Lectin-Positive Spherical Deposits (SPD) Detected in the Molecular Layer of Hippocampal Dentate Gyrus of Schizophrenia

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Abstract

Lectins are proteins which specifically bind (or crosslink) carbohydrates. Recently, importance of glycosylation has been emphasized in pathogenesis of diseases. Lectin-positive spherical deposits (SPD), 3-10 micron in diameter, have been detected in the molecular layer of dentate gyrus of hippocampus in schizophrenia, dementia, Down's syndrome, and aged individuals. In schizophrenia, SPD was observed without exception, regardless of having history of pharmacotherapy. By using multi-labeling histochemical methods, single strand DNA was co-localized in hippocampal SPD of schizophrenia with lectin, including GSI-B4 for galactose, and UEA-1 for fucose, suggesting that SPD formation in schizophrenia is related to apoptotic process. The molecular basis of SPD formation should further be investigated in brains with neuropsychiatric illnesses.

Keywords: Sugar chain; Postmortem brain; Apoptosis; Dentate gyrus; Hippocampus; Schizophrenia

Introduction

Hippocampal neuropathology, as a result of pathognomonic procedures of molecular basis related to memory and cognitive disturbance, has been described in mental disorders such as schizophrenia [1]. Glycoconjugates have been implicated to play a major role in the process of cell-cell recognition during development. Recently, increasing number of diseases come to be known to be caused by abnormalities in the biosynthesis of sugar chains [2]. Abnormal accumulation or deposition of the sugar chains in brains of patients with neurodegenerative diseases has been reported [3].

Lectin staining is able to reveal several kinds of carbohydrate-related depositions in addition to the conventional degenerative changes including senile plaques, neurofibrillary tangles and corpora amylacea (Table 1, Figure 1) [4]. According to an old definition,"Lectins are multivalent carbohydrate-binding proteins or glycoproteins except for enzymes and antibodies." As a significant number of exceptions are evident now, such a narrow definition, however, seems no longer relevant. To date, "lectins are defined as proteins which specifically bind (or crosslink) carbohydrates." The authors detected lectin-positive spherical deposits (SPD) in the molecular layer of hippocampal dentate gyrus of schizophrenia (Figures 1-3), dementia, Down's syndrome, and aged individuals (Figure 4) [4]. In the present mini-review, significance of lectin-positive SPD would be discussed.

Materials and Methods

Brain tissue sections from hippocampus were obtained at autopsy for pathological diagnosis in compliance with the ethical code of the Ethical Committee of the Japanese Society of Legal Medicine. In the present study, hippocampal sections from 51 individuals were examined, i.e., 16 cases of clinically diagnosed or under treatment of schizophrenia, 2 Down's syndrome, 6 dementia of Alzheimer type, 5 tangle's dementia, and 22 individuals without having clinical history of neuropsychiatric diseases.

Procedures of preparation of human brain materials and immunohistochemistry have been described elsewhere [5]. The hippocampal sections were made by using a microslicer (DTK-3000, Dosaka EM) in 40 micron thick in coronal planes.

In multi-fluorescent staining, antibodies against glial fibrillary acidic protein (GFAP), CD45, neuro-filament (NF), and single strand DNA (ssDNA) and SYBR Green were used. DBA, GSI-B4, and UEA-1 were used as lectins (Table 1). Details of procedures were described elsewhere [6].

In immuno-electron microscopical method, after embedding in Luveak-812, 60nm ultrathin sections were stained with uranyl acetate and lead citrate sequentially, and examined using Hitachi H-600 electron microscope. A floating method of lectin histochemical staining before embedding in Luveak-812 was used in the same three lectins as multi-fluorescent staining. Details of procedures were described elsewhere [6].

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Table 1: Interaction between lectins and antibodies, abbreviation, and the carbohydrate binding specificity is shown.

| Lectin and antibodies | Abbreviation | Carbohydrate binding specificity |
|-----------------------|--------------|---------------------------------|
| Archis hypogaea agglutinin | PNA | Galβ(1,3)GalNAc |
| Canavalia ensiformis agglutinin | Con A | Branched α-Man (Manα1-6 (Manα1-3) Man) |
| Datura stramonium | DSA | GalNAc |
| Dolichos biflorus agglutinin | DBA | α-GalNAc |
| Erythrina cristagalli | ECA | Galβ(1,3)GalNAc |
| Glycine max agglutinin | SBA | GalNAc(α1,3)Gal |
| Griffonia simplicifolia iso agglutinin I-B4 | GSI-B4 | α-Gal |
| Pismum sativum agglutinin | PSA | Fucα(1,6)Gal, α-Man |
| Trifolium vulgar | WGA | Manβ(1,4)GalNAc(1,4) GlcNAc |
| Ulex europaeus agglutinin | UEA-1 | α-Fuc |

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Figure: 1 (A): The hippocampal formation stained by Hematoxilin Eosin. g: granular layer p: pyramidal neuron ml: molecular layer f: fimbria (B): SPDs detected by lectin stains. GSI-B4. (C-a): Corpora amylacea are intensely stained by PAS. (C-b): SPDs are weakly stained by PAS. (D-a): SPDs show intense reactivity with anti-chondroitin sulfate. (D-b): Corpora amylacea show intense reactivity with anti-tau protein antibody.

Figure: 2 The hippocampal formation (HE) (a) CA2~CA4: cornu ammonis 2~4 DG: dentate gyrus (b) Lectin-staining by GS1-B4 Normal subject, 24y.o. male (c) Lectin-staining by GS1-B4 Schizophrenia, 44y.o. male
Lectin-positive spherical deposits (SPD) in the molecular layer of hippocampal dentate gyrus

Lectin immunohistochemistry using various antibodies that detect sugar chains (Table 1), combined with conventional staining methods, revealed SPD, 3-10 micron in diameter, in the molecular layer of the dentate gyrus of the hippocampal formation (Figures 1-3) from patients with dementia of Alzheimer type, tangle's dementia, Down's syndrome and schizophrenia, and aged individuals (Figure 4) [2].

The lectin-positive SPD did not show immunoreactivity for antibodies against ubiquitin and tau-protein, though the corpora amylacea showed clear immunoreactivity for these substances (Figure 1D-b) [4]. The SPD contained fucose (α-Fuc), galactose (α-Gal) (Figure 2C, 6), N-acetyl galactosamine (α-GalNAc) (Figure 5), N-acetyl glucosamine (GlcNAc), sialic acid, mannose (Man) and chondroitin sulfate (Table 1). Ultrastructural observation by electro-microscope showed that SPD morphology varied according to types of lectins (Figures 5-7).

Lectin-positive SPD in schizophrenia

In the brains of patients with schizophrenia, the lectin-positive SPD was observed in the molecular layer of the dentate gyrus of the hippocampal formation, without exception, regardless of previous medication of antipsychotics (Figure 8). In the lectin-positive SPD, partially disrupted nucleus with decreased staining properties by SYBR Green, a marker of DNA, were detected. Single strand DNA and lectin, including BSI-B4 for galactose (Figure 7B-a, 7B-b), UEA-I for fucose (Figure 7C-a, 7C-b), and DBA for N-acetylgalactosamine (Figure 7A-a, 7A-b) were co-stained in the portion of partially disrupted nucleus (Figure 7, Table 1). In immuno-electron microscope method, lectin-
positive structures were detected in the portion of partially disrupted nucleus (Figure. 5, 6, 7A-b, 7B-b, 7C-b).

**Lectin-positive SPD in dementia**

In dementia of Alzheimer type, and tangle's dementia, some cases did not contain hippocampal lectin-positive SPD, being different from schizophrenia (Figure 4).

**Discussion**

Recent studies have shown more and more importance of the involvement of altered glycosylation in pathological procedures of neuropsychiatric diseases. In the superior temporal gyrus, altered N-glycosylation of GABAA receptor has been shown in schizophrenia [7].

The SPD contained fucose (α-Fuc), galactose (α-Gal) (Figure 2C, 6), N-acetyl galactosamine (α-GalNAc) (Figure 5), N-acetyl glucosamine (GlcNAc), sialic acid, mannose (Man) and chondroitin sulfate, suggesting that there might be unusual glycometabolism (Table 1), possibly related to the process of neurogenesis in the molecular layer of hippocampal dentate gyrus (Figure 5-7). The lectin-positive structures, detected by electro-microscopic observation in the portion of partially disrupted nucleus (Figure. 5, 6, 7A-b, 7B-b, 7C-b) suggested that these

![Figure 5](image1)

**Figure 5** Ultrastructure of a lectin-stained cell DBA-positive structures are shown by arrows.

![Figure 6](image2)

**Figure 6** Ultrastructure of SPD stained by GSI-B4. The SPD shows more variety aspect than corpora amylacea. (A: x 6,000, B: x 10,000, C: x 8,000).

![Figure 7](image3)

**Figure 7** Lectin-positive SPD in hippocampus of schizophrenia (25 y.o. male) Immunofluorescence triple-staining and ultrastructural observation.
Conclusion

The molecular layer of hippocampal dentate gyrus of schizophrenia contained lectin-positive SPD, regardless of the history of pharmacotherapy. It was shown that hippocampal lectin-positive SPD formation in schizophrenia might be related to apoptotic process. To newly establish biomarkers of schizophrenia and other neuropsychiatric illnesses, the molecular basis of formation of lectin-positive SPD should further be analyzed.

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