four showed responses interpretable as impaired speech comprehension. In all such cases, the electrophysiologically determined location matched the area depicted by the combined method, although MEG-depicted receptive language areas covered relatively broad areas of the temporal lobe. The regions determined by the combined method were always broader, but had the border within the adjacent gyri of those determined by electrophysiological mapping.

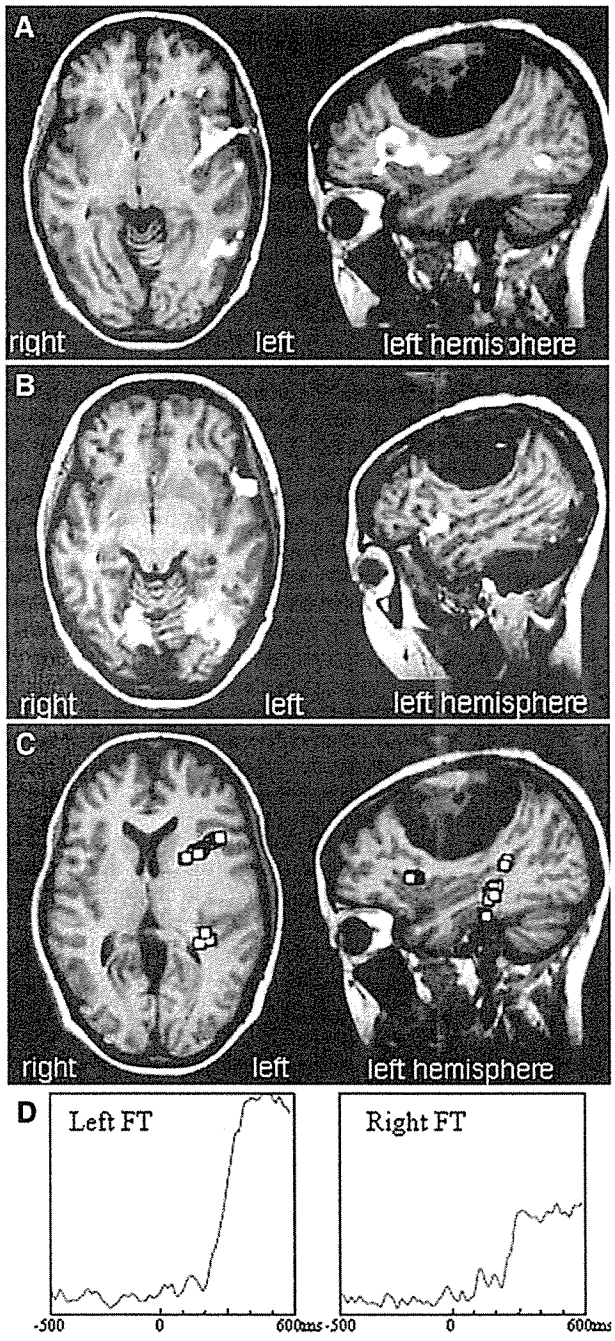


FIGURE 2. *A 16-year-old, right-handed female patient with a large meningioma in the left frontal region. The patient had no impairment of language or motor functions.* A, *fMRI with the verb generation task showed activations mainly in the left IFG and MFG that shifted inferiorly by the tumor.* B, *fMRI with the abstract/concrete categorization task demonstrated activations in the bilateral occipital regions in addition to small active spots in the left IFG.* C, *square root mean field profiles of language-MEG responses demonstrated that the left FT responses, peaking at 400 milliseconds, were markedly larger in amplitude than the right FT.* D, *source localization of the late deflections showed predominant dipole clusters in the left posterior temporal region. The left and right hemispheres contained 117 and 30 dipoles, respectively. The combined fMRI plus MEG method indicated left language dominance, which was confirmed by Wada test.*

ILLUSTRATIVE CASES

Patient 1

A 16-year-old, right-handed female patient had experienced transient numbness in her left upper extremity with a 2-month history. T1-weighted MRI scans demonstrated an extra-axial cystic lesion in the left frontal region. Although the lesion markedly compressed the frontal lobe, she had no impairment of language and motor functions. fMRI with the verb generation task demonstrated obvious activation in the left IFG and MFG shifted inferiorly by the lesion (*Fig. 2A*). The A/C categorization task activated a small area of the left IFG, but mainly the bilateral occipital lobes. Concerning MEG with the *Kana* reading task, RMS of the left FT was much higher than that of the right, and numbers of semantic dipoles were 117 and 30 in left and right hemispheres, respectively. The main dipole clusters were located in the left IFG and STG. The tumor was totally removed and histopathological diagnosis was meningioma.

Patient 2

A 24-year-old, right-handed male patient had a large AVM in the left frontal lobe. fMRI detected little activation in the IFG or MFG, although a part of the left angular gyrus was activated by the verb generation task (*Fig. 3A*). MEG, however, disclosed numerous dipole accumulations in the left superior temporal region. In the MEG examination, the left and right hemispheres contained 130 and 45 dipoles, respectively, suggesting left language dominance (*Fig. 3B*). Auditory comprehension and letter-reading were suppressed by administration of amobarbital into the left carotid artery, although motor language function was preserved. These findings suggested that the steal effect caused by the AVM partly interfered with functional brain mapping of fMRI and the Wada test. In this case, MEG was helpful to decide language dominance (*Fig. 3*).

Patient 3

A 32-year-old, right-handed man experienced amnesia for several minutes. T1-weighted MRI scans and brain computed tomographic scans disclosed a hypointense and hypodense mass in the right insular cortex involving the surrounding white matter. Computed tomographic scans performed 6 years earlier, however, revealed no abnormality. These findings suggested that a low-grade astrocytoma might

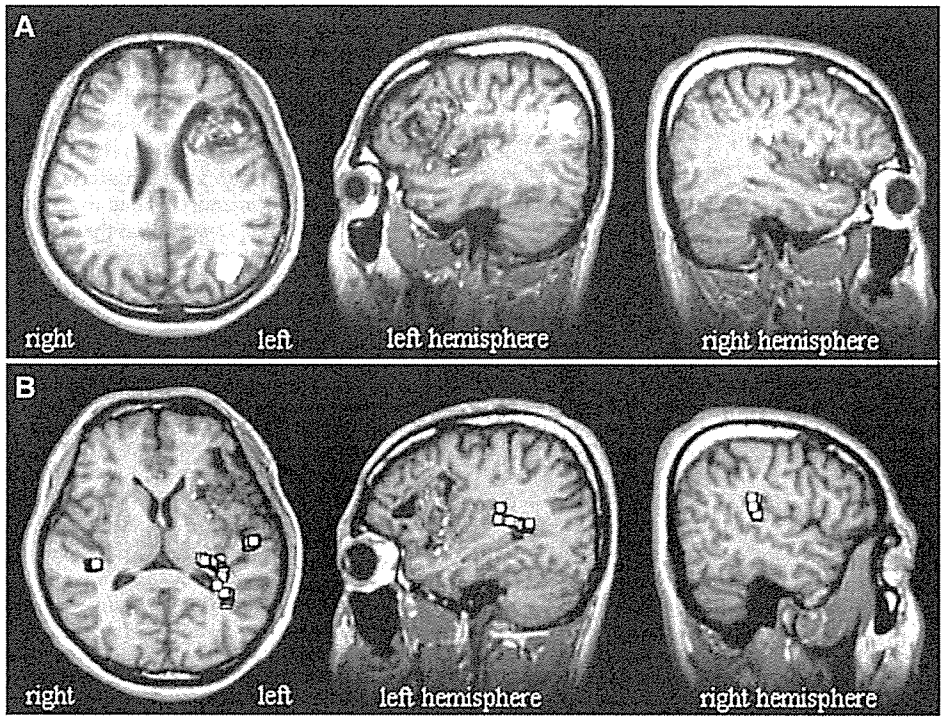


FIGURE 3. *A 24-year-old, right-handed man with a large AVM in the left frontal lobe.* A, *fMRI with the verb generation task showed little activation in the left frontal lobe where the AVM was located.* B, *source localization of the late FT and TO deflections on MEG showed predominant dipole clusters in the left posterior STG. The left and right hemispheres contained 123 and 51 dipoles, respectively.*

have slowly developed during the past 6 years. In the results of the verb generation task, the left hemisphere had obvious activations in the IFG, MFG, precG, and the angular gyrus, indicating that this patient had left dominance of motor-language functions (*Fig. 4A*). In contrast, estimated dipoles of the FT responses were concentrated in the posterior part of the right STG and MTG (138 dipoles) and another dipole cluster (64 dipoles) of the TO region was localized in the right FuG. The total dipole number of the left hemisphere (48 dipoles) did not reach even a quarter of that of the right hemisphere, suggesting right-sided dominance of temporal language functions (*Fig. 4*).

During the Wada test, he stopped counting (0 out of 4 points; 0%) and failed to name objects (6 out of 20 points; 30%) after left intracarotid injection, whereas letter-reading (21 out of 28 points; 75%), auditory comprehension (12 out of 12 points, 100%), and pointing objects tasks (16 out of 16 points; 100%) were well preserved. In contrast, after right intracarotid injection, letter reading (13 out of 28 points; 45%), auditory comprehension (3 out of 12 point; 25%), and pointing objects (4 out of 16 points; 25%) tasks were markedly suppressed, although he continued to count correctly without speech blockade (4 out of 4 points; 100%) and could perform naming (17 out of 20 points; 85%). These findings suggested that language functions were distributed separately over the bilateral hemispheres, and the expressive and receptive language functions were dissociated in the left frontal and right temporal lobes, respectively. A striking fact was that the combination of fMRI and MEG predicted the special profiles of language functions noninvasively.

DISCUSSION

We demonstrated that our method using both fMRI with the verb generation task and MEG with the *Kana* reading task is highly reliable in determining the language dominance in patients with brain lesions. The accuracy of the dominance laterality was confirmed by a 100% match with the results from the Wada test. fMRI and MEG compensated each other's disadvantages. The tasks of fMRI were rather simple and could be accomplished even by patients with mental dysfunctions, whereas MEG results were seldom affected by cerebral blood flow abnormalities. Reliable data on language functions were also obtained by combining the advantageous features of fMRI and MEG. fMRI with the verb generation task well depicted the expressive language area as activations in the frontal lobe, most commonly in the IFG. MEG, on the other hand, showed dipole clusters predominantly in the superior temporal regions representing the receptive language area. In the epilepsy group, left and bilateral dominance were approximately 85% and more than 6%, respectively, whereas, in the non-epilepsy group, left and bilateral dominance were more than 90% and less than 2%, respectively. The combined method, including the Wada test, fMRI, and MEG, clearly demonstrated bilateral dominance is more often observed in the epilepsy group than in the non-epilepsy group.

In our study, two out of 87 patients analyzed (2.3%) were found to have dissociation of the expressive and receptive language functions by co-utilization of fMRI and MEG, verified by the Wada test, which best described the usefulness of our method in identifying the areas of the two language functions separately. In both cases, neither modality alone demonstrated the dissociation. Although several cases have been reported that dissociated language functions were found by fMRI, none of those was proven by the Wada test (2, 8, 21, 23). Our results show that neither fMRI nor MEG alone is sufficient to accurately locate the expressive and receptive language areas, and the combined use is the key to obtaining high reliability.

The results from electrophysiological investigation via a subdural electrode implantation in 12 patients further confirmed the accuracy of the present method. Pouratian et al. (22) reported that the sensitivity and specificity of language-fMRI

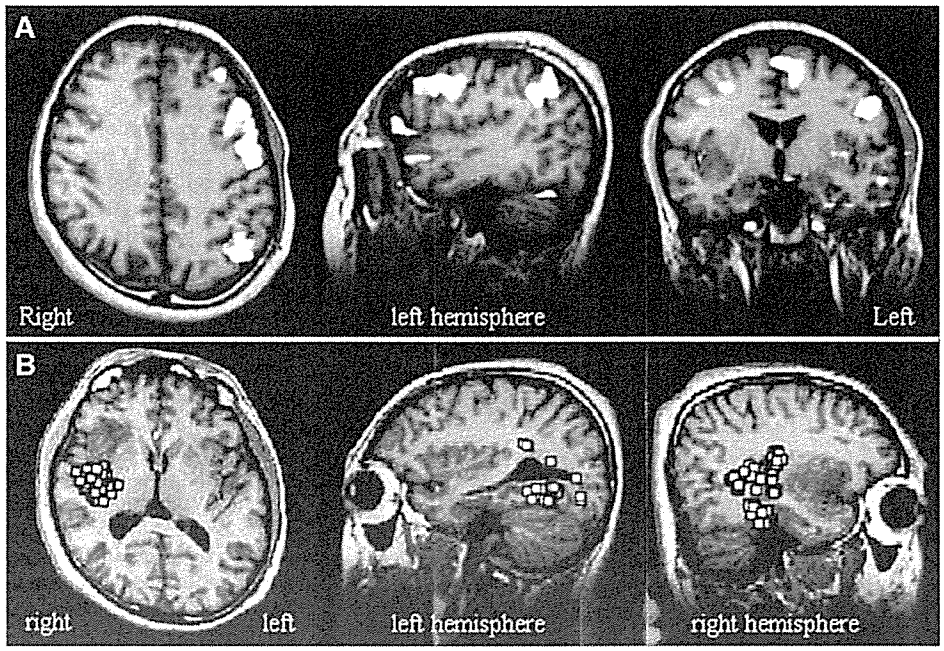
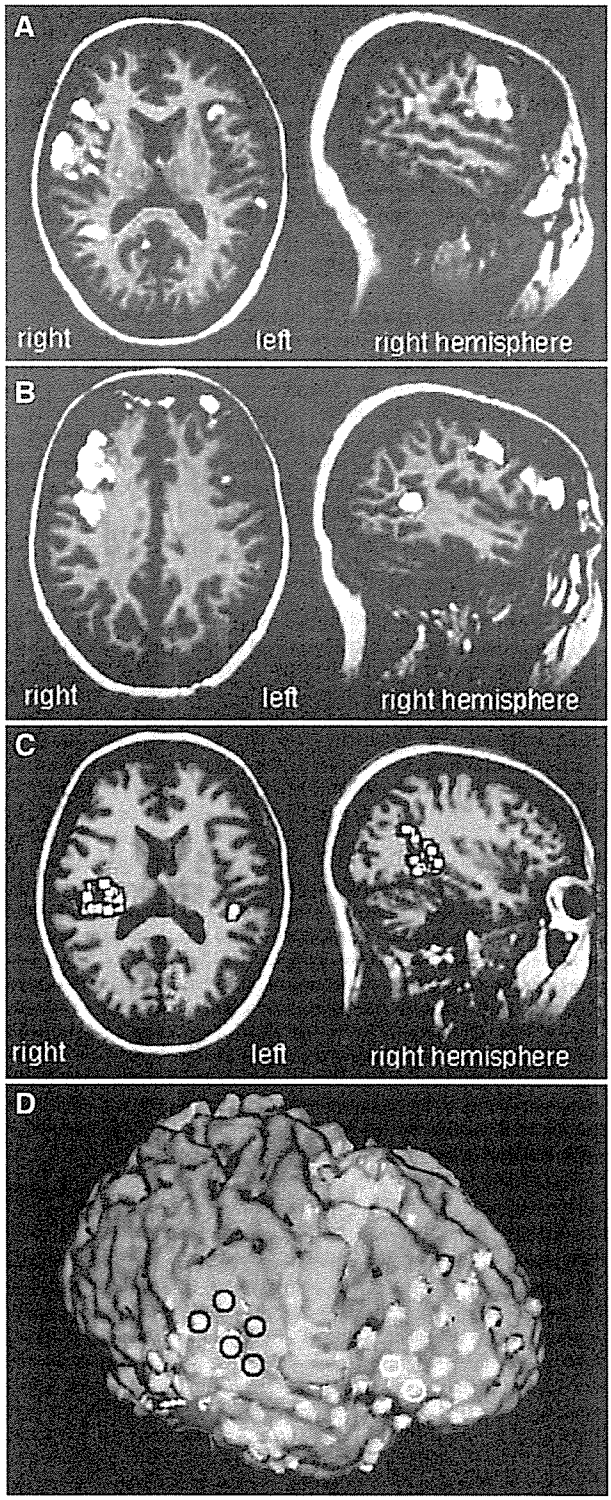


FIGURE 4. *A 32-year-old, right-handed man with astrocytoma in the right insular cortex and the surrounding white matter.* A, *fMRI with the verb generation task showed main activations in the IFG, MFG, precG, and AG, indicating left dominance of the expressive language function.* B, *in contrast to the fMRI results, source localization of the late FT deflections on MEG showed predominant dipole clusters in the right temporal lobe. The left and right hemispheres contained 48 and 202 dipoles, respectively. The combined fMRI plus MEG method thus indicated dissociated frontal motor and temporal receptive language functions. This result was confirmed by the Wada test. The patient showed impaired counting and object naming after amobarbitol injection into the left carotid artery. In contrast, letter reading, auditory comprehension and object pointing tasks were markedly suppressed, without counting impairment and speech blockade, after amobarbitol injection into the right carotid artery.*

were dependent on the task, lobe, and matching criterion. The sensitivity and specificity of fMRI activations during expressive linguistic tasks in the frontal lobe were found to be up to 100 and 66.7%, respectively, in the frontal lobe. FitzGerald et al. (6) reported that sensitivity and specificity for all multiple language tasks ranged from 81 to 53% (6). On the other hand, several groups have reported that the language map obtained from fMRI poorly matched the intraoperative electrical stimulation mapping (6, 25). In our study of language-fMRI, every electrical stimuli to the IFG, where the fMRI-activation was observed, caused speech arrest. However, the stimulation to MFG caused language-related symptoms in only half of patients. Although the sensitivity of fMRI might be high, there are still several issues of individual variability of fMRI activation and semantic tasks. The discrepancy can be partly accounted for by the fundamental differences in methodology such that the electrical stimulation directly blocks the specific language functions, whereas fMRI picks up all activated areas involved in the language tasks. Therefore, fMRI-based mapping largely depends on the design of the performing task. We tested two different tasks for fMRI and found the verb generation task better suited for language mapping than the A/C categorization task. The significance of activations depicted on fMRI is still under debate. Language-fMRI activations may be related to various semantic components of the task, including the will to retrieve verbal materials and the memory related to articulations. Despite that the A/C categorization task was designed to detect the receptive language area, activations in the temporoparietal region was less frequently observed than in the frontal region. Neural activities in the temporoparietal area are considered relatively scarce (25), and the discrepant activities of the frontal and temporoparietal regions may be owing to physiological variations of brain regions. Alternatively, the frontal and temporal lobes may have different oscillations (brain rhythms) of brain activity in response to verbal tasks, which are reflected in changes in neuronal currents and cerebral blood flow.

Our study demonstrated that dominance of the receptive language function could be accurately determined by MEG. For that purpose, we originally designed the task of three-letter word reading and silent categorization and used the dipoles calculated from late deflections to process the MEG results. It has been reported that cortical evoked potentials recorded by subdural electrodes showed responses at approximately 200 (early) and 400 (late) milliseconds in the left temporal lobe cortex after letter presentation (1, 17). The late potentials have been noted especially in tasks involving decisions based on visually presented words (13, 14). In this study, the sources of late responses (250–600 ms) were located mostly in the posterior temporal region, and the laterality of dipole clusters accurately reflected the receptive language dominance. It has been reported that dipoles in the superior temporal region showed an excellent agreement with an intraoperative electrical mapping (27). We also included dipoles in the FuG for language dominance determination based on our experience with a case in which an injury of FuG resulted in pure dyslexia (12). These contrivances in our method may have led to improvement in accuracy on language dominance determination over previous reports (20). Basic technical issues of the MEG investigation still remain. Eye movement artifacts were strong enough to distort the baseline of the MEG data. In our study,



we asked patients to keep gazing at the center of the screen during the semantic decision without blinking. As a result, artifacts were observed at later than 600 milliseconds after letter presentation and usually did not affect the early and late semantic responses. It is, however, important to prevent artifacts by monitoring eye movements and using rejection thresholds.

In conclusion, by co-utilizing fMRI and MEG, we established a method to determine language dominance with a high reliability. The fMRI activations with the verb generation task identified the expressive language area, whereas the language MEG dipoles located the receptive language areas. Our institution is now routinely using the combined technique to identify the language dominance. If it does not produce data on cerebral dominance, we additionally perform the Wada test before surgery. This non-invasive and repeatable method may be an effective alternative to the Wada test and may be useful in the management of patients with brain lesions.

Disclosure

This work was supported in part by the Japan Epilepsy Research Foundation, Takeda Promotion of Science Foundation, a grant-in-aid No.17591502 for scientific research from MEXT, a Research Grant of the Princess Takamatsu Cancer Research Fund, Terumo Promotion of Science Foundation, Brain Science foundation, and Grant-in-Aid No. 18020010 for Scientific Research on Priority Areas Integrative Brain Research from MEXT.

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FIGURE 5. *A 40-year-old, left-handed woman with epilepsy. A, fMRI with the verb generation task showed activations predominantly in the right IFG and MFG. B, fMRI with the A/C categorization task demonstrated activations in the right MFG and the posterior STG. C, source localization of the late deflections on MEG showed predominant dipole clusters* (white squares) *in the right posterior temporal region. The left and right hemispheres showed 44 and 144 dipoles, respectively. D, three-dimensionally reconstructed MRI scans fused with activation of the verb generation-fMRI* (orange) *and dipoles of language-MEG* (blue). *After implantation of subdural electrodes* (gold), *cortical mapping was performed with 50Hz bipolar electrical stimulation. Stimulation with intensity of 7mA to the right IFG caused speech arrest* (white circles), *whereas stimulation to the posterior STG caused impairment of auditory comprehension and reading capability* (black circles).

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COMMENTS

This is an interesting article evaluating the complementary features of functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) to assess language lateralization in 87 patients. Whereas any test of language lateralization is suspect if 100% correlation is found, the authors have carefully described their techniques and the analysis of results. It is quite apparent that fMRI with verb generation tasks is best at activating anterior language areas, whereas abstract versus concrete naming tasks can be less robust. This is a good article and a large experience worthy of publication.

G. Rees Cosgrove
Burlington, Massachusetts

The authors have applied fMRI and MEG techniques to localize speech function in a large number of patients with different brain lesions. They were able to supplement the two noninvasive tests with the Wada test in 80% of the patients. They were able to obtain useful data with the co-utilization of fMRI and MEG in 95.6% of the patients and found a somewhat surprisingly good match with the results of the Wada test in 100% of those. In the results section, the authors discuss a few differences to the localization of language areas by electrophysiological means. They point out the fact that atypical language dominance or bilateral language representation is more frequent in patients with chronic epilepsy than in those without epilepsy. This is an important fact not known to many neurosurgeons who are not ordinarily involved with epilepsy cases. The results of this study make it more likely that, in the future, the invasive Wada test procedure might be abolished in those institutions at which MEG is available. This constitutes a notable limitation of this noninvasive technique. If fMRI is used alone, the success rate for obtaining useful data is 84.6% for word generation tasks and only 67% for the abstract/concrete categorization task. This is quite an interesting study and the results are very promising; however, the limitations are not economical. A number of patients cannot complete all the tasks necessary for fMRI study, and MEG studies can be disturbed by eye movement artifacts. We look forward to other reports confirming these promising results.

Johannes Schramm
Bonn, Germany

The authors present some very interesting data in the realm of functional imaging to determine cerebral dominance for language. Currently, the standard modality for determining cerebral dominance is the venerable Wada test. In this study, the authors use both MEG and fMRI to determine language dominance based on activation in the inferior frontal gyrus and middle frontal gyrus using fMRI and dipole moments reflecting or indicating receptive language fields in the temporal lobe. As expected, they had some difficulty with the fMRI data owing to the underlying deficit in the patient, which suggests that fMRI is not always as good as one might expect in terms of determin-

ing cerebral dominance using a verb generation silent language task. We know that fMRI is not a good choice for defining receptive language fields that correspond to intraoperative stimulation mapping. However, when fMRI was used together with MEG, the authors were able to demonstrate 100% concordance with data from the Wada test. Thus, this is a very important study indicating that, in the near future, it may be possible to bypass the Wada test with these two powerful functional imaging modalities. That being said, not every institution is going to be able to obtain both of these functional tests. Therefore, it is unlikely that this strategy is going to replace Wada tests completely. Yet, this is a very important line of investigation and a novel observation that points out the frailties of functional imaging for cerebral dominance localization and the potential power when the different functional tests are combined.

Mitchel S. Berger
San Francisco, California

脳腫瘍の遺伝子治療

Gene therapy for brain tumors

藤堂具紀(写真)　宮本伸哉
Tomoki Todo and Shinya Miyamoto
東京大学大学院医学系研究科脳神経外科学

◎悪性脳腫瘍は手術，化学療法，放射線療法を併用した集学的治療を行っても一般的に予後が悪く，とくに悪性グリオーマはここ数十年治療成績の向上がみられない．遺伝子治療の悪性脳腫瘍への応用は開発早期から期待され，積極的に開発が進められてきた．初期の自殺遺伝子治療の臨床結果から既存のベクターの遺伝子導入効率の低さという問題点が明らかになり，最近は少ない発現から大きな抗腫瘍効果を期待できる免疫遺伝子治療と腫瘍特異的に複製する増殖型ウイルスを用いるウイルス療法が注目されている．とくに後者は，ウイルスゲノムに治療遺伝子を組み込むことによりウイルス複製に伴う直接的な殺細胞作用に加え，腫瘍内で増幅された治療遺伝子発現による抗腫瘍効果の増強が期待できる．欧米では増殖型ウイルスの脳腫瘍内投与の安全性が臨床試験で確認され，わが国でも膠芽腫に対する臨床試験の準備が進められている．

Key Word　悪性グリオーマ，ウイルス療法，増殖型ウイルスベクター，自殺遺伝子治療，免疫遺伝子治療

1988年に世界ではじめて遺伝子マーキングの臨床プロトコールが承認され，1990年にはアメリカでadenosine deaminase(ADA)欠損症患者にする遺伝子治療が行われて，遺伝子治療の臨床開発が本格的に幕開いた．現在までに世界で1,000件以上の遺伝子治療の臨床プロトコールが施行されている．わが国では1993年に厚生省(当時)と文部省(当時)でガイドラインが作成され，1995年に北海道大学でADA欠損症に対するわが国初の遺伝子治療が行われたが，現在までに実施された遺伝子治療プロトコールはわずか21件にすぎない．最近，遺伝子治療臨床研究に関する指針が改定され，わが国でも遺伝子治療の臨床が行える環境が整備されつつある．

初期の臨床試験で，動物実験結果から期待された成果がみられていない最大の原因は，治療遺伝子(transgene)の運び屋であるベクターの生体内における遺伝子導入効率が予想以上に低く，対象とする細胞のごく一部にしか治療遺伝子の発現がみられなかったことと考えられ，近年はより効率のよいベクター開発に力が注がれるようになった．1999年9月にアメリカで18歳のJesse Gelsingerがアデノウイルスベクターを用いたornithine transcarbamylase欠損症の治療の結果死亡したことや[1]，フランスでX-linked severe combined immune deficiencyに対するレトロウイルス

サイドメモ　ウイルス療法に有利なHSV-1の特徴——アデノウイルスとの比較

① ほぼあらゆる種類のヒト細胞に感染．
② 強力な殺細胞作用．
③ 抗ウイルス薬の存在．
④ ウイルスゲノムが大きいため，複数の外来遺伝子を挿入可能．
⑤ ウイルス自体の免疫原性が比較的低い．
⑥ 宿主免疫が抗腫瘍効果に有利に働く．
⑦ 血中抗HSV抗体が細胞間ウイルス伝搬に影響しない．

ベクターを用いた治療の約3年後(2002)に乳児2例が[2]，さらに最近あらたな1例が白血病様症状を呈したことをきっかけに，遺伝子治療の臨床応用の進め方が見直されたが，一方で開発を継続することの重要性が再確認された．現在，欧米を中心に基礎と臨床両面で積極的な研究が進められ，単一遺伝子疾患をはじめ悪性腫瘍領域においても，遺伝子治療による治療革新に期待が寄せられている．

自殺遺伝子治療

悪性脳腫瘍は高度に分化した非増殖細胞に囲まれた増殖細胞の集団であること，定位脳手術により比較的容易に腫瘍内直接投与が行えること，遠隔転移がまれであること，著効を示す治療法が現時点で存在しないことなどから，遺伝子治療の対象として適している．アメリカでは遺伝子治療開発早期より積極的に脳腫瘍を対象とした臨床試験が行われ，その大半はいわゆる自殺遺伝子治療であった．自殺遺伝子治療とは，ウイルスや細菌由来の特定の代謝酵素遺伝子を“自殺遺伝子”として腫瘍細胞に導入した後，抗ウイルス薬や抗生物質などのプロドラッグを全身投与し，腫瘍細胞のみ選択的に死滅させる遺伝子治療法である．用いられるプロドラッグは導入された遺伝子が発現する酵素でのみ代謝・活性化され，細胞毒性を有する物質に変換されるため，遺伝子導入細胞を選択的に死滅させることができる．

脳腫瘍に対する自殺遺伝子治療の臨床試験のほとんどは単純ヘルペスウイルス(HSV)のチミジンキナーゼ遺伝子(tk)を腫瘍細胞に導入し，プロドラッグとしてガンシクロビル(ganciclovir：GCV)を全身投与する方法である．チミジンキナーゼは，GCVをリン酸化して毒性のヌクレオチドアナログに変え，これがDNA合成を阻害しアポトーシスを起こす．この治療の最大の特徴は，HSV-tk遺伝子を導入された腫瘍細胞数よりはるかに多くの腫瘍細胞が死滅する現象が認められることで，周囲の細胞が巻き込まれるという意味で，bystander効果とよばれる．その機序として，*in vitro*ではHSV-tk発現細胞で生じたGCV-三リン酸がギャップジャンクションを介して隣接する細胞に流入して細胞毒性を発揮するとされるが，*in vivo*ではHSV-tk/GCV療法の殺細胞効果に伴って惹起される抗腫傷免疫の関与が大きいとされる．

遺伝子分配の媒介としてレトロウイルスベクター産生細胞，アデノウイルスベクターが用いられてきたが，遺伝子導入効率が*in vivo*では予想よりはるかに悪いこと，ベクター産生細胞やウイルスの免疫原性，それにより惹起される炎症と脳浮腫，GCVを投与するとベクター産生細胞も死滅することなどの問題が明らかとなった．レトロウイルスベクター産生細胞を用い，初発膠芽腫患者を対象とした第Ⅲ相臨床試験では，手術＋放射線治療の標準治療群に比べ，HSV-tk遺伝子治療を加えても付加的な治療効果がまったく得られなかった[3]．

初期の反省から，自殺遺伝子治療を従来の治療法と併用したり免疫遺伝子治療と組み合わせたりして，相互に治療効果を増強する試みがなされている．ヌードマウスの脳に植えたヒト膠芽腫に対して，HSV-tk, tumor necrosis factor αを組み込んだ非増殖型の単純ヘルペスウイルスⅠ型(HSV-1)ベクターを投与した後，ガンマナイフを併用すると著明な延命効果があることが報告された．また，改変型アデノウイルスベクターを用いてHSV-tkを導入し，通常の放射線治療と併用する臨床試験が膠芽腫患者を対象に開始された．インターロイキン2(IL-2)遺伝子治療と組み合わせて膠芽腫患者を対象とした臨床試験では，HSV-tk遺伝子治療単独より高い治療効果が示唆された[4]．

免疫遺伝子治療

免疫遺伝子治療とは免疫系を調節する蛋白の遺伝子を腫瘍細胞などに導入し，抗腫瘍免疫を惹起させる治療をいう．導入した遺伝子を発現する細胞が少なくても，いったん抗腫瘍免疫が確立されれば，大きな抗腫瘍効果が期待できる．脳は従来immune privileged siteと考えられてきたが，脳腫瘍に対しても腫瘍特異的な細胞傷害性Tリンパ球(cytotoxic T lymphocyte：CTL)の活性化を誘導することにより抗腫瘍効果が期待できることが明らかとなってきた．治療遺伝子としてはサイトカ

イン(IL-2, IL-4, IL-12, IL-18, GM-CSF, IFN-γ)や共刺激分子(B7-1, B7-2), 免疫原性を増強する因子(TGF-βアンチセンス, IGF-Ⅰアンチセンス, 同種組織適合性抗原)などが用いられている.

初期の免疫遺伝子治療は *ex vivo* 法とよばれ, 摘出した腫瘍組織より培養した細胞に免疫調節遺伝子を導入し, それを患者の皮下などに投与して特異的抗腫瘍免疫の誘導を期待するワクチン療法であった. しかし, 悪性脳腫瘍の進行の早さに比較し, *in vitro* での遺伝子導入細胞の確立過程に要する時間が長すぎるという欠点から, 培養が容易な線維芽細胞株や自己線維芽細胞に遺伝子導入をして, より短期間で準備する試みもなされている.

Ex vivo 法に対しベクターを直接腫瘍に到達させて *in situ* にて腫瘍細胞に遺伝子を導入する *in vivo* 法は, 効果が示されればより実用性が高い. 脳腫瘍実験モデルではIL-12や可溶型B7-1を用いた遺伝子治療の有効性が示されており[5,6], 臨床試験では悪性グリオーマを対象に, アデノウイルスベクターを用いてinterferon-β遺伝子を脳内の腫瘍細胞に直接導入する2件の臨床試験がアメリカで施行された[7]. わが国でも脳腫瘍に対するはじめての遺伝子治療臨床試験として名古屋大学において, カチオニックリポソームを利用したinterferon-β遺伝子治療が2000年から行われた. 従来の治療を行った後に再発した悪性グリオーマ5例に対し腫瘍を可及的に切除した後, 残存腫瘍に対してカチオニックリポソーム包埋interferon-β遺伝子を直接腫瘍内に注入し, さらに術後1～6回追加投与を行った. 有害事象はみられず, 病気進行までの期間が3～16カ月(中間値9カ月), 再発後生存期間が6～29カ月(中間値17カ月)であった[8].

増殖型ウイルスベクターを用いた治療

遺伝子分配効率の悪い非増殖型ウイルスベクターや非ウイルスベクターに代わり, 増殖型(replication-competent)ウイルスベクターの利用が注目されている[9]. ウイルスゲノムに遺伝子操作を加えることによって腫瘍細胞内で選択的に複製可能にし, 正常組織での病原性を抑制することができる. そのような遺伝子組換えウイルスに治療遺伝子を組み込むと, 従来の非増殖型ベクターに比べ, 腫瘍細胞内でのウイルス複製により治療遺伝子が増幅され, 広範な遺伝子分配が期待される.

また, 増殖型ウイルスに感染した腫瘍細胞はウイルス複製に伴う直接的な殺細胞効果により最終的に死滅するため, 治療遺伝子を導入せずとも腫瘍を治癒させることが可能である(図1). 治療遺伝子の発現効果ではなく, ウイルス複製による腫瘍細胞融解をおもな治療機序とした治療法をウイ

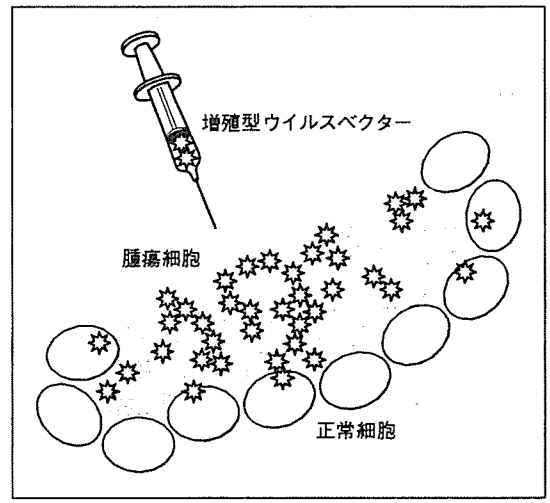

図1 ウイルス療法の原理を示す模式図

腫瘍治療用の増殖型ウイルスは腫瘍細胞での複製能を保ちつつ, ヒト正常組織での病原性が最小限であることが必要である. 元来, ヒトを宿主とせずヒトでは無症候あるいは弱毒性のウイルスを用いる場合(レオウイルスやニューカッスル病ウイルス)と, ヒトを宿主とするがウイルスゲノムに遺伝子操作を加えてヒト正常細胞に病原性を呈さないようにした遺伝子組換えウイルスを用いる場合(HSV-1やアデノウイルス)がある. いずれも腫瘍細胞ではウイルスに対する防御機構が障害されていることを利用するが, 後者の場合はウイルスゲノムをデザインすることにより意図的に正常細胞と腫瘍細胞におけるウイルス複製能の差を広げて治療域を拡大することや, 治療遺伝子を組み込むことが可能で, ウイルス療法の"薬"として発展する可能性が高い. 腫瘍細胞に感染した治療用の増殖型ウイルスは細胞内で複製し, その過程で感染細胞は死滅する. 増殖したウイルスは周囲に散らばってふたたび腫瘍細胞に感染し, その後, 複製→細胞死→感染を繰り返して抗腫瘍効果を現す. 一方, 正常細胞に感染した治療用ウイルスは複製しないため, 正常組織には害が生じない. HSV-1の場合はウイルス複製に伴う腫瘍細胞破壊が腫瘍特異的な細胞傷害性T細胞の活性上昇を伴う全身性抗腫瘍免疫を誘導し, 直接的殺細胞作用に抗腫瘍効果を上乗せする.

ルス療法(oncolytic virus therapy)とよび，遺伝子治療と区別する．

1. 単純ヘルペスウイルスⅠ型

脳腫瘍治療の分野では増殖型 HSV-1 を用いたウイルス療法の開発研究が進んでいる．HSV1716 は比較的病原性の強い野生型 HSV-1 17 株を親株とする第一世代の遺伝子組換え HSV-1 で，γ34.5 遺伝子(病原性に関連)の欠失を有する．脳腫瘍をはじめとする腫瘍細胞に対して比較的強い殺細胞効果を呈するが，正常組織における病原性もある程度残している．第二世代の代表 G207 はやや弱めの野生型 HSV-1 F 株を親株とし，γ34.5 遺伝子の欠失に加え，ICP6 遺伝子(リボヌクレオチド還元酵素の大サブユニット)が不活化されて二重変異を有する．ヒトの脳内に投与することを目的に，安全性を重視して開発された[10]．

イギリスでは再発悪性グリオーマ患者を対象とした HSV1716 の第Ⅰ相臨床試験が行われた．10^3 から 10^5 plaque-forming units(pfu)まで脳腫瘍内に定位的に投与し，安全性を確認した．2 回目の臨床試験では再発または初発の膠芽腫患者に 10^5 pfu を腫瘍内投与し，後に腫瘍切除を行ってウイルスが腫瘍内で複製していることを確認した．3 回目の臨床試験では膠芽腫患者を対象に，10^5 pfu を腫瘍摘出腔壁に投与し，放射線治療や化学療法を併用した．安全性が確認され，3 例が術後 15，18，22 カ月の時点で生存と報告された[11]．

G207 は再発悪性グリオーマの患者 21 例を対象に，第Ⅰ相臨床試験がアメリカで行われた[12]．1×10^6 pfu から 3×10^9 pfu まで 3 例ずつ段階的に増量され，造影 CT の増強部位に腫瘍内投与された．G207 に起因する中等度以上の有害事象はなく，脳内投与の安全性が確認された．6 例で Karnofsky スコアの改善を認め，20 例中 8 例で MRI 上腫瘍の縮小を認めた．脳梗塞で死亡した 1 例を除いた全例で腫瘍の再増大を認めたが，2 例が 5 年以上生存した．つぎに行われた第Ⅰb 相臨床試験では腫瘍摘出腔壁に G207 を直接投与する方法でも安全であることが確認された．G207 とテロゾロマイドを併用すると相乗効果を示すことが最近基礎実験で明らかとなり，アメリカで臨床試験が計画されている．

G207 の開発研究から，HSV-1 を用いたウイルス療法の抗腫瘍効果にはウイルスの殺細胞作用に加えて特異的抗腫瘍免疫が大きく関与していることがわかっている[13]．藤堂らは，G207 の抗腫瘍作用を増強し，治療域が広く実用性の高い増殖型 HSV-1 を開発すべく，G207 ゲノムにあらたな変異(α47 遺伝子と US11 プロモーターの欠失)を加えた第三世代(G47Δ)を作製した[14]．本来 HSV-1 の感染を受けると低下する宿主細胞の MHC class Ⅰ発現が維持されて免疫細胞に対する刺激が増強することと，腫瘍細胞に限定してウイルス複製能が改善することにより G47Δは G207 に比べ，安全性を変えることなく抗腫瘍作用が格段に増強した．現在，文部科学省がんトランスレーショナルリサーチ事業の一環として，膠芽腫患者を対象とした G47Δの臨床試験の計画が進んでいる．G47Δの変異個所をすべて欠失変異とした改良型(T-01)も最近開発された．

HSV-1 の利点のひとつは，比較的大きな治療遺伝子をウイルスゲノムに直接組み込むことができることである．とくに IL-12 などの免疫刺激因子を発現して抗腫瘍免疫を強力に惹起する機能を有した増殖型 HSV-1 ベクターが複数開発され，HSV-1 を治療遺伝子で“武装”することにより抗腫瘍効果を増強しうることが示された[5,15-17]．また，腫瘍や臓器特異的に発現する蛋白のプロモーターでウイルス遺伝子を制御することにより，特定の腫瘍に限定したウイルス複製能を有する HSV-1 を作製する試みもなされている[18]．元来，外来遺伝子を HSV-1 ゲノムに組み込むには相同組換え法が用いられ，多大な労力と時間を要したが，最近 bacterial artificial chromosome と DNA 組換え酵素を利用して，G47Δなどのゲノムの特定部位に任意の外来遺伝子を的確に組み込んで短期間に遺伝子組換え HSV-1 を作製できるシステムが開発され，HSV-1 を用いたウイルス療法開発に拍車がかかると期待される[19,20]．

2. アデノウイルス

増殖型遺伝子組換えアデノウイルスの利用は HSV-1 より後発であるが，遺伝子治療用ベクターとしてすでに普及していたことと遺伝子組換えの簡便性から，ウイルス療法用として急速に開発が

進んだ．とくにE1B領域に欠失変異をもつONYX-015はもっとも早くから，種々の癌を対象として臨床開発が行われた．E1B-55K蛋白は，p53産物と直接結合してこれを不活化させ，宿主細胞がアポトーシスに陥るのを抑制してウイルス複製を可能とする．したがって，ONYX-015はp53正常の細胞ではp53を不活化できないためにウイルスが複製できないが，p53機能を喪失している腫瘍細胞に限りウイルスが特異的に増殖し殺細胞作用を現すと考えられた[21]．アメリカで頭頸部癌をはじめ種々の癌を対象に臨床試験が進められたが，ONYX-015単独投与では特記すべき効果はみられていない．脳腫瘍に関しては放射線治療後に再発した悪性グリオーマ24例を対象に，第Ⅰ相臨床試験がアメリカ多施設で行われた[22]．10^7から10^{10} pfuまでの段階的増量で，腫瘍摘出腔壁10カ所に直接投与された．ONYX-015に起因する重度の有害事象は1例も認められなかったが，病気進行までの中間値が46日，生存期間中間値が6.2カ月であった．増殖型遺伝子組換えHSV-1に比べ，投与されたウイルス量のわりに腫瘍に対する殺細胞作用が弱く，悪性グリオーマのような増殖能・浸潤能の高い腫瘍細胞には不向きである印象がある．最近，中国の企業にONYX-015の開発権が譲渡され，肺癌患者を対象に第Ⅲ相臨床試験が終了して，近々ウイルス療法用として製品化される見通しである．

HSV-1と同様にアデノウイルスでも腫瘍/臓器特異プロモーターによるウイルス遺伝子の制御が利用されており，たとえば前立腺癌用アデノウイルスとして，E1Aをprostate specific antigenプロモーターで制御したCV706が開発された[23]．また，ONYX-015と同様の原理によりアデノウイルスのE1A(CR2領域)を欠失させると，pRB蛋白と結合してこれを不活化するE1A蛋白がないためpRB正常細胞では複製できないが，pRB経路異常の腫瘍細胞では複製できるようになる．さらに，このウイルスのE1AとE4のプロモーターをヒトE2F-1プロモーターに置換したONYX-411は，pRB経路異常腫瘍に限って野生型ウイルスと同等の強い殺細胞効果を示すようになる[24]．しかし，アデノウイルスは受容体(CAR)を有する細胞にしか感染しないことや，ウイルスそのものの免疫原性が高いために非特異的炎症反応を強く引き起こし，反復投与も有効でないことなど，不利な点も多い．ウイルス療法用ベクターとしては，HSV-1のほうが実用性，発展性ともに高い印象がある．

おわりに

わが国では遺伝子組換え技術全般に対する危険性が過度に強調され，遺伝子治療の将来性が過小評価される傾向がある．しかし，元来発現されていない遺伝子を人為的に導入する手法は，ポストゲノム時代の現在，もっとも発展性のある治療分野のひとつである．とくに増殖型ウイルスベクターはウイルス療法のみならず，遺伝子分配のツールとして遺伝子治療に広く利用されつつある．脳腫瘍をはじめあらゆる癌の治療に画期的な変革をもたらすポテンシャルを有しており[25]，わが国においても積極的な開発研究が推し進められることが望まれる．

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お知らせ

第 31 回日本外科系連合学会学術集会

テーマ：「外科，これから」
会　期：2006 年 6 月 22～23 日
会　場：石川県立音楽堂，ホテル日航金沢，金沢市アートホール
会　長：高島茂樹(金沢医科大学消化器外科治療学講座部門)
プログラム：
特別講演
1) Hartwig Bauer Secretary General German Surgical Society, Germany
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3) Wu Yilong Director Chinese Society of Lung Cancer, China
4) Russell William Stitz the President Royal Australasian College of Surgeons, Australia
市民公開講座：『がんとたたかう』
シンポジウム
1) 新初期臨床研修医制度を終えて
2) 思い出の他科協同手術(症例)
3) 拡大手術と縮小手術の標準化
4) 胃癌の術前術中所見からみた術式選択
5) 急性肺動脈血栓塞栓症に対する治療戦略―血栓摘除術，IVR，薬物療法
6) 頭頸部癌に対する外科的治療―機能温存手術から拡大手術まで
7) 膣・子宮先天異常に対する各種治療法の検討
8) 胸部腫瘍に対する胸腔鏡下手術の進歩
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10) 脳神経外科領域における術中画像診断と術中モニタリング
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CLINICAL INVESTIGATION

Head and Neck

RELATION BETWEEN ACUTE AND LATE IRRADIATION IMPAIRMENT OF FOUR BASIC TASTES AND IRRADIATED TONGUE VOLUME IN PATIENTS WITH HEAD-AND-NECK CANCER

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Purpose: Taste loss is a major cause of morbidity in patients undergoing head-and-neck irradiation. The relationship between the time course and the degree of taste disorder was studied in both acute and late phases.

Methods and Materials: Taste ability was measured by the taste threshold for the four basic tastes using a filter paper disc method in patients before, during, and after radiotherapy. The subjects were divided into two groups. In Group A, the radiation fields included most of the tongue ($n = 100$), and in Group B the radiation fields did not include the tip of the tongue ($n = 18$).

Results: In Group A, there was a significant impairment of the threshold of all four basic tastes at 3 weeks after starting radiotherapy (RT), and this impairment remained at 8 weeks ($p < 0.05$). This was not seen in Group B. In Group A, there was no significant difference in the patterns of taste sensitivity change between the high-dose (>20 Gy) and low-dose (≤20 Gy) groups. In the late phase, recovery of taste loss was seen in both groups since 4 months after completing RT.

Conclusions: Unless the anterior part of the tongue was irradiated, taste loss was not observed during RT. When the anterior part of the tongue was irradiated, a difference by radiation dose was not observed in the taste loss pattern. Additionally, radiation-induced taste dysfunction appears to be a temporal effect. © 2006 Elsevier Inc.

Taste threshold, Taste loss, Radiotherapy, Head-and-neck cancer, Filter paper disc.

INTRODUCTION

Patients with head-and-neck cancer frequently complain of changes in their taste perception, dry mouth, and some other distresses during radiotherapy (RT). Some studies, therefore, have been directed at investigating the four basic taste intensities (sweet, salty, sour, and bitter) in head-and-neck cancer patients treated with RT (1–15).

Several of these reports identified a diminished threshold sensitivity for at least one taste quality. However, a consensus is still lacking on the severity and pattern of taste impairments. Some studies reported that the ability to detect sweet solutions diminished whereas sensitivity for bitter ones increased (9, 10). Others, however, argued that the bitter and salty qualities showed the earliest and greatest impairments whereas the sweet quality showed the least (11–13). Schwartz *et al.* (15), however, reported a near normal suprathreshold intensity perception of the four basic tastes in patients after head-and-neck irradiation.

The four modalities of taste (salt, sour, bitter, and sweet) are mediated via taste buds, which are present predominantly on the tongue. A taste map of the tongue represents a misconception that is still seen in many publications. Such maps show that the tip of the tongue is most sensitive to sweet or salty stimuli, whereas the lateral edges and posterior aspects of the tongue respond predominantly to sour and bitter substances respectively (16, 17). It is now known that all four taste qualities can be perceived in all areas of the tongue and palate where taste buds are located (18, 19). Additionally, cranial (facial) nerve VII innervates the fungiform papillae through the chorda tympani branch; it innervates taste buds on the palate through the greater superficial petrosal branch. Cranial nerve IX (glossopha-

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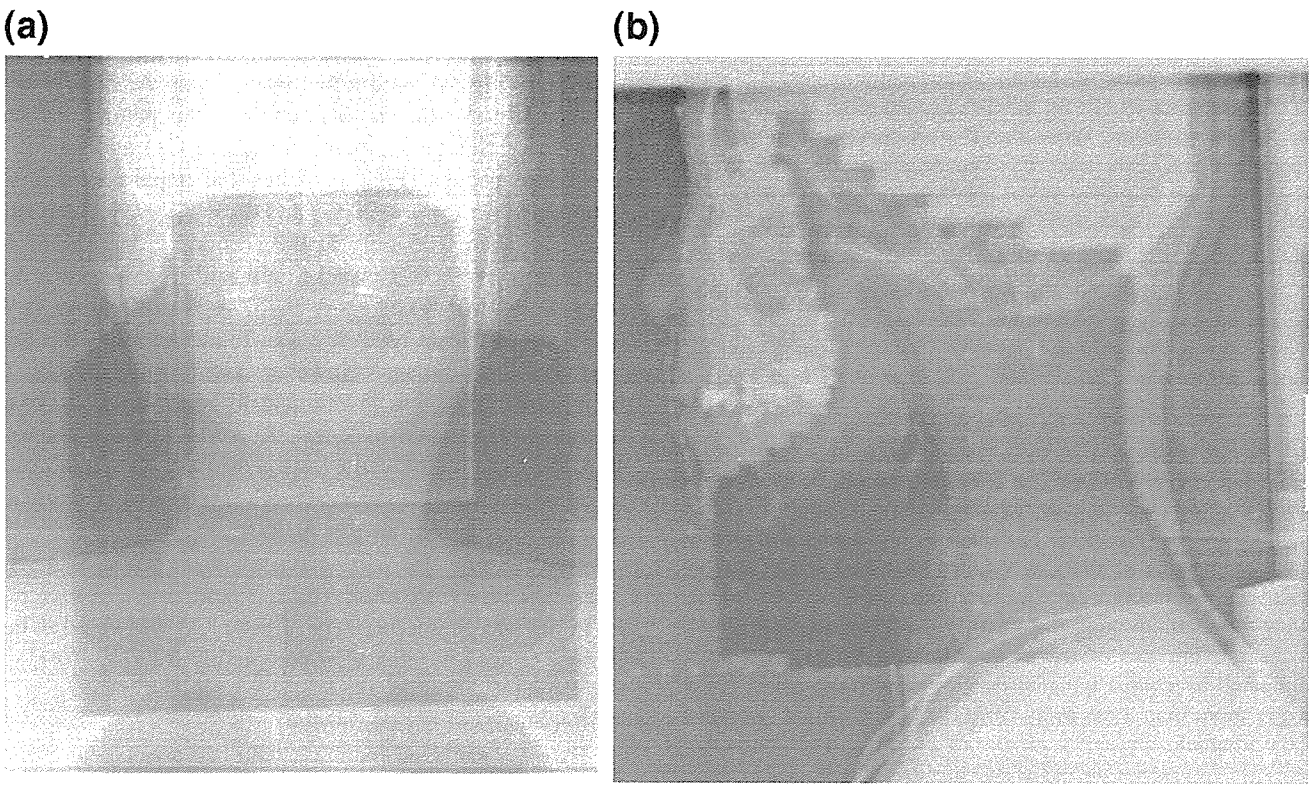

Fig. 2. Linac-graphy for checking radiotherapy field in a typical case. One monitor unit irradiation. The dense area stands for radiation field. (a) Group A. From start to 43.2 Gy/24 fractions anteroposterior direction. (b) Group B. Upper lateral field for patients with cervical nodal or Waldeyer's disease for lymphoma.

and Waldeyer's ring (Figs. 1b and 2). The submental nodes were included in the irradiation field for lymphoma, but the anterior tongue was never given any irradiation after checking the dose–volume histogram information of computed tomographic plans for all cases.

Concurrent chemotherapy was allowed in this study, and 80 subjects (68%) underwent chemotherapy combined with RT for only squamous cell carcinoma. No additional chemotherapy was given to all of the 118 patients.

Two groups

In Group A, the radiation fields included most of the tongue (between 77% and 100% of the tongue in the radiation field, n = 100), whereas in Group B the radiation fields did not include the tip of the tongue (between 30% and 64% of the tongue in the radiation field, n = 18) (Fig. 1). The tip of the tongue starts from the apex of the tongue and covers at least the front one-third of the tongue (i.e., the regions mainly dominated by the chorda tympani nerve). All 15 malignant lymphoma patients were included in Group B. RT treatment periods were 25–63 days (median, 34.5) in Group A and 12–93 days (median, 46) in Group B (unpaired t test; p = 0.0075).

Taste testing

The filter paper disc method (using taste-disc: Sanwa Kagaku Kenkyusho Co., Ltd, Nagoya, Japan) was used to measure thresholds of the four basic tastes under each gustatory innervation (20–22). For the four basic tastes, purified sucrose was used for sweet, sodium chloride was used for salty, tartaric acid was used for sour, and quinine hydrochloride was used for bitter. Each basic taste test solution was prepared with distilled water, and the tests were performed at room temperature. Consistent with the basic studies (23, 24), the taste concentrations of the test solutions were arranged in five concentration levels (T1–T5) for each taste and are shown in Table 1. According to Tomita, who created the taste-disc, convenience was an important requirement in the clinical examination. The T2 level was set as the median value of normal persons in all four taste categories (measured using electrical taste detection for 461 healthy subjects from 11 to 94 years old). T3 was set as the upper threshold of healthy subjects, and T4 (the standard of abnormal level) as the double concentration of T3. T5 was set as the double of T4 in salty and sour, as the quadruple in sweet, and as the eightfold in bitter (in which there are great differences between individuals) (25). In short, T2 or T3 answers indicated normal, whereas T1 indicated hyperreactive and T4 or T5 was for hypoactivity.

Table 1. Threshold concentrations used for taste testing

	Solute			
	NaCl	Sucrose	Tartaric acid	Hydrochloric acid quinine
Taste sensation	Salt	Sweet	Sour	Bitter
Threshold concentration (mmol)				
T1	52	8.8	1.3	0.25
T2	216	73	13	0.50
T3	862	292	133	2.52
T4	1724	585	267	12.59
T5	3448	2339	5333	100.76

In determination of test results, sensibility of taste was scored as "5" when the subject recognized the T1 concentration (see Table 1) of each taste solution, as "4" for the T2 concentration, as "3" for the T3 concentration, as "2" for the T4 concentration, and as "1" for the T5 concentration. When the T5 test concentration was not recognized, the score was "0". The details of the taste testing procedure were described in our previous study (1).

Threshold measurements

Taste recognition threshold measurements were performed once before RT and weekly from the first week up to 10–16 weeks, and monthly from 4 months to 14–24 months after the start of RT.

Filter paper disc

The filter paper disc was 5 mm in diameter. In conducting the test, the subjects were initially asked to extend the tongue as far as possible, and the disc was put on the back of the extended tongue. However, because of pharyngeal reflex (vomiting reflex) and because many subjects were unable to open their mouths widely owing to radiation-induced stomatitis, the paper disc was placed on the moving part of the tongue, i.e., on the region mainly dominated by chorda tympani nerve (the lateral portion of the tongue more than 2 cm away from the central line in the apex of the tongue).

Statistical analysis

The mean value of taste sensitivity threshold was calculated each time from the start of irradiation at every taste test. Statistical analyses were performed using StatView Dataset File version 5.0J for Windows computer software (SAS Institute, Cary, NC). Associations between the mean value of taste sensitivity in preirradiation and during/after irradiation were examined using the nonparametric Wilcoxon signed-rank test. Associations between with and without chemotherapy and between <20 Gy and ≥ 20 Gy at each week after the start of irradiation were examined by using both unpaired t test and Wilcoxon signed-rank test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Dose–volume histogram information

In Group A, the mean and median total doses of RT for tip of the tongue were 21.6 Gy and 14.0 Gy (range, 11.0–75.0 Gy) and for the posterior part of the tongue were 61.2 Gy and 70.0 Gy (range, 30.0–80.0 Gy). In Group B, the mean and median total doses of RT for the posterior part of the tongue were 43.3 Gy and 40.0 Gy (range, 30.0–72.0 Gy).

Acute and subacute objective taste acuity

The means ± standard deviations (SD) for the recognition thresholds of each taste are shown in Fig. 3 by the concentration numbers.

In Group A, all four taste qualities showed remarkably increased mean thresholds when radiation doses were accumulated to 3 weeks (Fig. 3). In Group A, a significant difference was observed between thresholds at pre-RT and with those at 3–8 weeks for all four taste qualities (all $p < 0.05$) (Fig. 3). In Group B, on the other hand, a statistically significant difference was not observed between thresholds at pre-RT and with those at any week for all four taste qualities (Fig. 4). Additionally, in Group A, there was no statistically significant difference in the patterns of taste sensitivity change between high (>20 Gy) and low (≤ 20 Gy) dose groups (Fig. 5). When the unpaired t test was performed for those >20 Gy and those ≤ 20 Gy; the p value was less than 0.05 only at the second ($p = 0.0463$) and seventh ($p = 0.0071$) weeks, and with the Wilcoxon test, only at the fifth week ($p = 0.0077$). No difference in the quickness of taste recovery was found between the high- and low-dose groups. However, for those >20 Gy, the number of cases was insufficient to perform the Wilcoxon matched-pair signed-rank test. According to the Wilcoxon test, for those ≤ 20 Gy, the taste sensitivity was significantly decreased during the third and eighth weeks when compared with preirradiation (all $p < 0.0001$).

Late objective taste acuity

In the late phase, recovery of taste loss was noted in both groups from 4 months after completing RT (Fig. 6).

With or without chemotherapy

The data analyzed for those who had concurrent chemoradiation therapy vs. radiation alone are shown in Fig. 7. An unpaired t test was performed between those with and those without chemotherapy, and the p value was less than 0.05 only at the fifteenth week ($p = 0.0046$). According to the Wilcoxon matched-pair signed-rank test, the p value was less than 0.05 only at the eighth week ($p = 0.0446$). Consequently, the overall effect with and without chemotherapy was without any difference. There was no significant difference in the patterns of taste sensitivity change between the "with" and "without" groups.

DISCUSSION

In the present study, the four basic tastes experienced temporary and insignificant impairments during treatment, which concurred with the results reported by Tomita and Osaki (5). Radiation-induced taste loss increased rapidly between a period of radiation dose at 10–40 Gy and reached maximum approximately 4–5 weeks later (40 Gy) (12). During a course of curative RT of 60–70 Gy in 6 to 8 weeks, taste function was observed to become measurably impaired by the second week of treatment (11, 26, 27). Continued RT exacerbated this complication to an extent that, by the end of therapy, the ability to taste even a concentrated taste solution was impaired (11). Unless the anterior part of the tongue was irradiated, even though the base of the tongue was included in the high-dose region, acute taste loss was not observed during and after RT. When the anterior part of the tongue was irradiated, even low radiation doses such as ≤ 20 Gy resulted in temporal taste loss. Acute taste loss pattern was independent of radiation dose (Fig. 5). Consequently, in order not to incur radiation-induced acute taste dysfunction, efforts were made to com-

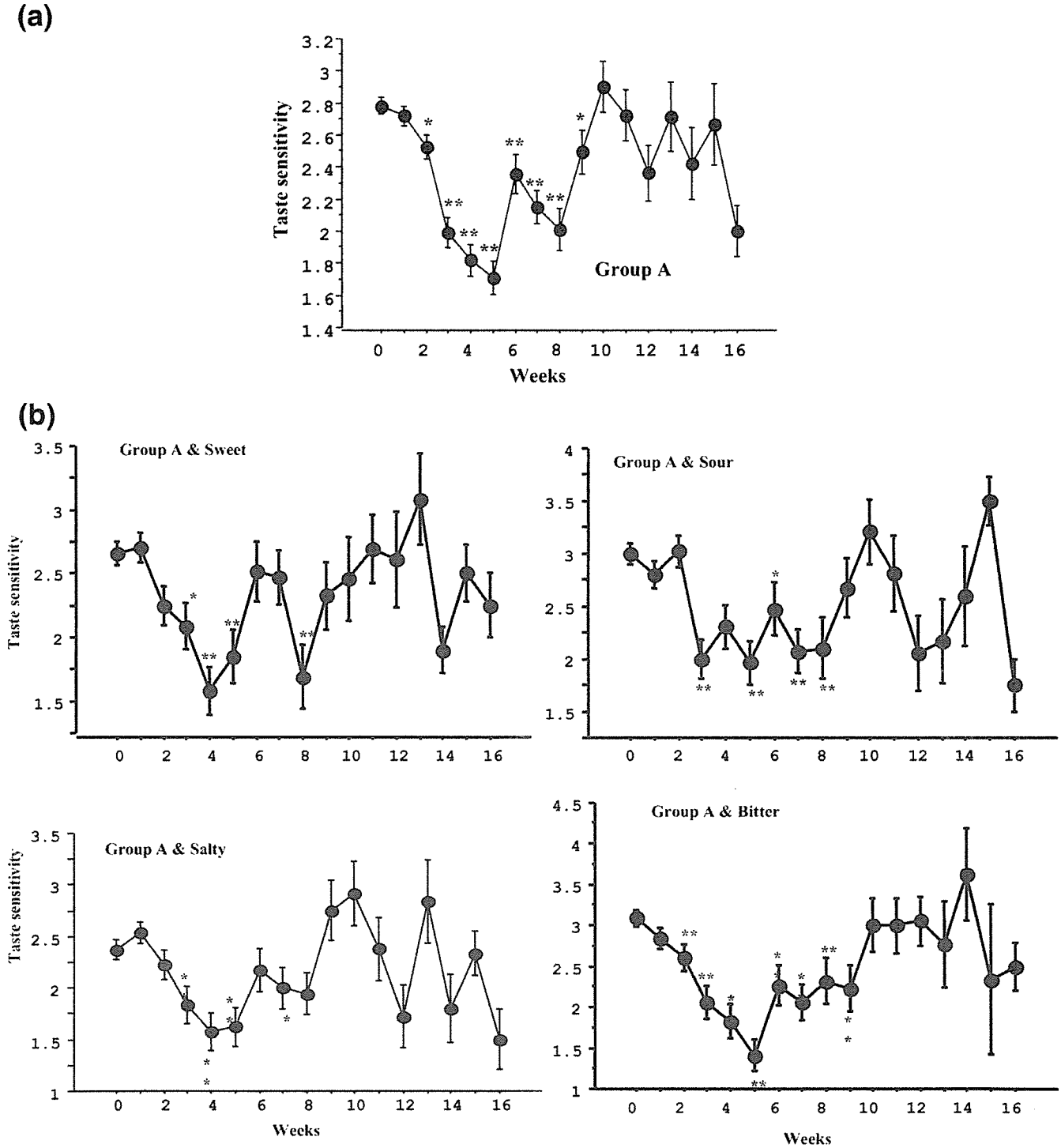

Fig. 3. In acute and subacute phase, recognition thresholds of the four tastes at different irradiation dose intervals in Group A, shown by mean ± SD of the concentration numbers (Wilcoxon signed-rank test comparing with 0 weeks, $*p < 0.01$; $**p < 0.05$). (a) All four tastes, and (b) each taste.

pletely remove at least the anterior part of the tongue from the radiation field and to avoid reduction of fraction dose to the tongue by using multidirection beams. Fifteen of 18 patients in Group B were lymphomas. This meant that they almost uniformly received a lower dose than Group A, and could well have received a very different chemotherapy. These may be confounding factors, given the definition of Groups A and B by volume of tongue irradiated. However, in this study, the contribution of chemotherapy to taste loss seems to be very low with respect to the time course of taste loss.

The role of salivary flow in affecting taste sensitivity is still open to debate (3). Salivary glands were damaged

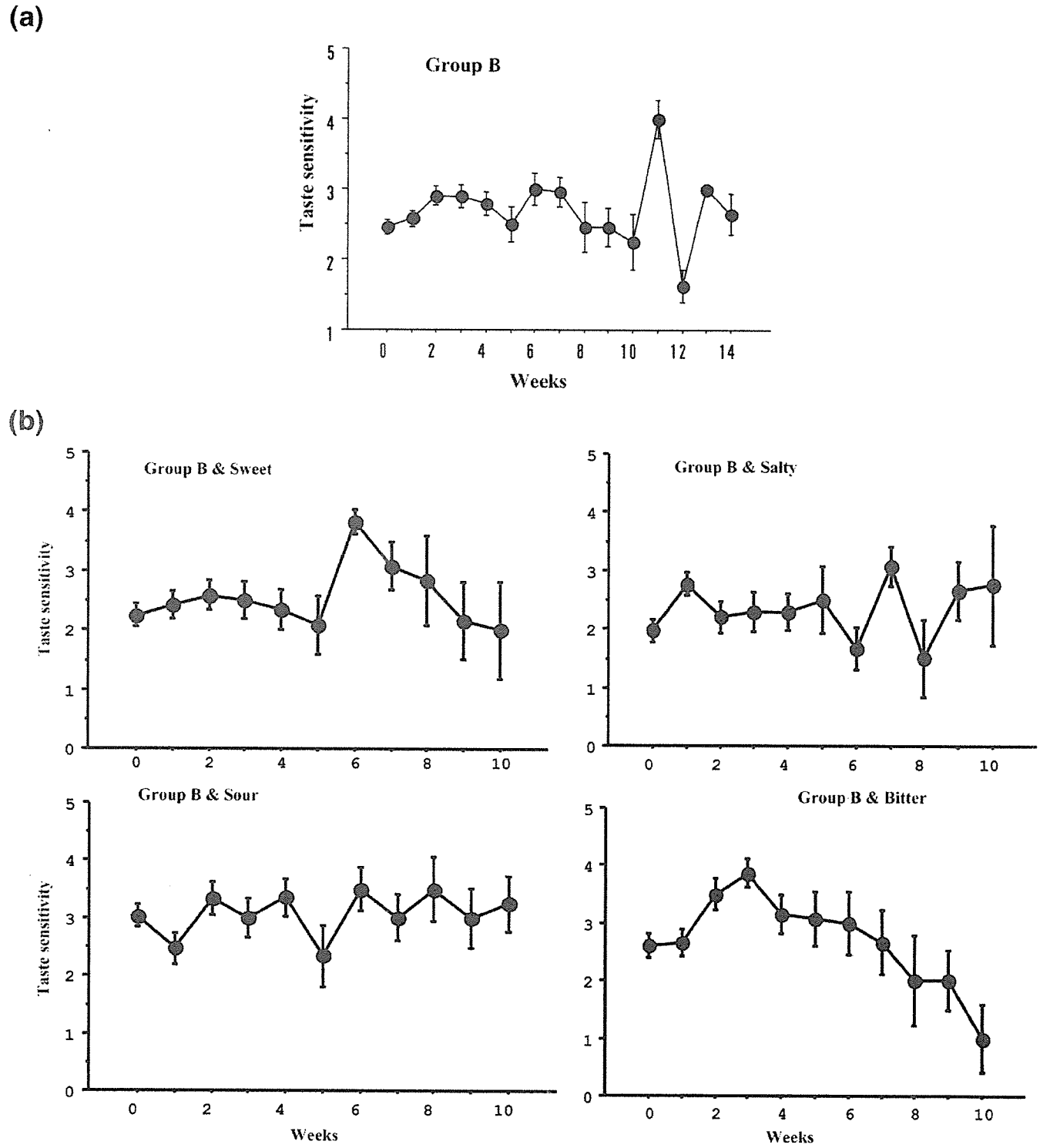

Fig. 4. In acute and subacute phase, recognition thresholds of the four tastes at different irradiation dose intervals in Group B, shown by mean ± SD of the concentration numbers. (a) All four tastes, and (b) each taste.

permanently by the radiation, whereas taste sensitivity only suffered a temporary impairment (5). Salivary secretions reached a minimum 6 months after irradiation, but taste sensitivity did not show further deterioration after RT (28). The relationship between salivary flow and taste disturbance was recently reviewed by Inokuchi (29), who concluded that salivary dysfunction was not likely to be the major factor in affecting taste disturbance. According to a previous study (30), the impairment curves of salivary secretion and taste sensitivity were not comparable both during and after head-and-neck irradiation, which strongly suggested a negative relationship between salivary flow and taste disturbance.

Some reports suggest that taste sensitivity was decreased

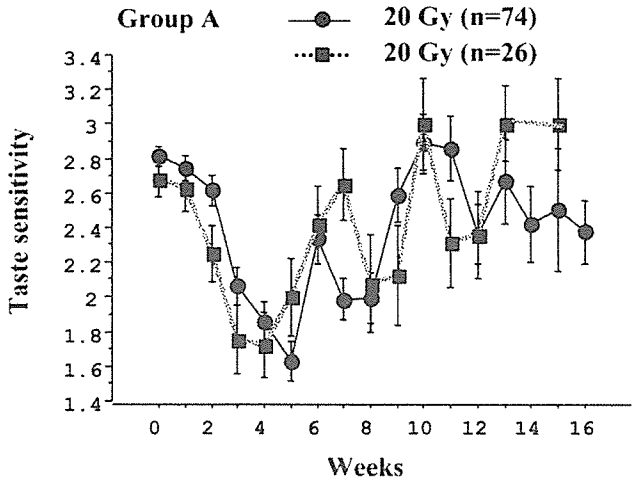

Fig. 5. Changes in taste sensitivity between two groups: those >20 Gy and those ≤20 Gy in the anterior part of the tongue.

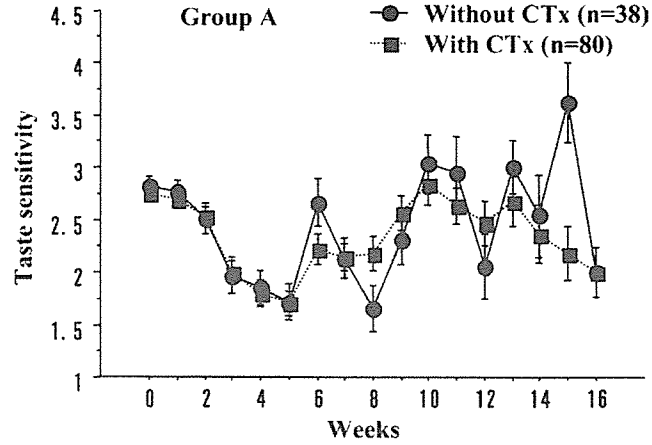

Fig. 7. Changes in taste sensitivity between two groups: those with and those without chemotherapy (CTx).

in cancer patients receiving chemotherapy (31–34). The drugs affecting taste sensitivity included cisplatin, carboplatin, cyclophosphamide, doxorubicin, fluorouracil, and methotrexate (35). In our study, as shown in Fig. 6, cancer drugs did not affect taste sensitivity more than irradiation to the tongue.

Results comparable to ours were reported by Fernando *et al.* (7), who showed that taste loss was significantly associated with the proportion of tongue contained within the radiation treatment field. They suggested that the volume of tongue within the radiation treatment field should be kept to a minimum, with the proviso that this did not risk compromising adequate local control. These data are in agreement with that of Conger and Wells (36, 37), who suggested that taste loss related more to area of tongue irradiated than of mouth dryness. These reports have indicated that the mechanisms involved are either via a direct antiproliferative effect on the taste cells or on their microvilli.

The mechanisms by which long-term changes in taste occur are not well understood. Results of studies of the time and extent of recovery of normal taste function in humans following RT are contradictory. Several investigators (13, 26, 36) have observed complete recovery of taste function in patients 1–3 months after treatment. Schwartz *et al.* (15) investigated the upper limit of taste detection thresholds from 6 months to 19 years (median, 2.4 years) after completing RT (total radiation dose range, 36–72 Gy; median, 60 Gy) in 15 patients with head-and-neck cancer. They concluded that nearly normal suprathreshold taste intensity perception was observed. This is consistent with our results, though our study investigated the late recognition threshold of the four basic tastes at 4–24 months after the start of RT. This suggests that radiation-induced taste dysfunction is only a temporal effect. In contrast, others have not observed complete recovery of taste function in patients (11, 27). Mossman *et al.* (6) indicated that impairment of taste function may persist in some patients up to 7 years after RT and

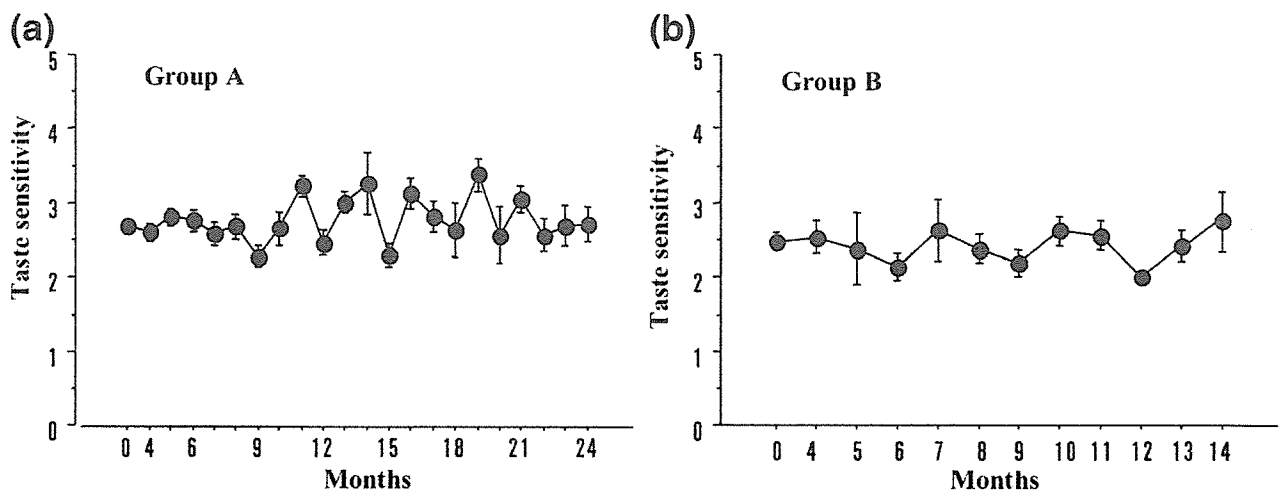

Fig. 6. In late phase, recognition thresholds of the four tastes at different irradiation dose intervals in both groups, shown by mean ± SD of the concentration numbers. (a) Group A and (b) Group B.

concluded that curative intent of RT for tumors of the head and neck may result in long-term changes in taste function.

CONCLUSIONS

Unless the anterior part of the tongue was irradiated, even though the base of the tongue was included in the high-dose region, acute taste loss was not observed during RT. When the anterior part of the tongue was irradiated, the difference in radiation dose did not affect the taste loss pattern. Whether or not acute taste loss occurred, taste loss was not observed in the late phase. This study provides evidence for near normal suprathreshold taste intensity perception in patients who have received head-and-neck irradiation in the late phase. Radiation-induced taste dysfunction appears to be a temporal effect.

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Stereotactic Radiosurgery Plus Whole-Brain Radiation Therapy vs Stereotactic Radiosurgery Alone for Treatment of Brain Metastases

A Randomized Controlled Trial

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Context In patients with brain metastases, it is unclear whether adding up-front whole-brain radiation therapy (WBRT) to stereotactic radiosurgery (SRS) has beneficial effects on mortality or neurologic function compared with SRS alone.

Objective To determine if WBRT combined with SRS results in improvements in survival, brain tumor control, functional preservation rate, and frequency of neurologic death.

Design, Setting, and Patients Randomized controlled trial of 132 patients with 1 to 4 brain metastases, each less than 3 cm in diameter, enrolled at 11 hospitals in Japan between October 1999 and December 2003.

Interventions Patients were randomly assigned to receive WBRT plus SRS (65 patients) or SRS alone (67 patients).

Main Outcome Measures The primary end point was overall survival; secondary end points were brain tumor recurrence, salvage brain treatment, functional preservation, toxic effects of radiation, and cause of death.

Results The median survival time and the 1-year actuarial survival rate were 7.5 months and 38.5% (95% confidence interval, 26.7%-50.3%) in the WBRT + SRS group and 8.0 months and 28.4% (95% confidence interval, 17.6%-39.2%) for SRS alone ($P=.42$). The 12-month brain tumor recurrence rate was 46.8% in the WBRT + SRS group and 76.4% for SRS alone group ($P<.001$). Salvage brain treatment was less frequently required in the WBRT + SRS group (n = 10) than with SRS alone (n = 29) ($P<.001$). Death was attributed to neurologic causes in 22.8% of patients in the WBRT + SRS group and in 19.3% of those treated with SRS alone ($P=.64$). There were no significant differences in systemic and neurologic functional preservation and toxic effects of radiation.

Conclusions Compared with SRS alone, the use of WBRT plus SRS did not improve survival for patients with 1 to 4 brain metastases, but intracranial relapse occurred considerably more frequently in those who did not receive WBRT. Consequently, salvage treatment is frequently required when up-front WBRT is not used.

Trial Registration umin.ac.jp/ctr Identifier: C000000412

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BRAIN METASTASES OCCUR IN 20% to 40% of all patients with cancer and are generally associated with a poor prognosis.[1,2] The most common route of metastatic dissemination resulting in brain metastases is hematogenous, and it is therefore presumed that the entire brain is "seeded" with micrometastatic disease, even when only a single intracranial lesion is detected. Consequently, whole-brain radiation therapy (WBRT) has been a mainstay of treatment.[1,2]

Recently, the assumption that the entire brain is seeded with micrometastases in all patients with overt brain metastases has been questioned, prompting

For editorial comment see p 2535.

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