REVIEW

Are lectin positive spherical deposits detected in the molecular layer of the hippocampal formation related with neuronal apoptosis?

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Abstract : Previously, multi-lectin positive spherical shaped deposits were detected in the hippocampal formation of degenerative demented and schizophrenic brains and reported they possessed some possibility as a predominant tool of postmortem diagnosis, more detected in schizophrenia cases than age-matched control cases. Multi-fluorescent immunohistochemical and lectin histochemical method and immuno electron microscope method were performed on 51 forensic autopsied brains containing 16 cases of schizophrenia. In multi-fluorescent staining, partial disrupted nucleus with decreased staining properties by mean of SYBR green were detected, and lectin and single strand DNA were co-stained in the portion of partial disrupted nucleus. In immuno electron microscope method, lectin positive structures were also detected in the portion of partial disrupted nucleus. These neurons were suspected in the process of apoptosis by their distinguishable features. Some experimental studies were reported that a kind of therapeutic products of major tranquillizers induced neuron apoptosis in dentate gyrus. As the lectin positive spherical shaped deposits were detected in not only 5 schizophrenia cases without drug treatment but also in 11 schizophrenia cases with drug treatment in this study, they might be detected as the intrinsic pathological change of schizophrenia. The lectin positive spherical shaped deposits detected in the hippocampal formation were suspected as the histopathological marker of the postmortem diagnosis for schizophrenia. Further examination for specifying group of neurons detected them in and initiated apoptosis are necessitated. J. Med. Invest. 57 : 183-190, August, 2010

Keywords **:** *lectin, apoptosis, postmortem diagnosis for schizophrenia, dentate gyrus, spherical deposit*

INTRODUCTION

The development during embryogenesis is a most complex morphogenetic process in cell-cell recognition in which glycoconjugates have been implicated to play a major role (1). However, the number of diseases known to be caused by abnormalities in sugar chains has expanded tremendously in recent years. A distinguishing trait of these newly described diseases is that they are related to abnormalities in the biosynthesis of the sugar chains (2). Abnormal accumulation or deposition of the sugar chains in brains of patients with neuro-degenerative diseases was also reported (2). In our previous study we showed that amorphous depositions, which are composed of vascular and stratiform type,

Received for publication June 30, 2010 ; accepted July 23, 2010.

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associated with sugar chains were observed in the white matter of brains of patients with Alzheimer type dementia and Down's syndrome, in addition to the existence of sugar chains detected in senile plaques, neurofibrillary tangles and corpora amylacea in the brains of patients with the diseases and aged persons (3). And, we also reported that the glycoconjugate deposits with spherical shape were detected in the molecular layer of the dentate gyrus of the hippocampal formation of patients with schizophrenia, Down syndrome, and Alzheimer type and tangles type dementia, which were named "the spherical deposits (SPDs)" after their shape (4, 5) (Fig. 1, 2). In the present study, the histological characteristics of SPDs of the hippocampal formation of schizophrenic patients were examined by

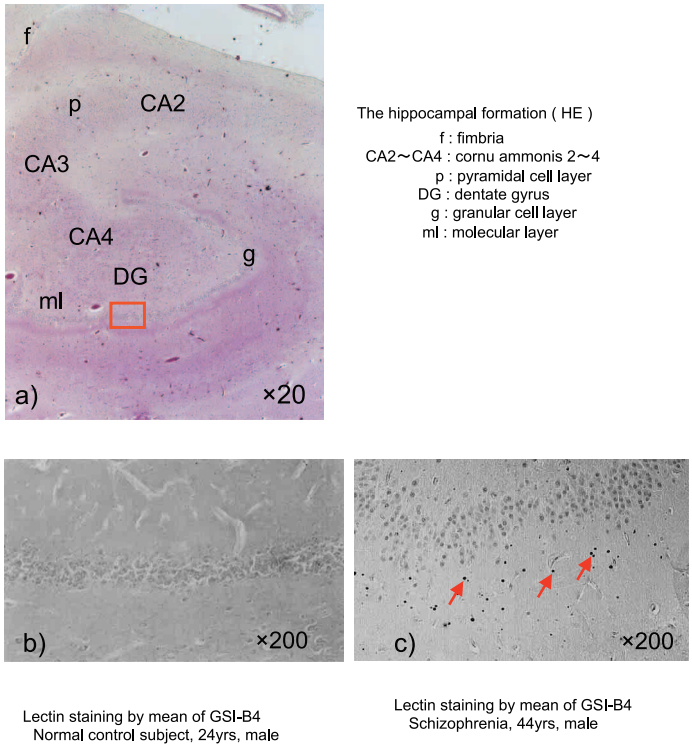

Fig. 1. a) The hippocampal formation stained by Hematoxylin Eosin. Many the spherical deposits (SPDs) were stained by mean of GS-I-B4 lectin in the red open square area of schizophrenia, which was shown as c). b) No SPD was stained in the same area of normal control.

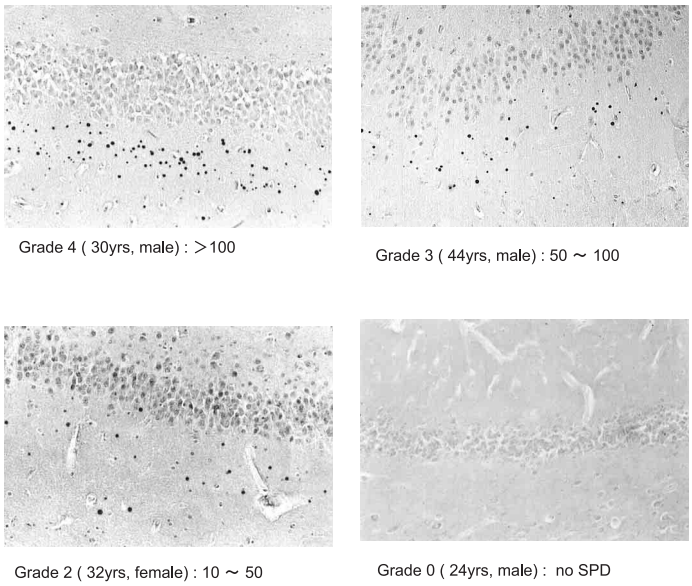

Fig. 2. Lectinhistochemical localization of the spherical deposits (SPDs) in the dentate gyrus of hippocampal formation from 4 typical cases using GS-I-B4 lectin. (×100)

means of immuno-electron microscopical technique and multi-fluorescent immunohistochemical and lectin histochemical methods.

MATERIALS AND METHODS

A flow chart of material and methods for this study is shown in Fig. 3. Brain tissue sections from the hippocampus were routinely obtained at autopsy to prepare tissue sections for the pathological diagnosis in compliance with the ethical code of the Ethical Committee of the Japanese Society of Legal Medicine. The brains were immediately sliced into 1 cm coronal slabs and immersed in the fresh fixative (pH 7.4) containing 4% paraformaldehyde in 0.01 M phosphate buffer (PB) at 4°C for 2-3 days. The slices were then transferred to PB containing 15% sucrose and 0.1% sodium azide for storage at 4°C. The hippocampal sections were made by means of a microslicer (DTK-3000, Dosaka EM) in 40 μm thick in coronal planes. In this study we examined the hippocampal formation from 50 individuals.

The cause of death, postmortem interval, symptoms and episode of the individuals are given in Table 1, of which 16 (cases 1 to 16) had been clinically diagnosed and under medical treatment for schizophrenia. Two individuals were clinically diagnosed as having Down's syndrome (cases 28 and 29) and the rest were patients with dementia, (cases 17 to 27), and individuals with other diseases or unknown episodes (cases 30 to 51). In these cases, we found no genetic disorder, e.g., Krabbe's disease, Gaucher's disease, CDGS (Carbohydrate deficient glycoprotein syndrome) etc. in which a metabolic enzyme deficiency or an altered glycoprotein

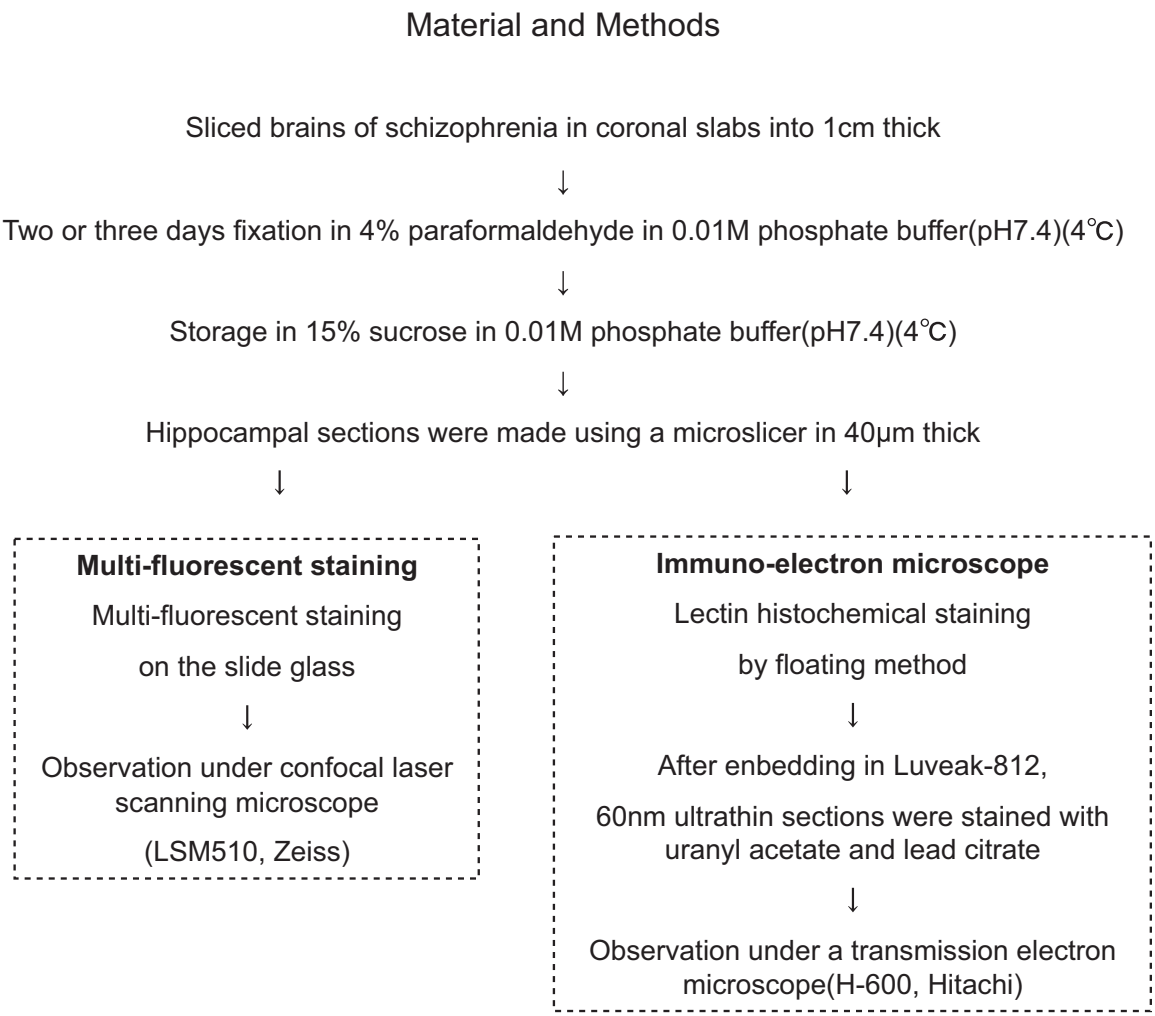

Fig. 3. Flow chart of Materials and Methods

Table 1 Summary of clinical and/or pathological diagnosis of individuals and results obtained by histochemical staining in the hippocampal formation.

Case No.	Autopsy No.	Age at Death, y	Sex	Cause of Death	Postmortem Interval, hrs	Expression grade of SPD
Schizophrenia, with clinical diagnosis						
1	N-116	21	M	Malignant syndrome	10	3+
2	N-221	25	M	Asphyxia, accident	21	2+
3	98-44	30	M	Asphyxia, homicide	11	4+
4	44154	32	F	Poisoning, suicide	9	3+
5	N-80	32	F	Burned in a fire, suicide	9	3+
6	N-147	32	M	Abdominal wound, suicide	20	3+
7	N-97	32	M	Poisoning, suicide	15	3+
8	N-90	33	M	Wound in the neck, suicide	12	2+
9	N-163	37	M	Bronchopneumonia	9	4+
10	99-21	44	M	Asphyxia, homicide	9	4+
11	N-130	44	F	Cardiac death	32	3+
12	N-34	45	M	Cardiac stab wound, suicide	13	3+
13	N-214	47	F	Asphyxia, accident	36	4+
14	N-212	48	M	Bronchopneumonia	17	4+
15	44153	55	M	Pneumonia	11	3+
16	14-7	66	M	Abdominal wound, homicide	24	4+
Pathologically diagnosed Alzheimer type dementia						
17	42208	63	F	Cardiac death	16	-
18	N-157	74	M	Pneumonia	16	4+
19	N-0	76	F	Cardiac death	11	3+
20	N-29	80	F	Cardiac death	6	2+
21	HS	84	F	Cardiac death	20	4+
22	98-17	91	F	Cerebral contusion, homicide	24	1+
Pathologically diagnosed dementia with neurofibrillary tangles						
23	43220	62	M	Cerebral hemorrhage	10	-
24	43051	69	M	Cardiac death	4	4+
25	N-86	82	F	Cardiac death	6	3+
26	97-47	83	M	Hypothermia, accident	72	2+
27	N-53	88	F	Cardiac death	10	4+
Down's syndrome						
28	41520	32	M	Cardiac death	12	3+
29	99-76	44	F	Cardiac death	8	1+
Adult individual, with unknown episode						
30	99-29	22	F	Brainstem laceration, accident	9	-
31	99-27	24	M	Drowning, accident	96	-
32	97-43	31	M	Cardiac death	7	-
33	97-42	33	F	Pulmonary embolism	11	1+
34	42206	35	M	Pneumonia	38	1+
35	N-13	35	M	Cardiac death	5	1+
36	41700	36	M	Cerebral hemorrhage	33	-
37	43052	38	M	Cardiac death	4	-
38	N-153	43	M	Subarachnoidal hemorrhage	13	-
39	42014	45	M	Cardiac death	12	1+
40	97-12	46	M	Aortic laceration, accident	8	-
41	N-129	47	M	Cardiac death	8	-
42	99-04	48	M	Burned in a fire, accident	22	-
43	98-02	55	F	Drowning, suicide	40	-
44	42171	62	M	Cardiac death	15	-
45	97-33	64	F	Cerebral contusion, accident	12	-
Aged individual, with unknown episode						
46	43502	73	M	Pneumonia	5	-
47	N-164	73	M	Subarachnoidal hemorrhage	10	2+
48	42313	73	M	Cardiac death	17	-
49	42349	79	M	Cardiac death	36	-
50	N-68	80	M	Drowning, accident	14	-
51	N-33	83	M	Hepatic cancer	5	-

M : male, F : female, SPD : Spherical shape of carbohydrate deposits. Number of carbohydrate depositions from SPD was expressed in figures, i.e. 4+ : over 100 deposits, 3+ : over 50 deposits, 2+ : over 10 deposits, 1+ : not greater than 10 deposits and - : no deposit in a slice section as shown in Fig. 2 of the molecular layer of the dentate gyrus.

has been characterized as the cause.

In multi-fluorescent staining, anti GFAP (glial fibrillary acidic protein), anti CD45, anti NF (neurofilament) and single strand DNA (ssDNA), and SYBR Green were utilized, and DBA, GSI-B4 and UEA-I were also used as lectins. For triple labeling of GFAP, SYBR Green and lectins, the sections were incubated in a mixture of mouse anti-GFAP antibody (DAKO, diluted 1 : 100) and biotinated lectins (UEA-I (EY LABORATORIES, diluted 1 : 100), DBA (EY LABORATORIES, diluted 1 : 100) and GSI-B4 (EY LABORATORIES, diluted 1 : 100) for a day, and in a mixture of Alexa546 labeled goat anti-mouse IgG (Invitrogen, 1 : 100), Alexa633 labeled streptoavidin (DAKO, diluted 1 : 100) and SYBR Green (Invitrogen, 1 : 10000) for 5 hours subsequently. For triple labeling of CD45, SYBR Green and lectins, sections were incubated in a mixture of mouse anti-CD45 antibody (Invitrogen, 1 : 50) and biotinated lectins for a day, and in a mixture of Alexa546 labeled goat anti-mouse IgG, Alexa633 labeled streptoavidin and SYBR Green for 5 hours subsequently. For triple labeling of NF, SYBR Green and lectins, sections were incubated in a mixture of mouse anti-NF antibody (DAKO, 1 : 100) and biotinated lectins for a day, and in a mixture of Alexa546 labeled goat anti-mouse IgG, Alexa633 labeled streptoavidin and SYBR Green for 5 hours subsequently. For triple labeling of ssDNA, SYBR Green and lectins, sections were incubated in a mixture of mouse anti-ssDNA antibody (Chemicon, 1 : 100) and biotinated lectins for a day, and in a mixture of Alexa546 labeled goat anti-mouse IgG, Alexa633 labeled streptoavidin and SYBR Green for 5 hours subsequently.

In immuno-electron microscopical method, after embedding in Luveak-812, 60 nm ultrathin section by mean of diamond knife were conventionally stained with uranyl acetate and lead citrate sequentially, and examined using Hitachi H-600 electron microscope (Fig. 3). A floating method of lectin histochemical staining before enbedding in Luveak-812 was performed using same three lectins as the multi-fluorescent staining. After lectin histochemical staining, the sections were postfixed in 0.2% glutalaldehyde and 1% OsO_4 sequentially. Then they were dehydrated and flat embedded in Luveak-812 between silicon-coated slide glasses. Regions in the molecular layer of the dentate gyrus of the hippocampal formation were dissected and the section was remounted on an epon stage and cut with an ultramicrotome using a diamond knife. Ultrathin sections were conventionally stained with uranyl acetate and lead citrate sequentially, and examined using Hitachi H-600 electron microscope.

RESULTS

Triple fluorescent staining by mean of NF, GSI-B4 and SYBR Green showed that the spherical deposit (SPD) existed in the neurons nearby nucleus with faint stained by SYBER Green. UEA-I and DBA also showed similar patterns (Fig. 4). And, no reactivity of GFAP and CD45 was found with reactivity of lectins. These findings suggested that SPDs existed in neurons but not in glias e.g. astrocytes or microglias.

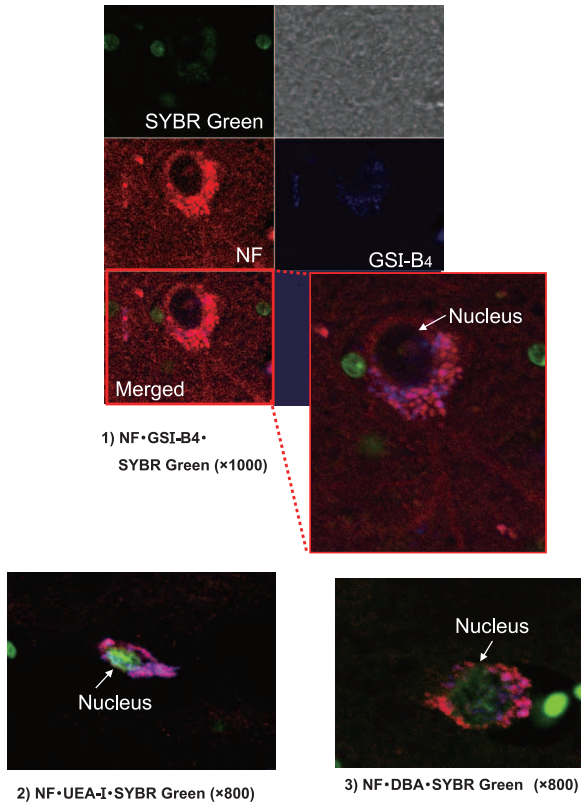

Fig. 4. Triple fluorescent staining by mean of NF (red), GSI-B4 (blue) and SYBR Green (green) in the hippocampal formation of 25yrs male schizophrenia patient. SPD existed in the neuron nearby nucleus with faint stained by SYBER Green. UEA-I (blue) and DBA (blue) also showed similar patterns.

In multi-fluorescent staining by mean of lectins, ssDNA and SYBR Green, partial disrupted nucleus with decreased staining properties by mean of SYBR green were detected, and UEA-I and single strand DNA were co-stained in the portion of partial disrupted nucleus (Fig. 5). In immuno-electron microscopical method, DBA positive structures were also

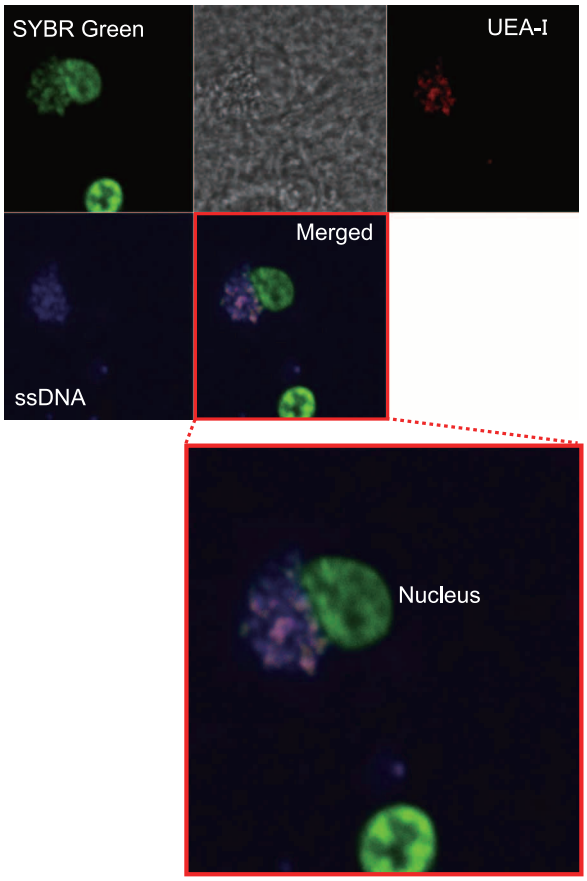

Fig. 5. Triple fluorescent staining by mean of ssDNA, UEA-I and SYBR Green in the hippocampal formation of 25yrs male schizophrenia patient. SPDs have both activities of ssDNA and UEA-I bordered by the nucleus (×1000).

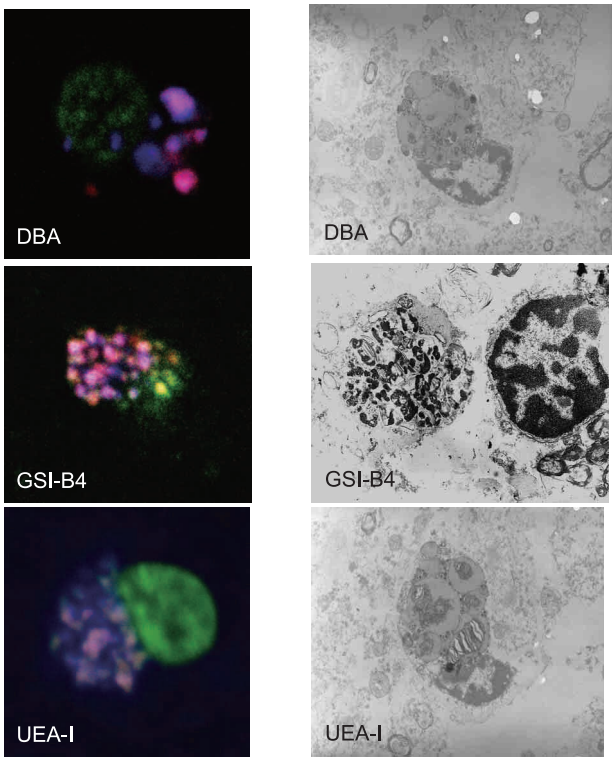

Fig. 7. Comparison between results of multi-fluorescent staining and immuno-electron microscope using DBA, GSI-B4 and UEA-I in the hippocampal formation of 25yrs male schizophrenia patient. Triple fluorescent staining by mean of lectins (red), ssDNA (blue) and SYBR Green (green). SPDs have both activities of ssDNA and UEA-I bordered by the nucleus and immuno-electron microscopy showed lectin positive structures were also detected in the portion of partial disrupted nucleus.

detected in the portion of partial disrupted nucleus (Fig. 6). Results of multi-fluorescent staining and immuno-electron microscope using DBA, GSI-B4 and UEA-I in the hippocampal formation were shown in Fig. 7. These neurons were suspected in the process of apoptosis by their distinguishable features.

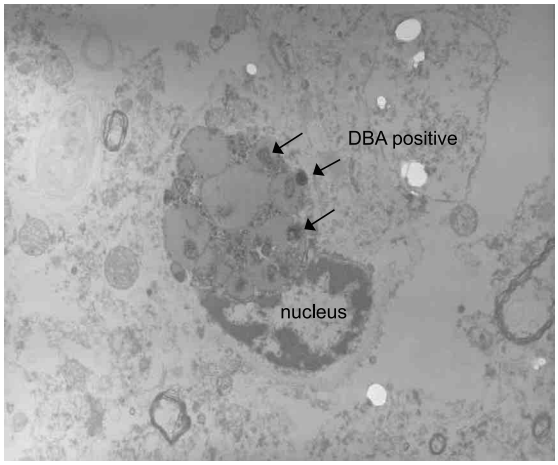

Fig. 6. Ultrastructure of SPDs stained by DBA in the hippocampal formation of 25yrs male schizophrenia patient. Several round or meniscus-shape structures without immunoreactivity were shown among of numerous phagosome/lysosome-like bodies with strong immunoreactivity bordered by nucleus. (×6,000)

DISCUSSION

Although the shape and histochemical reactivity of the spherical deposits (SPDs) were similar to those of the corpora amylacea (CA) showing reactivities with Con A, PSA, GS-I-B4, UEA-I and DBA lectins, the former could be distinguished from the latter obviously by PAS, HE, K-B and G-B staining. At electron microscopical level, SPDs could clearly be distinguished from corpora amylacea. Being different from the ultrastructure of corpora amylacea, homogeneous and round-shape structure with a patch-like immunoreactivity around their envelopes, the spherical deposits contained numerous phagosome/lysosome-like bodies with lectin immunoreactivity (6). Although the number of SPD and/or CA in the hippocampal formation varied among the patients or individuals, the appearance

was distinctive in the respective regions from all patients with schizophrenia, Alzheimer type dementia, dementia with neurofibrillary tangles or Down's syndrome and some aged individuals. The presence of SPD with a few CA was mainly observed in young patients with schizophrenia, and the co-localization of SPD and CA was recognized in middle-aged patients with schizophrenia and patients with Alzheimer type dementia, dementia with neurofibrillary tangles and Down's syndrome (4).

The molecular layer of the dentate gyrus in the hippocampal formation contains dendrite fibers of granular cells in the dentate gyrus, which is considered to be the first step in the intrinsic hippocampal circuit, perforant pathway from entorhinal cortex and glias (7). The perforant pathway synapses on the outer portion of the dentate that arises from the dentate gyrus granule cells. This subtends approximately two-thirds of the granule cell dendrite and the perforant pathway contributes 80-85% of the synaptic terminals that end in this zone. By contrast, the inner one-third of the molecular layer receives afferent nerves from the CA4 zone and from the septum (7). Eriksson et al (8) reported that new neurons were generated from dividing progenitor cells in the dentate gyrus of adult human, and indicated that the human hippocampus retained its ability to generate neurons throughout life. The physiological function of the hippocampus appears to be particularly concerned with memory and long-term potentiation (9). Although long-term potentiation is likely to serve as a mechanism for the storage of recent memory by the hippocampus, the formation of permanent memory traces is likely to involve the synthesis of new proteins and the formation of new synapses with the assistance of sugar chains. Since many lectin-positive spherical deposits were mainly observed in the inner one-third portion of the molecular layer of the dentate gyrus, it is suggested that there may exist a disadvantageous interaction between these deposits and dendrites of neurons of the C4 zone. The presence of spherical deposits was obvious in patients with schizophrenia, Alzheimer type dementia and Down's syndrome. These results indicate that spherical deposits may play a key role in formation of the neuronal network in the molecular layer of the hippocampal formation. Although the postmortem assignment of psychiatric diagnoses presents major practical difficulties (10), the presence of spherical deposits could be an indicator for psychiatric disorders of patients, since Grace (11) proposed that schizophrenia is a developmentally related disorder, in which disruption of the hippocampal influence over the limbic system during ontogeny results in a pathological alteration of cortico-accumbens interaction in the adult organism.

Some experimental studies were reported that a kind of therapeutic products of major tranquillizers induced neuron apoptosis in dentate gyrus (12-14). As the lectin positive spherical shaped deposits were detected in not only 5 schizophrenia cases (case No. 3, 4, 10, 13, 15) without drug treatment but also in 11 schizophrenia cases (case No. 1, 2, 5-9, 11,12, 14, 16) with drug treatment in this study, they might be detected as the intrinsic pathological change of schizophrenia. The lectin positive spherical shaped deposits detected in the hippocampal formation were suspected as the histopathological marker of the postmortem diagnosis for schizophrenia. Further examination for specifying group of neurons detected them in and initiated apoptosis are necessitated.

ACKNOWLEDGEMENT

This work was supported in part by a Grand-in-Aid for Scientific Research (C) (20590683) from Japan Society for the Promotion of Science.

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