Acta Paediatrica Hungarica 31 (4), pp 423-432 (1991)

BIOCHEMICAL AND ULTRASTRUCTURAL DIAGNOSTIC PROBLEMS IN MUCOLIPIDOSES

Aranka LÁSZLÓ[1], Z. LÁSZIK[2], L. KLUJBER[3], Z. HAVASS[1]

[1]A. Szent-Györgyi Medical University, Paediatric Department,
[2]Department of Pathology and Pathohistology, Szeged;
[3]Paediatric Department, Medical University, Pécs, Hungary

Received 2 October 1990

Biochemical and ultrastructural investigations were made in 2 children suffering from mucolipidosis type III. Among the lysosomal hydrolases the activity of beta-galactosidase and alfa-fucosidase diminished in the homogenate of the peripheral leukocytes in case I. The activity of serum and leukocyte arylsulfatase was normal.

By electron microscopy typical storage organellums for mucolipidosis were detected in different biopsy materials - liver, skin, conjunctival ones - and in the cytoplasm of the peripheral lymphocytes and leukocytes.

Definitive diagnosis was given by the specific electron microscopic investigations detecting the typical storage patterns for mucolipidosis.

INTRODUCTION

Mucolipidoses (ML) are lysosomal storage disorders with recessive autosomal inheritance. Clinical symptoms are different, Hurler-like dysostosis can be observed in ML type II. (I-cell disease). The cells of patients with I-cell disease and pseudo-Hurler polydystrophy (ML III) are characterized by a deficiency of N-acetylglucosaminyl phosphotransferase. This defect results in an inability of the cells to synthesize the mannose-6-phosphate recognition marker. As a consequence newly synthesized lysosomal enzymes are unable to bind to the mannose-6-phosphate receptors. In the fibroblasts from these patients, the lysosomal enzymes are excreted into the extracellular milieu and a generalized secondary deficiency of lysosomal enzymes in many cells of the body occurs /8/.

Akadémiai Kiadó, Budapest

Fibroblasts from patients with ML-II have extremely low or undetectable phosphotransferase activity whereas fibroblasts from patients with ML-III have partial phosphorilating activity.

Lysosomal sialidase deficiency, increased ganglioside content in autopsy tissues of sialidosis patient (ML-I) were detected by Ulrich-Bott /16/. Biochemical heterogeneity in ML-II was determined with sucrose-loading test classifying two distinct subtypes /14/.

Biochemically ML – IV. is characterized by accumulation of gangliosides, phospholipids and acidic mucopolysaccharides /11/.

By electron microscopy storage organelles typical of the mucolipidosis group are seen in cells /12/. The deficiency of ganglioside sialidase was reported as a metabolic defect in ML-IV. /16, 15/.

Our aim was to summarize the biochemical and ultrastructural investigation made in 2 children suffering from ML type III.

CASE REPORTS

Case 1. Dóra K. (female) was investigated at her 7 year-old age as our patient. Hurler-like phenotype, dysostosis, mild mental retardation, without any cornea opacity, vacuolated and metachromatically granulated peripheral lymphocytes were the pathognomic signs for mucopolysaccharidosis or mucolipidoses.

Urinary glycosaminoglucans were analysed: total glycosaminoglucans: 21.66 µmol uronic acid day, macromolecular GAG fragment GAG = 0.2, hyaluronic acid 84.4, K-heparansulphat 10.5, keratansulphat 1.4, chondroitinsulphat 3.1, dermatansulphat 0.6%. - Oligosaccharid-chromatography: negative.

Case 2. Peter N. (male) was admitted to our clinic at 11 year-old-age after a tetaniform attack with carpo-pedal spasmus and GM epilepsy attack suspecting for metabolic disease according to his Hurler-like course face and mental retardation. He attends at IV. class or special school for mentally retarded children. Mild Hurler-like vertebral dysostosis has been proven by X-ray examination.

Ophthalmological investigation: visus 0.7-0.8 D, without any corneal opacity.

GAG-uria: was normal, 29.8 mg/l g kreatinine.

EEG finding was suspected for temporo-parieto-occipital

irritative focus on the right side. As treatment Stazepine, Oradexon and Calcimusc were started.

Mucolipidosis (ML III. type) was diagnosed: vacuolated and metachromatically granulated peripheral lymphocytes, no pathological glycosaminoglucanuria (GAG-uria) and according to lysosomal enzyme analysis and the ultrastructural investigation of the different biopsy materials. Liver, skin and conjunctival biopsies were carried out for histochemical and ultrastructural (EM) specific investigations.

Biochemical investigations: lysosomal enzymes were determined from leukocyte homogenate with Griffith's /7/ method. The enzyme activities have been summarized in Table I.

TABLE I

Biochemical findings in patients suffering from mucolipidosis

Enzymes	nmol/mg/protein/h		Normal values SD± n = 20
Case 1.			
In leukocyte homogenate:			
Beta-galactosidase	114.0	D	888.0 388.8
Alfa-mannosidase	39.6	N	63.0 22.8
Alfa-fucosidase	7.2	D	231.6 157.2
Neuraminidase	47.4	N	36.0 7.2
In serum:			
Arylsulfatase-A	35.4 U/l	N	8-32 umol/l/h
Case 2.			
In leukocyte homogenate:			
Alfa-mannosidase	121.4	N	
Alfa-fucosidase	79.1	N	
Arylsulfatase-A	138.0	N	40-180 nmol/mg pr./h

N = normal

D = decreased

Ultrastructural examinations were carried out in both of the two cases on the skin, liver, conjunctival biopsy materials and on the peripheral lymphocytes. The lymphocytes were isolated

with the Ficoll-Hypaque method fixed in buffered glutaraldehyde, washed in the same buffer and postfixed in OsO_4. After a dehydration process the blocks were embedded in Araldite. The ultrathin sections were examined with Tesla BS 500 and a Zeiss EM 9S2 electron microscope.

RESULTS

Among the lysosomal hydrolases the activity of beta-galactosidase and alfa-fucosidase diminished in the homogenate of peripheral leukocytes in patient 1, while the activity of serum and leukocyte arylsulfatase was normal.

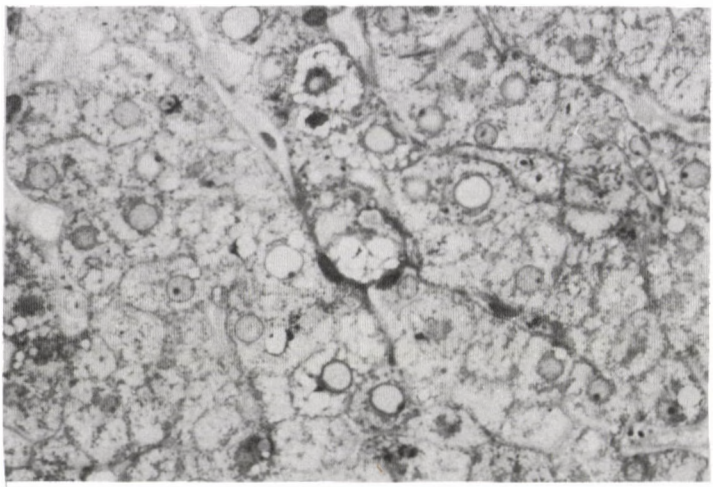
Result of the morphological investigations: liver biopsy material: light microscopy showed diffuse vacuolisation and in a small number lipid inclusions in the hepatocytes.

In the conjunctival biopsy material few epidermal cells were noted with both PAS and alciane blue positive large vacuoles in their cytoplasms. These vacuoles showed metachromasia with toluidine blue dye.

Electron microscopy revealed a great number of membrane limited inclusions in the conjunctival epidermal cells, in the endothelial cells and in the fibroblasts. These inclusions contained lamellar osmiophilic material often with a clear center. The latter component frequently contained fibrillar or fibrillogranular material. Similar inclusions were also seen in the hepatocytes (Fig. 1.a, b, Fig. 2.a, b) and in the cytoplasm of lymphocytes and polymorphonuclear leukocytes.

DISCUSSION

The different genetic types of mucolipidoses (ML) can be distinguished according to the manifestations, the onset of the



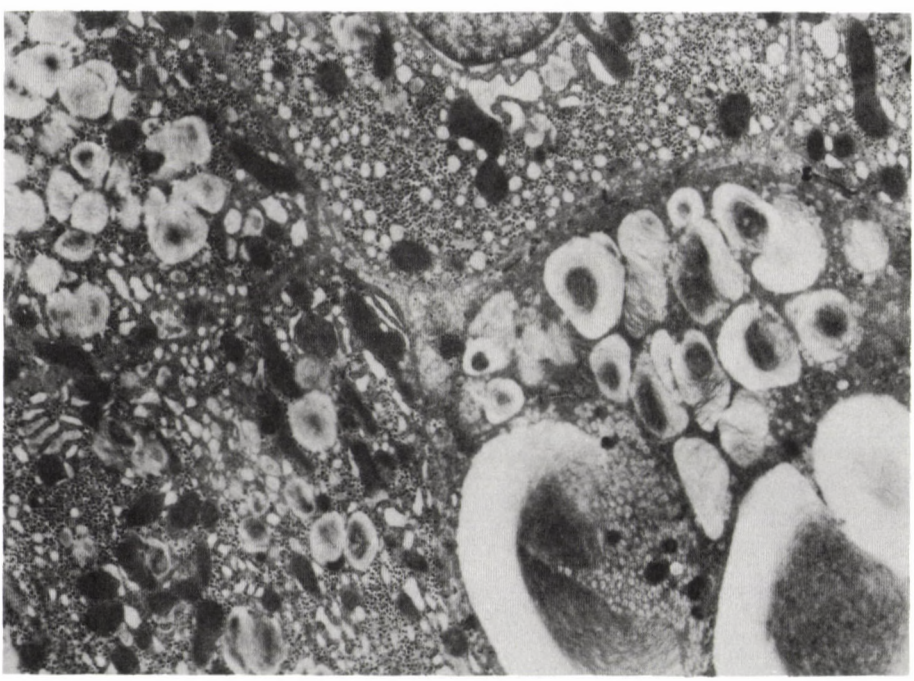


Fig. 1.a. Patient No 1.
Light micrograph of liver biopsy specimen.
Hepatocytes showed vacuolisation and a few lipid inclusions are also present x 320
Electron microscopy revealed a lot of number of membrane-limited inclusions filled with fibrillar or fibrillo-granular material x 3200

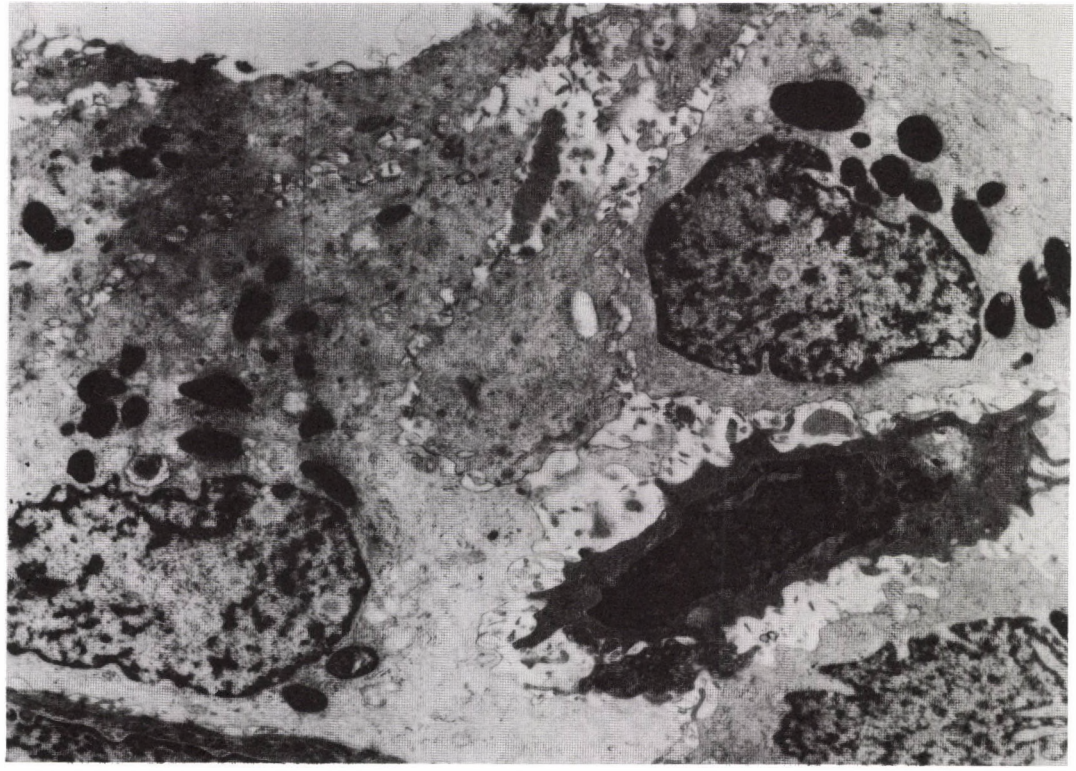


Fig. 2.a.b. Patient No 1
Conjunctival biopsy material.
Dense, lamellar material is seen in the epithelial cells, with fibroblasts in the endothel cells x 4800

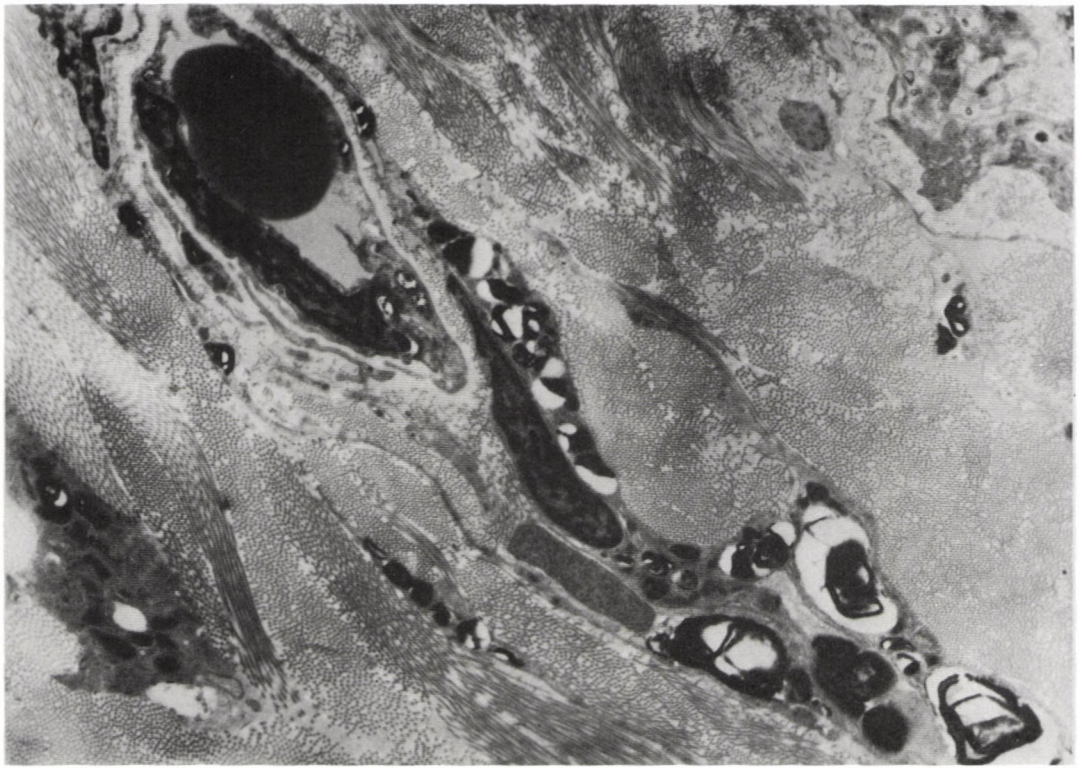


Fig. 2.a.b. Patient No 1
Conjunctival biopsy material.
Dense, lamellar material is seen in the epithelial cells, with fibroblasts in the endothel cells x 4800

disease, to the neurological deterioration, to the type of dysostosis, the lack of pathological GAG-uria, vacuolisation of lymphocytes, according to the stored substance and to the specific enzyme defects.

The type of the dysostosis is Hurler-like in ML type II, while in type III of ML there is a mild dysostosis multiplex, as we have noticed in our patients.

Cornea opacity is found only in mild degree in ML III. type but it is very frequently occurred in ML type IV. together with retinal degeneration. The split lamp finding was negative in our ML patients without any cornea opacity. The most severe mental retardation can be seen in ML type II, so-called inclusion cell disease.

Amir /1/ published the clinical spectrum and developmental features of ML-IV. analysing the data of 20 patients. The clinical manifestation of the disease - psychomotor retardation and visual impairment appeared during the first year of life.

The decreased lysosomal beta-galactosidase activity of the liver is a common biochemical feature in mucopolysaccharidosis (MPS) type I., ML type II. and III., but that is typically increased in ML type I. Multiple lysosomal enzyme deficiencies can be detected in fibroblasts and in the liver biopsy material in ML type II, so alfa-D-mannosidase, beta-D-mannosidase, beta-D-xylosidase and alfa-D-galactosidase; while beta-D-glucosidase, N-acetyl-beta-D-glucosaminidase, acid-phosphatase and alfa-D-glucosidase activities are not changed in the liver tissue.

The arylsulfatase-A activity was normal in the serum and leukocyte homogenate of our patients. The arylsulfatase-A activity is enhanced in ML type II. and contrary to the previous data it is decreased or normal in the type III. of ML. According to the clinical picture, the ultrastructural findings and the investigated lysosomal enzyme activities our patients proved to be III. type of ML manifesting in different severity of the same disease.

Specific enzyme analysis can be available for the different types of ML: sialidase for ML type I., glycoproteine-N-acetyl-

glycosamine-phosphotranferase for type II., sialoglycoprotein sialidase and sialoglangliosid sialidase for the type IV. of ML /6, 4/.

The lymphocytic vacuolisation and the storage of glycose-aminoglucans (GAG) can be detected in extreme amount in ML type I. and II., but these might be seen in type III., too.

GAG-s and glycolipids are stored in ML I. and II. types, GAG-s and neutral lipids in type III., while GAG-s, lipids and gangliosids are typical for the type IV. of ML /2, 3, 4, 5, 9/.

Ultrastructural findings of liver and conjunctival biopsy materials proved to be the most informative morphological diagnostic signs.

REFERENCES

1. Amir N, Zlotogora J, Bach G.: Mucolipidosis type IV: clinical spectrum and natural history. Pediatr 79: 953, 1987

2. Bach G, Cohen MM, Kohn G: Abnormal ganglioside accumulation in cultured fibroblasts from patients with mucolipidosis IV. Biochem Biophys Res Commun 66: 1483, 1975

3. Bach G, Zeigler M, et al: Mucopolysaccharide accumulation in cultured skin fibroblasts derived from patients with mucolipidosis IV. Am J Hum Genet 29: 610, 1977

4. Bach G, Zeigler M, Schaap P, et al: Mucolipidosis type IV: Ganglioside sialidase deficiency. Biochem Biophys Res Commun 90: 1341, 1979

5. Berman ER, Livni N, Shapira E, et al: Congenital corneal clouding with abnormal systemic storage bodies: A new variant of mucolipidosis. J Pediatr 84: 519, 1974

6. Caimi L, Tettamanti G, Berra B, et al: Mucolipidosis IV., a sialolipidosis due to ganglioside sialidase deficiency. J. Inherit. Metab. Dis. 5: 218, 1982

7. Griffiths PA, Milsom JP, Lloyd JB: Plasma acid hydrolase in normal adults and children and in patients with some lysosomal storage disease. Clin Chim Acta 90: 129, 1978

8. Durand P: Recent progress of lysosomal diseases. Enzyme 38: 256, 1987

9. Goutieres F, Arsenio-Nunes ML, Aicardi J: Mucolipidosis IV. Neuropaediatrie 10: 321, 1979

10. Ikeda K, Goebel HH, Burck U, et al: Ultrastructural pathology of human lymphocytes in lysosomal disorders: a contribution to their morphological diagnosis. Eur J Pediatr 138: 179, 1982

11. Lake BD, Milla PJ, Taylor DSI, et al: A Mild Variant of Mucolipidosis Type IV. in Cotlier E., Maumense IH, Berman ER /eds / Genetic Eye Disease. AR Liss, New York, 1982, pp 391.

12. Livni N, Merin S: Mucolipidosis IV. Ultrastructural diagnosis of a recently defined genetic disorder. Arch Pathol Lab Med 102: 600, 1978

13. Merin S, Livni N, Berman GR, et al: Mucolipidosis IV. Ocular, systemic and ultrastructural findings. Invest Ophthalmol 14: 437, 1975

14. Okada, S, Inui K, Furukawa M, et al: Biochemical heterogeneity in I-cell disease. Sucrose-loading test classifies two distinct subtypes. Enzyme 38: 267, 1987

15. Telles-Nagel I, Rapin I, Iwamoto T, et al: Mucolipidosis IV.-Clinical, ultrastructural, histochemical and chemical studies of a case, including a brain biopsy. Arch Neurol 33: 828, 1976

16. Ulrich-Bott B, Klem D, Kaiser R, et al: Lysosomal sialidase deficiency: increased ganglioside content in autopsy tissues of a sialidosis patient. Enzyme 38: 262, 1987

Aranka LÁSZLÓ, MD
P.O.Box 471
H-6701 Szeged, Hungary