (85.0)	2 (10.0)	1 (5.0)	0 (0.0)	0 (0.0)
	Granisetron	19	15 (78.9)	3 (15.8)	1 (5.3)	0 (0.0)	0 (0.0)
0–24	Ramosetron	20	14 (70.0)	4 (20.0)	1 (5.0)	1 (5.0)	2 (10.0)
	Granisetron	19	14 (73.7)	2 (10.5)	0 (0.0)	0 (0.0)	3 (15.8)
24–48	Ramosetron	20	16 (80.0)	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.0)
	Granisetron	19	14 (73.7)	0 (0.0)	2 (10.5)	0 (0.0)	3 (15.8)

*According to the National Cancer Institute (NCI) Common Toxicity Criteria[9]: grade 0, normal appetite; grade 1, ability to eat one-third of a meal; grade 2, ability to eat half a meal; grade 3, inability to eat and requiring intravenous fluids; grade 4, inability to eat and requiring hyperalimentation.

with water and reported that it was too hard to swallow.

To evaluate the effects of 5-HT3 receptor blockers, the records of 17 malignant glioma patients treated with the same ACNU protocol who had not received anti-emetic drugs were analyzed (Table 5). Ten patients had no vomiting episodes within 6 hours of ACNU administration. Only one patient experienced iterative vomiting (grade 2) during the first 24 hours, and 12 of the total of 14 episodes occurred during the first 6 hours. Nausea occurred in 12 patients during the first 24 hours, but 12

Table 5 Occurrence of vomiting, nausea, or anorexia after ACNU treatment without anti-5-HT3 receptor blocker

A: Vomiting

Time after ACNU (hr)	No. of patients	No. (%) of patients by NCI grades*				
		0	1	2	3	4
0-6	17	10 (58.8)	3 (17.6)	4 (23.5)	0 (0.0)	0 (0.0)
6-24	17	16 (94.1)	0 (0.0)	1 (5.9)	0 (0.0)	0 (0.0)
24-48	17	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

B: Nausea

Time after ACNU (hr)	No. of patients	No. (%) of patients by NCI grades*			
		0	1	2	3
0-6	17	7 (41.2)	6 (35.3)	2 (11.8)	1 (5.9)
0-24	17	5 (29.4)	5 (29.4)	6 (35.3)	1 (5.9)
24-48	17	12 (70.6)	2 (11.8)	2 (11.8)	1 (5.9)

C: Anorexia

Time after ACNU (hr)	No. of patients	No. (%) of patients by NCI grades*				
		0	1	2	3	4
0-6	17	6 (35.3)	4 (23.5)	3 (17.6)	4 (23.5)	0 (0.0)
0-24	17	2 (11.8)	6 (35.3)	6 (35.3)	3 (17.6)	0 (0.0)
24-48	17	6 (35.3)	5 (29.4)	3 (17.6)	3 (17.6)	0 (0.0)

*For NCI grading, see the legends to Tables 2-4. The patients received only dopamine D2 receptor antagonists for the prevention of gastroenterological side effects.

patients were nausea-free at 24-48 hours. Only two of the 17 patients could eat normally during the first 24 hours. Eleven patients were anorexic at 24-48 hours.

Discussion

In our control population, vomiting and nausea were most severe during the first 6 hours after ACNU administration and only one third of patients were able to eat normally after 48 hours.

Anticancer drugs such as cisplatin produce oxygen-free radicals in the enterochromaffin cells and the free radicals induce the release of serotonin which binds to 5-HT3 receptors on the afferent vagus nerve terminals, and consequently signal the vomiting center.[2] Both ramosetron and granisetron are 5-HT3 receptor blockers and potentially powerful anti-emetics.

Ramosetron is a specific 5-HT3 receptor blocker developed in Japan. Ramosetron tablets are half the price of injected drugs and can be taken without water. The intravenous concentration reaches the maximum within 2 hours and then decreases to half maximum at 6 hours.[7] Ramosetron inhibits the binding of serotonin to 5-HT3 receptors more potently than granisetron in vitro and in animal models.[5] Comparison of the anti-emetic effects of i.v. ramosetron and granisetron in patients treated with cisplatin showed that while the effects were not significantly different at 6 hours, although ramosetron but not granisetron continued to be effective at 18-24 hours.[6]

Fifteen of our 20 patients treated with ramosetron were nausea-free at 24 hours after ACNU administration compared to five of the 17 historical controls. However, four patients treated with ramosetron were anorexic at 24-48 hours. Ramosetron and granisetron were more effective in suppressing the emetic effects of ACNU than cisplatin, as 70% of patients receiving ramosetron and 73.7% of patients receiving granisetron were able to eat normally at 24 hours after ACNU administration compared to 48% and 33.7%, respectively, of patients after cisplatin administration.[3] The effect of i.v. ramosetron was longer than that of i.v. granisetron.[6] The present study found no significant difference between the effects of oral ramosetron and those of i.v. granisetron.

Our patients reported that the ramosetron tablets