Pathochemical Study of a Case of Hereditary Cerebral Degenerative Disease—Lipopigment Accumulation and Peroxidase Deficiency in Brain

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— Ultrastructural study of autaptic cerebral tissue from a patient with hereditary degenerative disease of the nervous system revealed lipofuscin-like bodies and variable sized osmiophilic granular masses in neurons. Autofluorescent granules were also found in neurons. Biochemical study demonstrated a deficiency of diaminobenzidine peroxidase and the accumulation of lipopigment in cerebral tissue. — peroxidase; lipopigment; ceroid lipofuscinosis

The neuronal ceroid lipofuscinosis (NCL) is a clinically heterogeneous group of disorders characterized by blindness, mental regression and motor incoordination and is classified according to the onset of symptoms and signs as follows: infantile type (Hagberg-Santavuori), late infantile type (Spielmeyer-Sjögren) and adult type (Kufs) (Ceuterick et al. 1976). Pathologically, autofluorescent lipopigments resembling ceroid and lipofuscin are accumulated in tissues such as neural tissue, skin, muscle and spleen (Siakotos et al. 1976). Another atypical form of NCL has also been reported (Greenwood and Nelson 1978). However, the pathogenesis of the NCL is still obscure.

The present report is concerned with clinical, morphological and biochemical findings on a patient with unusual form of NCL and also describes deficiency of peroxidase activity in this patient’s brain, which has not been reported previously.

Case Report

A female patient was born as a full term baby to non-consanguineous parents, and her developmental milestones were normal. The first convulsion was noticed

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when she was 10 years old and thereafter she progressed to status epilepticus; gait and speech deteriorated, dysphagia appeared, and she died of cachexia at the age of 12. During the course, no abnormality of the eye fundus was observed. An EEG exhibited multiple spikes in all over the areas and slow waves in the posterior area. Computerized tomography of the brain showed slight dilatation of the ventricles. Routine laboratory examinations of the blood and urine were normal.

A sister, two years older than the patient, showed a clinically similar course to that of the patient, and died of cachexia at the age of 12. Autopsy was not done on the sister.

**MATERIALS AND METHODS**

The cerebral tissues obtained at autopsy 1 hr after death were quickly frozen and stored at −80°C. Prolonged assisted respiration was not performed prior to death. Control brains were obtained from three age-matched patients who had not suffered from any known metabolic or neurological disorders.

The cerebral tissues were processed to extract lipids by the method of Folch et al. (1957), and subsequently the lipids were analyzed by the method of Suzuki (1965). The analysis of lipopigment was carried out by the method of Csallany and Ayaz (1976) as follows: The cerebral tissue was homogenized with 20 volumes of chloroform-methanol, 2:1 (V/V) in a glass homogenizer at room temperature. The homogenate was filtered and the filtrate was washed twice with distilled water; the water washes were combined and washed with chloroform-methanol, 2:1 (V/V). The combined organic layers were dried under a nitrogen stream. This extract was made up to 2 ml with chloroform-methanol, 1:9 (V/V). Chromatography was carried out on Sephadex LH 20 column (2.5×30 cm) equilibrated with chloroform-methanol, 1:9 (V/V) and lipopigment was eluted with the same solvent. Two-ml-fractions from the column eluate were collected and monitored for fluorescence intensity using a Turner fluorometer Model-111 at an excitation wavelength of 365 nm, and emission wavelength 435 nm.

Lysosomal enzymes were assayed in leucocytes and brain homogenate according to the methods of Suzuki (1978). For assays of β-galactosidase, β-N-acetylglucosaminidase, β-glucosidase, the respective 4-methylumbelliferyl substrates were used. Arylsulfatase A was assayed using p-nitrocatechol sulfate (Baum et al. 1975) and acid phosphatase using p-nitrophenyl phosphate (Bingham and Zittle 1963). Sphingomyelinase was assayed using a radiolabelled substrate as previously reported (Yamaguchi and Suzuki 1977). Peroxidase was assayed using diaminobenzidine as a hydrogen donor by the method previously reported (Yamaguchi and Eto 1983).

Brain peroxidase was purified by the procedure as described elsewhere (Yamaguchi and Eto 1983). The enzyme was applied on a Sephadex G-200 column (1.5×80 cm), eluted with 10 mM Tris-HCl buffer (pH 7.0) and 2.4 ml-fractions were collected. Protein determination was performed according to the method of Lowry et al. (1951). Catalase activity was assayed using the method of Beers and Sizer (1952). Leucocytes were prepared by the method of Kampine et al. (1966).

4-Methylumbelliferyl derivatives (Sigma) and 3,3’-diaminobenzidine (Tokyo Chemical) were obtained from commercial sources.

**Results**

The weight of the brain was 910 g. The cerebral cortex was markedly
atrophied. Light microscopic observations of the brain revealed distended and rounded neurons. The nucleus was pushed to the margin of the cell. Large amounts of autofluorescent granules were found in neurons. On electronmicroscopic examination, cytoplasmic inclusion bodies were found in almost all types of neuronal cells (Fig. 1). They were membrane bound, frequently with polycyclic contours; they contained osmiophilic granular masses and sometimes short lamellar profiles. In some inclusions, these lamellar profiles were more abundant. Large amounts of lipofuscin-like bodies were encountered in the cytoplasm (Fig. 2).

Total lipid content was increased two-fold compared with normal control, but the pattern of individual lipids was normal. Activities of lysosomal enzymes in brain tissues and in leucocytes were normal.

Lipopigment separated by Sephadex LH 20 column chromatography from pathological brain was compared with normal control. Lipopigment content was monitored by fluorescence intensity. In control brains, very low fluorescence intensity was observed (Fig. 3A). The pathological brain exhibited two peaks having high fluorescence intensity; the first small and the second high peak (Fig. 3B). These fractions were combined, dried, and re-dissolved in chloroform-methanol, 1:9 (V/V) to study the fluorescence spectra. The extinction maxima and emission maxima were 365 nm and 435 nm, respectively.

The activities of diaminobenzidine peroxidase were very low in crude
Fig. 2. Frontal cortex, neuronal perikaryon. High power electronmicrograph of an inclusion body disclosing variously-sized, electron dense deposits surrounded by a limiting membrane. (×14,000)

Fig. 3. Separation of lipopigment of brain from a normal control (A) and the patient (B) by Sephadex LH 20. Lipopigment was monitored by fluorescence intensity.
The enzyme source was partially purified by Sephadex G-200 gel chromatography. A typical elution profile of peroxidase for control brain is shown in Fig. 4A. After the void volume, one large peak of the enzymatic activity was observed. The enzyme fractions were combined and used for characterization. The optimal pH was 4.5. On heat treatment the enzyme was stable at 55°C for 5 min in citrate phosphate buffer, pH 4.5. But in pathological brain, the enzymatic activity was very low as shown in Fig. 4B.

A mixing experiment of peroxidase was performed using the enzymatically active fractions from control and pathological brain eluted from Sephadex G-200 column chromatography. The calculated enzymatic activity was not changed after mixing. This suggests the absence of inhibitors in the pathological brain. In solubilized enzyme sources, the activities of catalase showed no significant differences between normal controls and the patient.

**DISCUSSION**

Clinically this case is similar to NCL except for the absence of eye involvement. Light microscopic examination revealed the accumulation of substances in neurons. In white blood cells, no lymphocytic vacuoles were observed. In atypical ceroid lipofuscinosis, involvement of the eye may be absent as was reviewed by Greenwood and Nelson (1978). Dekaban et al. subdivided cerebral lipidosis with post infantile onset (CLPI) into four types; type 1A, 1B, 2 and 3 by clinical, histological and electronmicroscopical categories (Dekaban and Herman 1974). According to this subdivision, our case did not fit to any type
because of the different clinical and electronmicroscopical results. The accumula-
tion of lipofuscin in neurons is sometimes seen in lipidoses other than NCL and
CLPI (Dekaban and Herman 1974). The lysosomal enzyme activities in white
blood cells and the general lipid analysis of the brain tissue indicated no
significant changes. These results ruled out the possibility of gangliosidosis and
other lipidoses.

In NCL, the pathogenesis is still obscure, but the accumulation of retinoyl
complexes and dolichol derivatives in brain, and the abnormal excretion of
dolichol derivatives in urine of patients with Batten disease were reported (Wolfe

The fluorescence spectrum of the accumulated lipopigment in the present case
is similar to that of the aged brain (Siakotos et al. 1976). The pathogenesis of the
cerebral degeneration in our case could be explained by the similar phenomena
that occur in aging tissues. Aging phenomena are believed to be concerned to
abnormalities of lipid peroxidation by peroxidase and catalase (Siakotos and
Armstrong 1975; Wong-Riley 1976). The demonstration of the activity of
peroxidase using diaminobenzidine for assay in the brain has been described
previously (Yamaguchi and Eto 1983). The presence of inhibitors in the brain of
the patient may be ruled out by the mixing experiment. The possibility of
primary enzyme protein abnormalities is still open.

Further examination will be needed to clarify the pathogenesis of NCL,
other familial neurodegenerative disease such as CLPI, or that of the described
patient.

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