Research Article

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A Humoral Recognition-Behavioral Stress-Coping Glycolipid Considered As another Biomarker of Psychotic Symptoms of Schizophrenia

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Abstract

Background: *Mammalians have the recognition-behavioral stress-coping system regulated via the neuronal modules followed by some humoral glycolipids. A sulfated Galbeta1-4GlcNAc-lipid which promotes the serotonergic module, keeps physical strength by regulating emotional behaviors. GalNAcalpha1-3GalNAc-lipid which promotes the adrenergic module, induces stress-coping behaviors. A sulfated Fucalpha1-2Gal-lipid protects the cholinergic module maintaining stress-coping memories from the ischemic stress. Sialalpha2-3Gal-lipid which promotes the dopaminergic module, integrates these recognition-behaviors. It is considered stresses are closely related to onset of schizophrenia, and the psychotic symptoms are not necessarily deleted after long-time medication. Schizophrenic patients might abnormally produce the humoral recognition-behavioral stress-coping glycolipids even under medication.*

Materials and Methods: *I examined the humoral stress-coping glycolipids of medicated schizophrenic patients and those of medicated manic patients without psychotic symptoms for comparison.*

Results: *The medicated manic patients increased sulfated Galbeta1-4GlcNAc-lipid production. The medicated schizophrenic patients increased sulfated Galbeta1-4GlcNAc-lipid production, and remarkably produced Sialalpha2-3Gal-lipid. These indicate the manic patients and the schizophrenic patients had a stress to be coped with the serotonergic module activity, and psychotic symptoms of the schizophrenic patients would be induced via stress-coping Sialalpha2-3Gal-lipid production.*

Conclusion: *The stressors are not clear, however, I understood humoral Sialalpha2-3Gal-lipid would be considered as another biomarker of psychotic symptoms of schizophrenia.*

Keywords: Mammalian Recognition-Behavioral Stress-Coping System, Humoral Glycolipids, Biomarker, Psychotic Symptoms, Schizophrenia

Introduction

Mammalian brains work via the functional neuronal modules-network system [1,2]. The recognition-behavioral stress coping modules are followed by some humoral glycolipids. A sulfated Galbeta1-4GlcNAc-lipid (sG1-4GN) which promotes the serotonergic module, keeps physical strength by regulating emotional behaviors [3-5]. GalNac1-3GalNAc-lipid (GN1-3GN) which promotes the adrenergic module, induces stress-coping behaviors [6,7]. A sulfated Fucalpha1-2Gal-lipid (sF1-2G) protects the cholinergic module keeping stress-coping memories from the ischemia-stress, as an adaptogen does [8-10]. Sialalpha2-3Gal-lipid (S2-3G) which promotes the dopaminergic module, integrates the recognition-behaviors, however, the excessive production induces abnormal adaptation-behaviors by depriving compatibility of the integration [11-13]. On the other hand, it is considered stresses are closely related to onset of schizophrenia, and the psychotic symptoms are not necessarily deleted after long-time medication [14]. I hypothesized schizophrenic patients would abnormally produce the humoral recognition-behavioral stress-coping glycolipids even under medication. In the present study, I examine humoral recognition-behavioral stress-coping glycolipids of medicated schizophrenic patients and those of medicated manic patients without psychotic symptoms for comparison.

Materials and Methods

Subjects and the Sera Collection

According to ICD-10, 3 psychiatrists identified schizophrenic patients (SZ; 3 women and 3 men, aged from 33 to 59, average 43 years-old) medicated with antipsychotics and manic patients without psychotic symptoms (MA; 2 women and 4 men, aged from 45 to 62, average 46 years-old) mediated with lithium carbonate, from the

inpatients and the outpatients of Department of Neuropsychiatry, Akita University Hospital. They were successfully medicated for 4-12 weeks. They did not suffer from physical diseases at the identification. These patients and healthy volunteers not-suffering from the psychoses (Control; 3 women and 3 men, aged from 28 to 62, average 38 years-old) agreed to participate in the present study, under the intensive informed consent with preservation of their anonymity and guarantee of the withdrawal agreement.

A 2 ml of venous blood was individually collected from their arm vein by a medical doctor, under watching of the other medical doctors. The sera were pooled and restored at 4ºC. All of these procedures were conditioned in accordance with Clinical Study Ethics Committee, Graduate School of Medicine, Akita University (the approval number: 2042).

Established Experimental Methods

I and the colleagues have investigated humoral glycolipids which follow the recognition-behavioral stress-coping modules, and I established methods examining sugar-chain-structure of humoral stress-coping glycolipids in the previous report [15]. Also in the present study, I utilized the experimental methods.

Humoral Lipid Fractionation

Humoral lipid fractionation was performed as next. A 1.25ml of chloroform and 2.5ml of methanol were added to each 1ml of the pooled serum. The solution was intensively mixed for 3min and incubated for 10min at room temperature (RT). Then, 1.25ml of chloroform was added to the solution, and followed by intensive mixing for 30s. A 1ml of water was added to the solution, and followed by intensive mixing for another 30s. The mixture was then centrifuged at 150gravities for 10min at RT. The lower chloroform layer was collected, and the solvent chloroform was evaporated at RT. The extracted lipids were then suspended in 1ml of water. The solution was applied to 0.5 ml of an ion exchanger DE-52 (Whatman Co., Maidstone, UK) column, which had been saturated with 10mM NaHCO3, pH8.3, and washed with water. Samples were eluted with 0.5ml consecutive washes of 50, 100, 150, 200, 250, and 300mM-NaCl. Fractions eluted with 50, 100, 150, and 250mM-NaCl were then diluted to 1ml with water as the present samples.

Sulfate-Radical Elimination

Stress-coping humoral glycolipids fractionated with 100 and 250mM-NaCl are sulfated. Sulfate-radical was eliminated from the glycolipids for detecting the terminal sugar-chain reactivity as next. Lipids were extracted again from 800µl of the sample solutions by using methanol-chloroform method as described above. The extracted lipids were added 400µl of the reagent containing silyl-agents of TMS-HT kit (Tokyo Chemical Industry Co. Tokyo, Japan), and then, incubated at 90°C for 3h. The solutions were added water to 800µl, and intensively mixed for 30s.

Measurement of the Glycolipid Production

A modified Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) was performed for measuring the glycolipids production as next. The sample solution obtained from the fraction eluted with 50 or 150mM-NaCl, the sulfate-radical-eliminated sample solution obtained from the fraction eluted with 100 or 250mM-NaCl, and physiological saline (PS) as Blank control, were prepared to 50% ethanol solution. 100µl of the solution was poured into a well of a 96-well plastic plate (Sumitomo-Bakelite Co., Tokyo, Japan). The ELISA was performed with the use of 300µl of 5% bovine serum albumin (Sigma-Aldrich Co., St. Louis, MO, USA) as a blocker, a biotinized-lectin of Macckia amurensis recognizing Sialalpha2-3Gal, that of Recinus communis recognizing Galbeta1-4GlcNAc, that of Dolichos biflorus recognizing GalNAcalpha1-3GalNAc or that of Aleuria aurentia recognizing Fucalpha1-2Gal, peroxidase-conjugated-avidin (Seikagaku Co., Tokyo, Japan), and the coloring kit (Sumitomo Bakelite Co.). Then, the light absorbance was measured at the dual wavelength of 450/655nm. The ELISA procedure was individually performed on different 5 plates.

Statistical Analysis

Steel-Dwass test was used. A $p<0.05$ was considered as a significant difference.

Results

S2-3g Production

A S2-3G is produced in the fraction eluted with 50mM-NaCl. The production was detected in all of the samples, however, the SZ produced the glycolipid much more than the MA and the Control did [Table 1].

Table 1: Mean±SD of S2-3G reactivity in the samples obtained from Subjects

Subject	Light absorbance (450/655 nm)
Control (Healthy volunteers)	0.074±0.007
MA (medicated Manic patients)	0.087±0.013
SZ (medicated Schizophrenic patients)	※0.200±0.018
Blank (Physiological saline)	0.055±0.002

S2-3G: Sialalpha2-3Gal-lipid promoting the dopaminergic module ※$p<0.05$ (Steel-Dwass test)

A sG1-4GN production

A sG1-4GN is produced in the fraction eluted with 100mM-NaCl. The production was detected in all of the samples, however, the SZ and the MA produced the glycolipid the same, but more than the Control did [Table 2].

Table 2: Mean±SD of sG1-4GN reactivity in the samples obtained from Subjects

Subject	Light absorbance (450/655 nm)
Control (Healthy volunteers)	0.074±0.005
MA (medicated Manic patients)	※0.101±0.008
SZ (medicated Schizophrenic patients)	※0.107±0.005
Blank (Physiological saline)	0.044±0.006

A sG1-4GN: sulfated Galbeta1-4GlcNAc-lipid promoting the serotonergic module※$p<0.05$ compared to Control (Steel-Dwass test)

GN1-3GN production

GN1-3GN is produced in the fraction eluted with 150mM-NaCl. The production was detected in all of the samples. The production was not different in the SZ, the MA and the Control [Table 3].

Table 3: Mean±SD of GN1-3GN reactivity in the samples obtained from Subjects

Subject	Light absorbance (450/655 nm)
Control (Healthy volunteers)	0.100±0.007
MA (medicated Manic patients)	0.114±0.013
SZ (medicated Schizophrenic patients)	0.102±0.012
Blank (Physiological saline)	0.054±0.004

GN1-3GN: GalNAcalpha1-3GalNAc-lipid promoting the adrenergic module

A sF1-2G production

A sF1-2G is produced in the fraction eluted with 250mM-NaCl. The production was detected in all of the samples. The production was not different in the SZ, the MA and the Control [Table 4].

Table 4: Mean±SD of sF1-2G reactivity in the samples obtained from Subjects

Subject	Light absorbance (450/655 nm)
Control (Healthy volunteers)	0.115±0.014
MA (medicated Manic patients)	0.136±0.010
SZ (medicated Schizophrenic patients)	0.137±0.006
Blank (Physiological saline)	0.045±0.006

A sF1-2G: sulfated Fucalpha1-2Gal-lipid protecting the cholinergic module

Supplementary Data

"Raw data of light absorbance indicating the glycolipid production"

	Plate				
	1	2	3	4	5
sG1-4GN					
SZ	0.117	0.108	0.103	0.103	0.104
MA	0.117	0.101	0.096	0.096	0.096
Control	0.082	0.077	0.072	0.070	0.069
Blank (PS)	0.054	0.047	0.044	0.040	0.035
GN1-3GN					
SZ	0.123	0.109	0.095	0.093	0.091
MA	0.116	0.110	0.104	0.098	0.091
Control	0.098	0.104	0.110	0.100	0.089
Blank (PS)	0.056	0.052	0.048	0.054	0.060
sF1-2G					
SZ	0.135	0.133	0.145	0.130	0.143
MA	0.151	0.143	0.134	0.128	0.122
Control	0.123	0.129	0.125	0.108	0.092
Blank (PS)	0.050	0.045	0.039	0.043	0.047
S2-3G					
SZ	0.211	0.192	0.172	0.200	0.227
MA	0.109	0.092	0.079	0.079	0.078
Control	0.057	0.072	0.087	0.080	0.073
Blank (PS)	0.056	0.052	0.048	0.054	0.060

Discussion

The Control produced S2-3G, sG1-4GN, GN1-3GN and sF1-2G. This indicates healthy human always prepares these glycolipids for stress-coping. The MA produced sG1-4GN more than the Control did. This suggests the MA had a stress to be coped with serotonergic module activity in spite of the medication. The SZ produced sG1-4GN more than the Control did, and remarkably produced S2-3G. This suggests the SZ had a stress to be coped with serotonergic module activity and a stress to be coped with strong dopaminergic module activity in spite of the medication. The stressors are not yet clear, however, the present findings also suggest sG1-4GN production is related to manic symptoms, and S2-3G production is related to psychotic symptoms of schizophrenia. In fact, it was previously reported psychotic symptoms are related to sialic glycolipids Gangliosides in the brain [16]. Furthermore, according to PubChem Compounds, sugar-chain-structure of S2-3G is NeuAcalpha2-3Gal, and NeuAcalpha2-3Gal-ceramide is one of Gangliosides.

Some researchers have investigated humoral biomarkers of schizophrenia in the view of the proteins, the metabolites and the RNAs [17-18]. Mechanism producing S2-3G in the peripheral blood is not be clarified in the present time, however, I understood S2-3G would be considered as another humoral biomarker of psychotic symptoms of schizophrenia.

Results

The medicated manic patients increased sulfated Galbeta1-4GlcNAc-lipid production. The medicated schizophrenic patients increased sulfated Galbeta1-4GlcNAc-lipid production, and remarkably produced Sialalpha2-3Gal-lipid. These indicate the manic patients and the schizophrenic patients had a stress to be coped with the serotonergic module activity, and psychotic symptoms of the schizophrenic patients would be induced via stress-coping Sialalpha2-3Gal-lipid production.

Conclusion

The stressors are not clear, however, I understood humoral Sialalpha2-3Gal-lipid would be considered as another biomarker of psychotic symptoms of schizophrenia.

Ethical Approval and Informed Consent

All of the participants in the presented study were adults. They consented to participate in the study under the intensive informed consent with preservation of their anonymity and guarantee of the withdrawal agreement.

Funding

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Declaration of Competing Interest.

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