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Study of Glycolipid Profiles of Low Grade Astrocytomas

Kohshi TATEWAKI, Toshiaki YAMAKI,
Yukihiro IBAYASHI and Kazuo HASHI

Department of Neurosurgery, Sapporo Medical University School of Medicine, S-1, W-16, Sapporo 060, Japan

ABSTRACT

We analyzed the glycolipid composition of eight cases with low grade astrocytomas. In all of the cases the composition of neutral glycolipids was notably different from the normal brain. The composition of acidic glycolipids was almost the same as normal. An increase of minor gangliosides as well as some that are usually undetectable in the normal brain was recognized in acidic glycolipids. We classified those glycolipid alterations into three types: a) an increase in short chain gangliosides; b) novel expression of lacto series gangliosides; and c) both a) and b). Glycolipids did not correlate with microscopic findings, proliferation (Ki-index), or prognosis.

key words: Low grade astrocytoma, Glycolipid, Ganglioside

INTRODUCTION

It is usually impossible to predict the prognosis of a glioma on the basis of its histology. A biochemical or a molecular biological index which objectively shows biological characteristics of gliomas is needed. We have been examining glycolipids, which are most abundant in the central nervous system, with particular focus on the compositions of glycolipids of gliomas. In this report, we present our findings on the glycolipid profile of low grade astrocytomas.

MATERIALS AND METHODS

1) Surgical Specimens

We analyzed specimens from eight cases (age 28-77, Table 1) which had been obtained at Sapporo Medical University hospital and its affiliated hospitals. They (0.28-1.73 g) had been preserved in a freezer at −80℃.

Table 1 *Summary of Cases with Low Grade Astrocytomas*

Case No.	age (y)	sex	site	therapy	Ki (%)	survival*
①	56	F	rt. parietal	R	3.6	1y3m
②	72	M	lt. frontal	R	<1.0	7m
③	77	M	lt. temporal	R	<1.0	3m
④	46	F	rt. frontal	—	3.0	>4m
⑤	44	F	rt. temporal	R+C	1.4	>3y7m
⑥	61	F	lt. frontal	R+C	3.2	4m*
⑦	28	F	lt. frontal	R	<1.0	1y10m*
⑧	57	M	lt. frontal	R+C	1.4	>2y10m

* after onset of symptoms * multiple organ failure
R: radiation, C: chemotherapy, Ki: Ki-index

2) Extraction of Glycolipids

The total lipid was extracted with chloroform/methanol/water (CMW)=4:8:3 (v/v/v). The whole extract was applied to an ion-exchange column (bed volume 13 m*l*; Sephadex A-25, acetate form, Pharmacia, Sweden) for separation into neutral and acidic fractions. The acidic fraction was desalted with a gel filtration column (bed volume 25-30 m*l*; Sephadex LH-20, Pharmacia, Sweden)

3) Thin Layer Chromatography

Both the neutral and acidic fractions of each sample were chromatographed on a high performance TLC plate (Merck, Silica Gel, Germany). The neutral fraction was also developed on a borate-impregnated TLC and CMH was further separated into glucosylceramide (GlcCer) and galactosylceramide (GalCer).

4) Densitometric Analysis

Chromatograms were examined with a densitometer (Shimadzu dual-wavelength TLC scanner, CS91) with a wavelength of 435 nm for the sample and 800 nm for the reference. The percent distribution of each band was calculated by integration.

5) TLC Immunostaining

The acidic fractions were two dimensionally chromatographed on a high performance TLC plate (Merck, aluminum Silica Gel 60, Germany). The TLC plates were reacted with monoclonal antibodies and visualized with a Konica immunostain kit. The antibodies used were Choleragenoid (anti GM1), M2590 (anti GM3), Mab126 (anti GD2), R24 (anti GD3), 3G5 (anti 9-O-acetyl GD3), and DUPAN2 (anti LM1).

RESULTS

1) Thin Layer Chromatography

The neutral fractions were composed of CMH, CDH, CTH, and globoside (or paragloboside). They were different from those of the normal brain, which is almost exclusively composed of CMH (Fig. 1). On borate-impregnated TLC, only GalCer is present in the normal brain, while GalCer and a small amount of GlcCer were recognized in these cases (Fig. 2).

In all cases but No. 2, GM1, GD1a, GD1b, and GT1b, which are the main gangliosides in the normal brain, were dominant components of the acidic fraction. Some components which are present in trace amounts (e. g. GM2, GM3, GD2, and GD3) or are not detectable in the normal brain increased or appeared (Fig. 3).

2) Densitometric Analysis

In the neutral fractions of all specimens, the percentage of CMH (especially GalCer) was the highest (49.8-82.3%) except case 6 (27.4%). At the same time, other components such as CDH, CTH, and globoside were recognized in shares of 6.2-29.6%, 6.6-36.8%, 3.2-12.5%, respectively (Table 2).

In the acidic fractions, we found changes in the minor ganglioside compo-

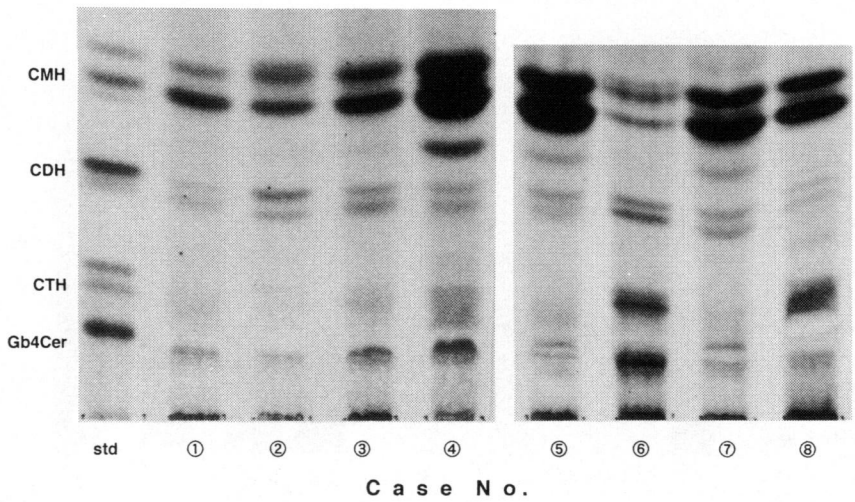

Fig. 1 Thin layer chromatogram (TLC) of neutral glycolipids. The bands were visualized with orcinol-H_2SO_4 reagent.

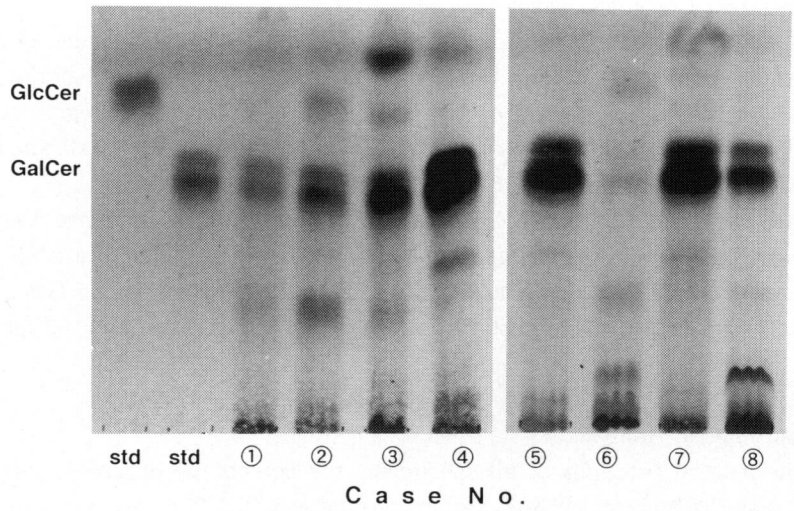

Fig. 2 Borate-impregnated TLC of neutral glycolipids. The bands were visualized with orcinol-H_2SO_4 reagent

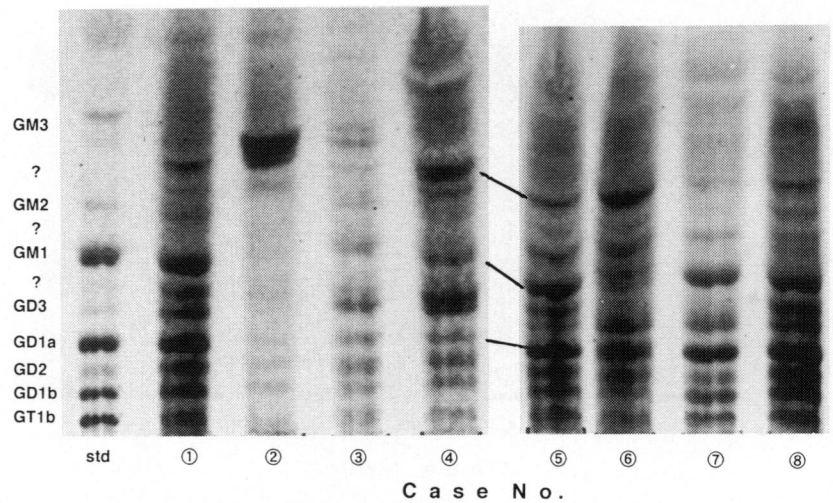

Fig. 3 TLC of acidic glycolipids. The bands were visualized with resorcinol-HCl reagent

Case No.	①	②	③	④	⑤	⑥	⑦	⑧
CMH	49.8	56.6	60.2	82.3	70.3	27.4	67.6	53.3
GalCer	100	66	75	100	92	61	100	95
GlcCer	0	34	25	0	8	39	0	5
CDH	22.2	29.6	21.4	6.2	10.6	19.9	18.2	9.3
CTH	15.5	7.3	10.5	8.2	6.6	36.8	6.6	26.4
Gb4Cer	12.5	6.5	7.9	3.2	12.4	15.9	7.6	11.0

Table 2 *Percent distribution of neutral glycolipids of eight astrocytomas. CMH : ceramidemonohexoside, GalCer : galactosylceramide, GlcCer : glucosylceramide, CDH : ceramidedihexoside, CTH : ceramidetrihexoside, Gb4Cer : globoside (including paragloboside)*

nents. Among the ganglio series gangliosides, GM2, GM3, GD2, and GD3, which have short sugar chains, were high in cases 3, 4, 6. Novel components (supposed to be lacto series gangliosides) emerged in cases 1 and 8. In cases 5 and 7, both series of gangliosides were mixed (Table 3).

Case No.	GM	①	②	③	④	⑤	⑥	⑦	⑧
GM3	2.7	7.1	38.5	12.1	12.7	6.7	18.3	5.1	5.5
?		5.2	8.8	4.7	0.0	0.0	3.7	3.8	4.6
GM2	4.1	7.0	7.0	9.2	8.2	8.4	10.0	6.7	4.1
?		4.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8
GM1	14.9	14.0	6.9	13.7	14.4	15.3	11.9	15.6	12.8
?		7.7	0.0	0.0	0.0	7.9	0.0	0.0	7.5
GD3	5.4	8.5	10.6	20.2	22.5	5.4	15.3	16.8	7.3
GD1a	21.7	15.4	6.6	10.1	8.4	14.5	12.4	17.3	14.4
GD2	8.0	9.2	6.0	11.5	11.3	12.4	9.7	8.3	12.0
GD1b	18.2	11.2	6.9	9.2	8.1	14.2	8.3	13.0	12.3
GT1b	16.3	10.6	8.6	9.3	8.9	15.1	10.3	13.4	15.9
SA1*		190.6	93.7	317.5	287.6	434.7	504.7	709.0	538.6
SA2**		4.9	4.9	19.4		35.5	39.6	153.5	68.3

* lipid-bound sialic acid content (μg)/g wet weight

** lipid-bound sialic acid content (μg)/mg protein

Table 3 *Percent distribution of acidic glycolipids of eight astrocytomas. GM (in the first row of the table) : gray matter of the normal brain. The left extreme column indicates each ganglioside component.*

Quantification of sialic acids in gangliosides using resorcinol-HCl method revealed that the quantity of sialic acid per wet weight or protein did not correlate with ganglioside profile. Only the decrease of gangliosides in case 2 was conspicuous (Table 3).

3) TLC Immunostaining

TLC immunostaining was carried out in cases 1, 2, 3 and 8 (Table 4). GM3, GD3, 9-O-acetyl GD3 were stained specifically in all cases. Reactivity to GM1, GD2 was not very specific and identification of those antigens is not yet complete. DUPAN2, which reacts against 3'-isoLM1 and 6'-LM1, which are reported as glioma associated antigens, did not stain them. We are now reconfirming the results.

4) Relevance of Glycolipid Profile to Clinical Data

The composition of glycolipids did not correlate with age, location of tumor, Ki-index, or prognosis (Table 1).

Table 4 *TLC immunostaining.*

Case No.	Ag. Ab.	GM1 cholera	GM3 M2590	GD2 Mab126	GD3 R24	$^{9-O}_{Ac}$ GD3 3G5	LM1 DUPAN2
①		○	○	△	○	○	×
②		×	○	○	○	○	×
③		△	○	×	○	○	×
⑧		○	○	△	○	○	×

○: specific △: broadly specific ×: negative Ag: antigen Ab: antibody.

DISCUSSION

We studied the glycolipid composition of eight cases with low grade astrocytomas.

The neutral glycolipids were composed of CMH, CDH, CTH, and globoside (or paragloboside), which is quite different from the composition of the normal brain. Only a few studies about neutral glycolipids of glioma have been published(1, 2, 4). Every article described the existence of globoside or paragloboside in glioma tissue, which was consistent with our results. This may be caused by activation of glycolipid biosynthetic pathways to the globo and lacto series. Singh *et al.*(1) recently reported that the percentage of CMH in the total of neutral glycolipids in anaplastic astrocytoma was high (73.5%) and that in low grade astrocytoma was 43.3%. They also reported that the percentage of GalCer

in CMH was higher than that of GlcCer, which did not account for more than 6%. Jennemann *et al.*(2), on the other hand, reported that GlcCer was higher than GalCer. Our results showed 27.4-82.3% (mean 58.4%) of CMH and 0-15% (mean 8.1%) of GlcCer, which rather supported the results of Singh's.

There are some possible interpretations for the high percentage of GalCer. First is contamination from the normal brain to the materials. We believe this should be minimal in the present materials because the specimens used were all described as the tumor center. Besides, the ratio of CMH in the neutral glycolipid suggested that the tumor comprised at least 50% of the tissue. Singh *et al.* described that GalCer was demonstrated within tumors immunohistochemically. They speculated that the change in biosynthetic pathway following aberrant gene expression with major genetic damage, and differentiation of tumor cells into oligodendrocyte (GalCer originates from myelin) were likely causes of the phenomenon.

As for the composition of acidic glycolipids, Seifert(3) first described the increase of GM3 in gangliosides in glioma. Subsequently, Eto(4), Yates(5), Traylor(6), and Jennemann(2) *et al.* have reported that the percentage of simple composite gangliosides such as GM2, GM3, GD2, GD3 is significantly increased as compared to the normal brain. Fredman *et al.*(7) described that a monosialolacto series ganglioside, 3'-isoLM1, could be a glioma marker detected specifically in astrocytoma grade 3 and 4. Recently Sung *et al.*(8) described that the appearance of 6'-LM1 and the quantity of the ganglioside 1b series were related to prognosis. We classified glycolipid alterations into three types: a) an increase in short chain gangliosides; b) novel expression of lacto series gangliosides; and c) both a) and b). In cases of type b), however, 3'-isoLM1, 6'-LM1 could not yet be recognized by immunostaining with antibody DUPAN2. Deactivation of glucosyltransferase or activation of breakdown enzyme of glucosyltransferase are thought to cause shortening of sugar chains.

We did not find any correlations among the composition of glycolipids, histology, proliferative ability (Ki-index), or prognosis.

We are in the midst of an investigation to identify some gangliosides recognized on TLC by immunological method and/or analyze their structures using FAB-MAS, NMR, etc. In addition, the glycolipid profile of high grade astrocytomas is also going to be studied to examine the relations between glycolipids and clinical manifestations.

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