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Lectin histochemistry of feline sphingomyelinosis

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Summary. The brain from a Siamese cat with sphingomyelinosis was examined with lectin histochemistry. Swollen neurons were stained with *Canavalia ensiformis* agglutinin (Con A). Some of them were also stained with *Ricinus communis* agglutinin-I (RCA-I) and *Ulex europaeus* agglutinin-I (UEA-I). A small number of axonal spheroids and glia cells were positive for Con A, RCA-I, UEA-I and wheat germ agglutinin. Control tissues were weakly stained with Con A, but not with any of the other lectins. These results indicate that affected neurons contain mannose and glucose residues in addition to sphingomyelin. This study points to the possibility that the characteristics of lectin histochemical study might be helpful for the diagnosis of sphingomyelinosis.

Key words: Lectin histochemistry, Sphingomyelinosis, Cat

Introduction

Sphingomyelinosis, commonly called Niemann-Pick disease (NPD), is an inherited storage disorder, which occurs in humans (Lake, 1984) and animals (Dorling, 1984; Baker et al., 1987). NPD is characterized by widespread sphingomyelin accumulation in intra- and extraneural tissues (Glew et al., 1985; Elleder, 1989).

Lectin histochemistry has recently been applied in order to identify the accumulated carbohydrate residues in various types of metabolic disorders in humans and animals (Faraggiana et al., 1982; Alroy et al., 1984, 1986, 1988). Lectin histochemistry of NPD has also been reported in human liver and spleen (Lageron, 1987), feline kidney (Castagnaro et al., 1987) and mouse brain (Weintroub et al., 1989). To our knowledge, however, there is no information available on lectin histochemistry of the brain of feline NPD.

Recently, we described a Siamese cat with NPD (Yamagami et al., 1989). The purpose of the present study is to detect specific carbohydrate residues of storage materials in the feline brain cells using lectin histochemistry.

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Materials and methods

Several parts of the brain were obtained from an 11-month-old female Siamese cat with NPD. Her clinical and pathological descriptions are available elsewhere (Yamagami et al., 1989). Corresponding brain tissues from normal cats served as a control. The tissues were fixed in 10% neutral-buffered formalin, dehydrated and embedded in paraffin according to conventional procedures. Sections, 3-6 μm thick, were stained with hematoxylin and eosin, and several lectins.

Lectin histochemistry was carried out according to the method of Hsu and Raine (1982). Deparaffinized sections were treated with 0.3% H_2O_2 to block endogenous peroxidase and then immersed in normal bovine serum to reduce background staining. The sections were incubated with one of seven biotinylated lectins (Vector Laboratories, USA) (Table 1), followed by incubation with avidin-biotin-peroxidase complex (ABC: Vector Lab.). Finally, the sections were treated with diaminobenzidine and then counterstained slightly with hematoxylin. Controls were as follows: 1) oxidation with periodic acid for 10 min prior to lectin staining; 2) substitution of unlabelled lectins for biotinylated lectins; 3) incubation with ABC alone; and 4) incubation with lectins containing 0.1-0.2M solution of their corresponding blocking sugar (Table 1).

Results

Light microscopic examination revealed swollen neurons in various sites of the affected brain sections

(Fig. 1). Axonal spheroids and glial ballooning were also found. However, cerebrovascular endothelial cells were not swollen.

The results of lectin histochemistry are summarized in Table 2. The cytoplasm of swollen neurons was stained with *Canavalia ensiformis* agglutinin (Con A) (Fig. 2), but not with *Dolichos biflorus* agglutinin (DBA), peanut agglutinin (PNA), soya bean agglutinin (SBA), or wheat

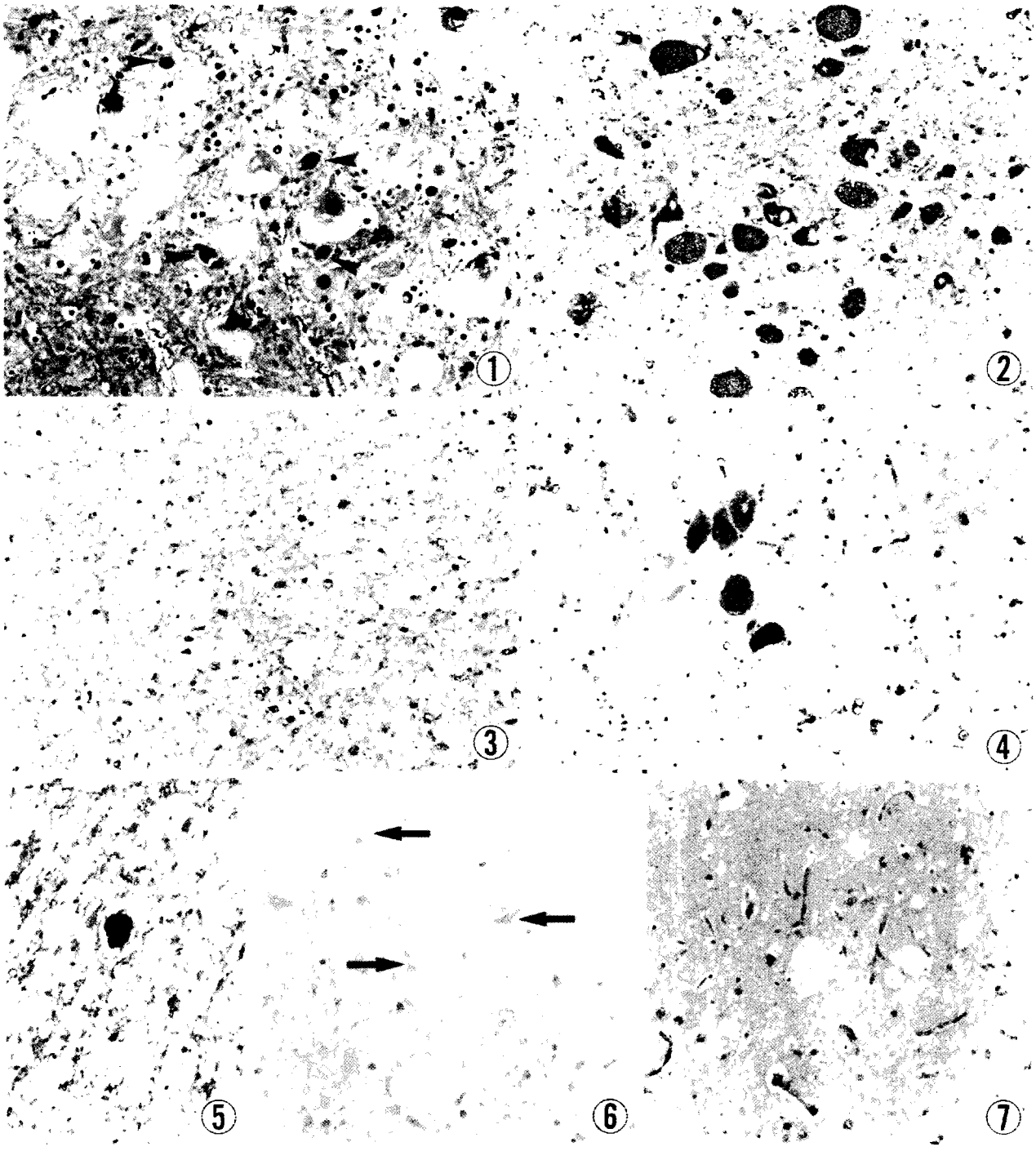

Fig. 1 Swollen neurons and spheroids (arrowheads) in Nucl. Ruber. Hematoxylin and eosin. × 175
Fig. 2. The cytoplasm of swollen neurons is stained with Con A. Putamen, × 150
Fig. 3. No reaction of swollen neurons to WGA. Putamen, × 150
Fig. 4. Several swollen neurons are stained with RCA-I. Olive, × 150
Fig. 5. Spheroid and myelin are stained with WGA. Nerve tract of brain stem, × 175
Fig. 6. Several glia cells (arrows) are positive for LIEA-I, while swollen neurons are negative. Putamen, × 300
Fig. 7. Endothelial cells of blood capillaries are positive for RCA-I, while swollen neurons are negative. Cerebral cortex, × 300

Table 1. Lectins used for detection of feline sphingomyelinosis.

Lectin (Acronym)	Concentration (μg/ml)	Sugar specificity	Binding inhibitor
Arachis hypogaea (PNA)	50	D-Gal-ß-(1-3)-D-GalNAc	D-Gal
Canavalia ensiformis (Con A)	10	α-D-Man, α-D-Glc	α-Methyl-Man
Dolichos biflorus (DBA)	50	α-D-GalNAc	D-GalNAc
Glycine maximus (SBA)	50	α-D-GalNAc, α-D-Gal	D-GalNAc
Ricinus communis (RCA-I)	20	ß-D-Gal	D-Gal
Triticum vulgare (WGA)	10	ß-D-GlcNAc, NeuNAc	D-GlcNAc
Ulex europaeus (UEA-I)	20	α-L-Fucose	L-Fucose

Abbreviations: Gal = galactose; GalNAc = N-acetyl-galactosamine; Glc = glucose; GlcNAc = N-acetyl-glucosamine; Man = mannose; NeuNAc = N-acetyl-neuraminic acid.

Table 2. Lectin staining of different cell types in the brain of feline sphingomyelinosis.

	Con A		RCA-I		UEA-I		WGA		DBA		PNA		SBA	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A
Neurons	±	+	−	+	−	+	−	−	−	−	−	−	−	−
Spheroids	−	+	−	+	−	+	−	+	−	−	−	−	−	−
Glia cells	−	+	−	+	−	+	−	+	−	−	−	−	−	−
Endothelial cells	−	−	+	+	+	+	−	−	−	−	−	−	−	−

Abbreviations: C = control; A = affected.

germ agglutinin (WGA) (Fig. 3). A small number of swollen neurons were stained with *Ricinus communis* agglutinin-I (RCA-I) and *Ulex europaeus* agglutinin-I (UEA-I) (Fig. 4). In addition, some of the axonal spheroids were positive for Con A, RCA-I, UEA-I and WGA (Fig. 5). Some of the swollen glia cells were stained weakly with Con A, RCA-I, UEA-I and WGA (Fig. 6). However, corresponding control tissues obtained from normal cats were stained either weakly or not all with Con A.

No staining difference between affected and control cats was noted for any lectin in neuropil, vasculature or myelin (Figs. 5, 7).

Discussion

Our previous communication showed that this Siamese cat had a progressive neurological disorder with widespread accumulation of sphingomyelin (Yamagami et al., 1989). The present study clearly demonstrated the binding of Con A to the neurons of the affected brain. Con A has an ability to bind specifically to α-mannosyl and α-glucosyl residues (Damjanov, 1987). This indicated that, in addition to sphingomyelin, mannose- and glucose-containing glycoconjugates are stored in the affected neurons. These results are in agreement with the previously demonstrated accumulation of heterogeneous storage material in human and animal NPD (Lake, 1984; Glew et al., 1985; Baker et al., 1987; Elleder, 1989). A few of the affected neurons were also positive for RCA-I and UEA-I. Although the molecules with which the reactive surgar residues bind with RCA-I and UEA-I are unknown, it should be considered that this heterogeneous accumulation of stored substance may be due to the inhibition of multiple lysosomal hydrolases by the primary stored sphingomyelin, as suggested by Lake (1984) and Elleder (1989).

On the other hand, the staining pattern of glia cells and spheroids was slightly different from that of neurons. These results suggest that the storage material found in them is different from that in neurons. It is suspected that such differences may depend on the metabolic status of the individual cell.

Lectin histochemical studies have indicated that affected neurons in the brain of mice with NPD were stained with Con A and succinyl-WGA (Weintroub et al., 1989). In the present study, however, the affected neurons were stained with Con A, RCA-I and UEA-I. These discrepancies cannot be explained exactly, but might be explained by a species difference. Alroy et al. (1989) stated that differences among species may occur in the molecular size of lysosomal hydrolase, in the presence or absence of the hydrolase, in the nature of the substrate to be degraded, in the character of the stored metabolite, and in the severity of the disease in the organ affected.

The present study demonstrated that staining pattern of affected neurons in feline NPD was different from that reported in the other storage diseases of humans and animals, such as G_{M1}- and G_{M2}-gangliosidosis, mannosidosis, fucosidosis, sialidosis, Fabry's disease and Hurler's disease (Virtanen et al., 1980; Faraggiana et al., 1982; Alroy et al., 1984, 1986, 1988; Lageron, 1987). In these cases, affected neurons were stained with several different lectins, such as DBA, PNA and SBA. In the present study, however, affected neurons of feline NPD were not stained with any of these three lectins. Thus, it is reasonable to assume that by using lectin histochemistry one can distinguish between various storage diseases that

have similar morphology and ultrastructure but which lack a biochemical diagnosis.

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