The Significance of Lipid Resonances in Proton MR Spectroscopy of Brain Tumors

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Abstract
We studied the significance of lipids (Lip) to confirm the effectiveness of proton magnetic resonance spectroscopy (1H-MRS) for brain tumors. Among the cases diagnosed as a brain tumor between April 2003 and June 2004 at our hospital, 24 cases underwent preoperative 1H-MRS. They were all primary cases and confirmed by pathological diagnoses and required surgical extirpation. The obtained specimens underwent 1H-MRS, were analyzed and prepared as frozen sections. All the sections were compared and examined with H&E staining, Ki-67 labeling index (MIB-1 index), and Sudan-II staining. There were 14 out of 24 (58.3%) Lip positive cases in 1H-MRS, and the positive cell rate of Sudan-II staining and MIB-1 indexes were higher in the cases with increased Lip, so it seemed that they indicated a rapid tumor growth rate. In contrast, there were some cases with a high MIB-1 index, no increase in Lip, and a low positive cell rate as seen with Sudan-II staining, so it seemed that they indicated that the tumor growth was detected at a relatively early stage.

With 1H-MRS, although there are problems such as a change of pattern due to settings and precision, it is a useful and valid test to determine the diagnosis and evaluate the grade of malignancy from a metabolic state. Furthermore, in this study, the developmental stage and growth rate of a tumor can be determined by knowing its biological behavior. This study may also be of help in predicting its prognosis and determining its therapeutic effects.

Key Words
proton magnetic resonance spectroscopy, lipid droplets, necrosis, biological behavior, brain tumor

Introduction
Proton-magnetic resonance spectroscopy (1H-MRS) uses the principal of MR and allows surgeons to non-invasively determine the body’s metabolic state and is currently employed for various disorders. In neurosurgery, it is used as a supplemental diagnostic tool, the effectiveness and importance of which have been reported with the development of recent imaging techniques. For brain tumors, among other applications, every facility uses it in clinical applications because it allows the preoperative determination of the kind of tumor and whether it is benign or malignant.

In 1H-MRS for brain tumors, the relative signals of choline (Cho) related to the production and disintegration of cell membranes, creatine (Cr) that is an intermediary product of energy metabolism, N-acetyl-aspartate (NAA) that is specifically contained in neurons and neural processes, lactate (Lac) that is a product of anaerobic glycolysis, and lipids (Lip) that are implicated in most pathological changes. Protons and these factors are measured and their patterns enable a correct diagnosis.
Though there are many reports on $^1$H-MRS related to the histological diagnoses of tumors\(^6\)\(^7\), there are few reports on the evaluation of grade of malignancy. And most of those reports are related to the Ki-67 labeling index that indicates the grade of histological malignancy and the change of Cho, Cr, and NAA, and those related to Lip are only found occasionally\(^8\)\(^-\)\(^11\).

Based on the examination of Lip obtained from $^1$H-MRS of brain tumors operated on at our hospital, of the 55 cases, the tumors confirmed by pathological diagnoses with more histological malignancy tended to show Lip frequently\(^12\). In general, there is not so much lipid in brain tissue as to appear in signals on $^1$H-MRS and its appearance reflects necrosis, and a tumor with more histological malignancy shows signals easily.

The studies on the appearance of lipid in brain tumors are usually carried out with rats\(^13\)\(^-\)\(^15\), and there are only a few surgical studies on humans. In this study, we will evaluate the correlation between the implication of the appearance of Lip in $^1$H-MRS and the grade of biological malignancy and examine its effectiveness.

**Methods**

**Subjects**

We studied 24 patients diagnosed as having a brain tumor from April 2003 to June 2004 who underwent preoperative $^1$H-MRS. They were primary cases and confirmed by histopathological diagnosis and judged to require surgical extirpation, including stereotacitic biopsy. However, the cases with a history of irradiation and the cases undergoing chemotherapy were excluded. Their ages ranged from 2 to 84 years (average age $\pm$ standard deviation: 59.8 $\pm$ 18.4), including 14 male cases and 10 female cases. Regarding the specimens used in this study, we obtained informed consent for the use of extirpated specimens from the patients or their family. This study was approved by the ethics committee of St. Marianna University Hospital.

**Imaging conditions**

In MR imaging, Gyroscan NT Intera/Master 1.5 T (Philips Medical Systems) was used, and the MRI was used for routine preoperative T1WI, T2 WI, FLAIR, and Gd-EDTA. The imaging condition of $^1$H-MRS was as follows: the PRESS (point resolved spectroscopy) method, TR/TE = 2000 ms/136 ms, 3 Hz Gauss function, -1.5 Hz exponential function. A part of the tumor lesion which showed an enhancing effect by Gd-EDTA was measured with a single voxel of 1 to 8 cm\(^3\). If the imaged part is smaller than the voxel, we tried not to include the surrounding normal brain as much as possible and aimed at inside a solid or cystic lesion. Signals were selected as: 3.2 ppm for Cho, 3.0 ppm for Cr, 2.0 ppm for NAA, 1.3 ppm downwards for Lac, 1.3 ppm upwards and 0.9 ppm for Lip (Fig. 1). The peak values of each signal were measured, and the two peak values of Lac and Lip were totaled. The Cho/Cr ratio was also calculated. Cho, Cr, NAA, and the Cho/Cr ratio were determined as increasing or decreasing by the comparison of the value of the opposite side. Lac and lip were determined as positive if a signal was confirmed.

**Pathological examination**

A specimen was extracted from the area of the voxel setting point as close as possible. The specimen was placed in an OTC compound and was frozen and sliced with a cryotome at $-20^\circ$C at a thickness of 10 $\mu$m. Each section was stained with hematoxylin and eosin (H&E), Ki-67 immunostaining, and Sudan-II.

**H&E staining:** For diagnosis and confirmation of necrosis and its localization.

**Ki-67 immunostaining (MIB-1 index):** Anti-human GFA mouse monoclonal antibody (DAKO Corp.) was used as a primary antibody, and Ki-67 immunostaining was performed with the LSAB2 (Labeled Streptavidin Biotin 2) method. The positive cell rate of each section, for more than 1,000 cells, was measured to calculate the MIB-1 index.

**Sudan-II staining:** A tumor cell which stained with red oil stain was regarded as a lipid droplet, and the tumor cells with such droplets were considered as positive. The positive cell rate of each section for more than 1,000 cells was then calculated for the MIB-1 index. Necrotic tissue was excluded from this measurement.

**Statistical tests**

Data were classified according to pathological diagnosis and analyzed. StatView 5.0 (SAS Institute) was used for data analysis, and the Student’s t-test was used to determine the significant differences among each group in which $p<0.05$ was considered significantly different. For the comparison of 2 variables, the Pearson’s correlation coefficient was used.

**Results**

The Lip positive cases in $^1$H-MRS were 14 cases
Classified by the pathological diagnosis, Lip positive cases were as follows: 7 cases of malignant brain tumor (WHO classification grade III, grade IV), 2 cases of malignant lymphoma, 3 cases of metastatic brain tumor, 2 cases of meningioma. However, there were no Lip positive cases for pituitary adenoma and acoustic neurinoma (Table 1).

The higher the histological malignancy, a higher percentage of lipid droplets stained with Sudan-II appeared in the cells (Fig. 2). Necrotic areas were stained with Sudan-II as well as in tumor cells. There were many positive cells adjacent to the necrotic areas (Fig. 3).

Correlations were confirmed both between Lip and the positive cell rate of Sudan-II staining, and the positive cell rate of Sudan-II staining and the MIB-1 index (r=0.49, r=0.59) (Figs. 4, 5). Regardless of the necrosis in H&E stained sections and high positive cell rate of Sudan-II staining, there were 5 cases (3 malignant glioma cases, 1 metastatic brain tumor case, and 1 meningioma case) with no increase in Lip. The pathological diagnoses were as follows.

1. Glioblastoma multiforme (WHO classification, grade IV)
   Three cases out of 6 were Lip positive (50%). The 3 cases showed a significantly higher MIB-1 index compared to the other 3 cases (p < 0.05) (Fig. 6).

2. Ganglioneuroblastoma (WHO classification, grade IV)
   Lip positive and both the positive cell rate of Sudan-II staining and the MIB-1 index were high.

3. Anaplastic astrocytoma (WHO classification, grade III)
   Three cases out of 4 were Lip positive (75%). One case (No. 20) with no Lip, showed a ring-like enhancement on the MRI, the Cho/ Cr ratio was high as was the MIB-1 index, but the positive cell rate of Sudan-II staining was low.

4. Metastatic brain tumor
   Three cases out of 4 were Lip positive (75%). One case (No. 1) with no Lip was a solid tumor on MRI and showed necrosis in H&E stained sections.

5. Malignant lymphoma (WHO classification, grade IV)
   Both cases were Lip positive. There was no necrosis. Both the positive cell rate of Sudan-II staining and the MIB-1 index were high.

6. Meningioma
   Two cases were Lip positive out of 5 (40%).
There was no correlation between Sudan-II staining and the MIB-1 index. Both cases with Lip positive (Nos. 12 and 18) showed low values in Sudan-II staining. In contrast, there was one case (No. 4) that showed no Lip but necrosis in H&E staining.

7. Pituitary adenoma and acoustic neurinoma
Two cases showed no Lip and increased only in Cho.

Discussion

There are many reports on 1H-MRS as pathological diagnosis of brain tumors. There are only a few reports on the evaluation of grading of histological malignancy, and most of them have compared the MIB-1 index and the increase in Cho, the decrease in Cr, the increase of the Cho/Cr ratio, the increase of the Cho/NAA ratio, or the appearance of Lac. Reports on the signal of Lip are only occasionally found.[6, 7, 16] Lipid droplets are lipids absorbed into a cell, which indicates the previous step of necrosis, i.e., the change of metabolism due to inadequate blood flow. Therefore, when lipid droplets are found in tumor cells, they are no doubt malignant cells with a high biological activity and a strong proliferation ability[6]. In the present study, the higher the histological malignancy, the darker the lipid droplets in tumor cell substrate were stained with Sudan-II.

In general, necrosis often occurs in a solid tumor, and tumor vessels are regularly arranged around the necrotic area both in vitro and in vivo[17]. Moreover, some tumors have vessels at their center and form cylindrical cord-like structures consisting of live cells surrounded by necrotic tissue, or some form a tumor nodule consisting of a central necrotic area and a peripheral network of vessels. Necrosis will occur when the nutrients leak from the tumor vessels and decrease substantially or will occur when the toxic cell degradation products increase[18]. Blood stagnation in capillary vessels due to infarction in a tumor may also cause necrosis of a tumor.
The proliferative rate of cells close to a vessel, which are well nourished, is higher than those of tumor cells close to a necrotic area. Some tumors do not become necrotic but instead form a watery cyst.

$^1$H-MRS is a means by which the growth stage and rate of tumors, along with the biological grades of the tumors can be precisely determined.

From the pathological examination at the early stage of tumor growth, it can be said that there is an increase of Cho, and a decrease of Cr and NAA.

With further growth of the tumor, a low nutrient and hypoxic state occurs and Lac appears. Moreover, with growth of the tumor, Lip from lipid droplets, micronecrosis, and necrosis appears (Fig. 7 a–e). When the setting of voxel is completely focused on a necrotic portion, Cho, Cr, and NAA will decrease or be eliminated, and the signals will consist of only Lac and Lip, and the findings of a cyst and/or infarction will become similar (Fig. 7 c–e).

As if supporting the relation between the bio-
logical grade of malignancy and the appearance of Lip, when the cases were surveyed according to the groups classified by histological diagnosis, more than half of the cases of malignant glioma showed Lip. This concept stemmed from the fact that the glioblastoma cases with Lip showed a high positive cell rate of Sudan-II staining and MIB-1 index. It is indicated that the biological activity and the proliferating capacity of tumors are high at those areas, vascular proliferation lags behind, and many cells utilize lipid droplets as a source of energy by absorbing them.

Ganglioneuroblastoma showed a similar trend. Contrarily, there was a case (No. 20) of an anaplastic astrocytoma that showed a high Cho/Cr ratio, about three times as high as the normal value, and was suspected of having a high tumor activity from an MIB-1 index of 16.3%, but did not show Lip and only a low positive cell rate of Sudan-II staining of 4.9%. This indicated that the early stage of tumor growth when the proliferating capacity of the tumor was high, but it was not with an inadequate blood supply.

In metastatic brain tumor and malignant lymphoma, Lip was observed in a higher incidence. These are malignant tumors with high activity as indicated by the MIB-1 index. Commonly, metastatic brain tumors growth rapidly and cause central necrosis earlier, and lipid droplets also appear in malignant lymphomas. However, one case without Lip was encountered in a metastatic brain tumor. Case No. 1 was a case in which a tumor was detected at a relatively early stage, because pathological necrosis was observed in spite of a solid tumor shown on the MRI. This was probably due to the voxel setting problem of the $^1$H-MRS and a problem of accuracy. It was detected by the NAA because it is in the normal brain tissue.

There was no correlation between Sudan-II staining and the MIB-1 index for a meningioma. But there were 2 cases with no necrosis and no positive Sudan-II staining despite the appearance of Lip. There are the possibility that there were lipid producing cells and benign microcyst which were degenerated in this tumors. Moreover it remains difficult to identify several subtypes of meningioma with $^1$H-MRS. Conversely, in cases with necrosis

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**Fig. 4.** The relation between lipid resonances (Lip) in $^1$H-MRS and Sudan-II stain positive cells rate (%) is shown. The correlation coefficient is 0.49.

**Fig. 5.** The correlation between MIB-1 index (MIB-1) and Sudan-II positive cells rate (Sudan-II) is shown. The correlation coefficient is 0.59.
Fig. 6. The comparison between Lip resonances and MIB-1 index in Glioblastoma is shown.

Fig. 7. The relation between process of tumor growth and $^1$H-MRS.

The early stage of tumor growth, increase of Cho, decrease of Cr and NAA can be observed (a). When hypoxia is occurred, appearance of Lac can be observed (b). Growth proceeded further, appearance of Lip can be observed (c). Only Lac and Lip can be observed in necrotic parts, cystic parts and infarction parts (c, d, e).
without the appearance of Lip, a characteristic pattern showing only an increase in Cho was observed. There were no cases with Lip appearance in pituitary adenoma and acoustic neurinoma. They are benign tumors according to the WHO classification, and their MIB-1 index are also low, therefore the result was as expected.

In this study, there were 5 cases (3 malignant gliomas, 1 metastatic brain tumor, and 1 meningioma) none of which showed Lip, despite the necrosis in H&E staining or a high positive cell-rate as observed with Sudan-II staining. The reason for this was that this study placed emphasis on the diagnosis, so the tests were carried out with the normal setting of TE = 136 ms. A means to enhance the accuracy of Lip detection is to shorten TE to 30 ms\(^{23,24}\). If this measure was adopted, identification might have been possible.

In \(^1\)H-MRS, though there are problems such as a change of pattern, due to settings and precision, it is a useful test for differential diagnosis and evaluation of grading malignancy from the state of metabolism. Furthermore, the developmental stage and the growth rate of a tumor can be determined by its biological behavior.

**Conclusion**

\(^1\)H-MRS, though there are problems such as change of pattern due to settings and precision, is a useful test for differential diagnosis and evaluation of grading malignancy from the state of metabolism. Furthermore, in this study, the developmental stage and the growth rate of a tumor can be determined by its biological behavior. The potential effectiveness for the prognoses and determination of therapeutic effects were indicated, proving that Lip resonances in \(^1\)H-MRS are important in brain tumors.

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**References**


抄録
脳腫瘍における proton magnetic resonance spectroscopy (1H-MRS) の有用性を確認すべく、脂肪 (Lip) の出現について検討した。対象は、2003 年 4 月から 2004 年 6 月までに当院にて脳腫瘍と診断された症例のうち、初発例で、術前に 1H-MRS 検査が施行され、手術（定位的生検を含む）にて組織診断、摘出が必要とされ病理確定診断に至った 24 症例である。方法は、1H-MRS を解析、採取した手術検体は H-E 染色、Ki-67 免疫染色（MIB-1 index）、Sudan-・染色を施行し比較、検討した。結果は、1H-MRS にて Lip の出現していた例は全 24 例中 14 例（58.3％）であり、Lip の出現している例では Sudan-・染色陽性細胞率、MIB-1 index が高く、腫瘍の成長速度が早いることを示唆していた。逆に MIB-1 index が高く、Lip の出現はなく Sudan-・染色陽性細胞率が低いものも存在し、腫瘍の成長の比較的早い段階をとらえていることを示唆していた。1H-MRS では設定によるパターンの変化や精度の問題等があるが、腫瘍の代謝の状態から疾患の鑑別、悪性度の評価ができる検査として有用である。さらに本研究において生物学的活性を捉えることにより、腫瘍の発育段階や成長の速度を知ることができ、予後の予測、治療効果判定に有用であることが示唆された。