Vol. 196, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1993 Pages 154-159

NIEMANN-PICK C DISEASE: CYSTINE AND LIPIDS ACCUMULATE IN THE MURINE MODEL OF THIS LYSOSOMAL CHOLESTEROL LIPIDOSIS

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Received August 17, 1993

Cystine levels in tissues of the murine BALB/C mouse model of type C Niemann-Pick disease were shown to be greatly elevated. Subcellular fractionation of liver homogenates by differential centrifugation suggested preferential accumulation in a fraction corresponding to lysosomes. Developmentally, a sharp increase in the accumulation of cystine in the mutant mouse liver occurs subsequent to a similar change in the accumulation of cholesterol, sphingomyelin and glucocerebroside. The lysosomal accumulation of cystine in this mutant mouse provides the experimental opportunity to study some aspects of the deficiency of lysosomal cystine transport noted in cystinosis.

In both the mouse (1) and human (2) forms of Niemann-Pick type C disease (NPC), an unidentified autosomal recessive mutation has been shown to be closely linked to a lesion that blocks the transport of endocytosed cholesterol from lysosomes. An array of abnormal secondary responses are known to be associated with the formation of cholesterol loaded lysosomes. Glycosphingolipids, sphingomyelin and lysobisphosphatidic acid accumulate. Spingomyelinase and glucocerebrosidase activities

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0006-291X/93 $4.00

are diminished and the activity of many other lysosomal enzymes is elevated (3,4). The present communication documents the additional accumulation of cystine in NPC mouse lysosomes. This accumulation of a nonlipid metabolite suggests that multiple functions of lysosomes may become affected when lysosomes store excess cholesterol.

Cystine accumulation in lysosomes has also been shown to be a hallmark phenotype of cystinosis, a metabolic disorder thought to be due to the dysfunction of a membrane transporter that inhibits egress of cystine from this oganelle (5). It has also been shown that both cystine and cholesterol accumulate in lysosomes of fibroblasts derived from patients with I-cell disease (6,7). No animal models describing lysosomal cystine accumulation have previouly been documented. Although cystinosis, NPC and I-cell diseases are clearly different metabolic disorders, the accumulation of cystine in the murine NPC model offers an experimental animal for the study of lysosomal cystine processing that may contribute to a better understanding of the cellular trafficking of cystine and the pathogenesis of cystinosis.

METHODS AND MATERIALS

The origin and characterization of the NPC mutation in BALB/C mice has been extensively described (1). Cystine was extracted from tissues and quantitated as previously described using a cystine binding protein assay (8). Proteins were assayed by the method of Lowry et al (9). Lipids were extracted from tissue samples and quantitated after thin layer chromatographic separation as previously described (3).

Cystine binding protein was purchased from Riverside Scientific Enterprises, Bainbridge Island, WA. E. Merck (Darmstadt) precoated Silica Gel 60 thin layer chromatographic plates, 0.25 mm thickness, were obtained from MCB Manufacturing Chemical, Inc., Cincinnati, OH. All other chemicals used were of the highest quality commercially available.

RESULTS

Cystine levels were assayed in homogenates prepared both from normal and mutant mouse tissues as well as normal and mutant human tissues (Table I). Certain tissues from the mutant mouse had substantially elevated levels of cystine as compared to normal mice. These same tissues were previously shown to accumulate large levels of cholesterol as well as glycolipids and sphingomyelin (3). Similar cellular cystine increases are seen in liver and kidney tissues from cystinotic patients as compared to normal humans. NPC human tissues in turn showed only slightly elevated levels of cystine.

Table I

Cystine levels in tissues tested. The indicated tissues were homogenized in a Dounce homogenizer in potassium phosphate buffer, 50 mM, pH 7.4, in the presence of N-ethylmaleimide, precipitated with 4% sulfosalicylic acid, proteins determined on the pellet and cystine assayed in the supernatant. Mouse tissues were freshly dissected while human tissues were from autopsy material stored at -70°C.

	nmol 1/2 cystine / mg cell protein				
	Mouse Tissues		Human Tissues		
	Normal	Mutant	Normal	Cystinotic	NPC
Liver	0.10±0.02	7.92±1.31	0.60±0.10	5.51±0.59	1.22±0.83
Lung	0.06±0.01	1.02±0.14			
Spleen	0.16±0.04	9.99±3.16	0.30±0.24	0.43	1.78±0.65
Kidney	2.29±0.58	6.52±0.93	0.57±0.04	10.70±1.5	1.62
Brain	0.03±0.01	0.03±0.01			
Muscle	0.02±0.01	0.05±0.01			

Normal and mutant mouse liver homogenates were subfractionated by differential centrifugation. The majority of accumulated cystine was recovered from the 17,000 xg pellet (Table II). This fraction contained lysosomal markers such as β-hexoseaminidase and accumulated lysosomal lipids such as cholesterol (data not shown).

The developmental progression in hepatic accumulation of cystine, cholesterol, glucocerebroside and sphingomyelin was monitored in affected animals from birth to the terminal stages of the murine disorder at 72 days (Figure 1). Sequestration of all of the above metabolites was higher

Table II

Subcellular distribution of cystine in mouse liver homogenate. Livers from normal and affected mice were dissected and used immediately. Tissue was homogenized in a Dounce homogenizer and cell fractionation carried out in a 0.25 M sucrose density gradient by differential centrifugation. The 17,000 xg pellet contained the majority of β-hexosaminidase (lysosomal) activity (17). Each fraction was analyzed for cystine and protein as described in Methods and the % of total cystine calculated.

	Normal Mouse			Mutant Mouse		
Fraction*	nmol 1/2 cys/mg	Total nmol	% of Total	nmol 1/2 cys/mg	Total nmol	% of Total
1	0.28	439	100	18.46	31031	100
2	0.12	43	9.8	3.98	2068	6.7
3	1.08	367	83.6	73.77	25892	83.4
4	0.03	28	6.9	7.32	2487	8.0

*1. Whole homogenate.
2. 700 xg pellet, nuclei and plasma membrane.
3. 17,000 xg pellet, lysosomes and mitochondria.
4. 17,000 xg supernatant, microsomes and cytosol.

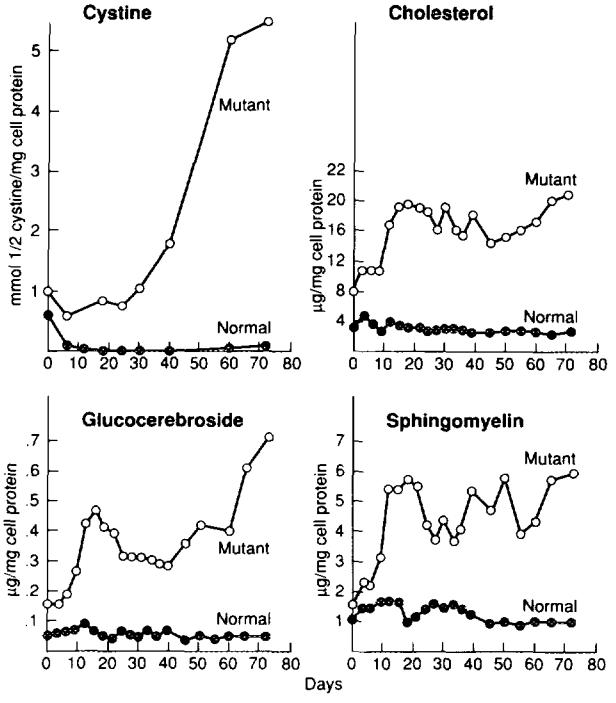

Figure 1. Accumulation of metabolites in liver of maturing lipid storage disease NPC mouse. Livers from normal and mutant mice were dissected on the days indicated and immediately extracted for lipids, cystine and protein and assayed for each parameter as outlined in methods.

than normal at all times tested. The accumulation of cystine accelerated rapidly after 30 days. In contrast, accumulation of the major lipids associated with lysosomal storage in the mutant mouse showed an earlier rapid rise such that their respective storage had essentially reached maximum levels by 15 days.

DISCUSSION

The lysosomal hypertrophy and hyperplasia associated with the murine form of NPC disease has been well documented (1). A pleotropic array of biochemical alterations parallel this cellular proliferation of lysosomes. These changes include the accumulation of several lipids such as cholesterol, glycolipids, sphingomyelin and lysophosphatidic acid (3). These lipids accumulate primarily in lysosomes filled with multilamelar inclusion bodies (11). The NPC mutation has been most closely linked to a lesion in the transport of exogenously derived cholesterol from such lysosomes (12). Consequently it has been suggested that NPC disease is primarily a lysosomal cholesterol lipidosis and that other developing

abnormal phenotypes represent secondary consequences of the accumulation of cholesterol in lysosomes (13). A number of specific transporters that regulate the relocation of hydrolyzed metabolites across the lysosomal membrane have been characterized (14). Enrichment of membranes with cholesterol has been shown to substantially alter the physical and biological properties of membranes including dysfunction of membrane receptors (15). With regard to the cellular pathogenesis of murine NPC disease the present studies have shown that cellular lysosomal accumulation of hepatic unesterified cholesterol accelerates rapidly during the postnatal period and essentially reaches maximum levels by 15 days. The kinetics of abnormal cystine accumulation in affected mouse liver were quite different. During the first 30 days of maturation, elevated cystine levels remained relatively constant. Accumulation of cystine began to rapidly increase after 30 days and continued to rise until 60 days where levels appeared to maximize. The sequence in which lipids and cystine accumulate in lysosomes may suggest a cause and effect relationship. However, high levels of accumulated cholesterol exist for a substantial period before cystine levels begin their largest rate of accumulation suggesting that increased sequestration of cystine may not be directly invoked by cholesterol accumulation.

Three human genetic diseases are known where cystine and/or cholesterol is stored in lysosomes. In cystinosis, cystine accumulates in lysosomes of most tissues to levels 50 to 100 fold of that seen in normal tissues (5). These patients fail to thrive, require kidney transplants at about 10 years of age and develop neurologic problems in the following decade. Cystinotics do not store cholesterol in lysosomes (unpublished data). In NPC disease, where patients develope neurologic defects, cholesterol is sequestered in lysosomes and is known to compromise the normal homeostatic responces of cells to cholesterol; cystine showed slight accumulation in such patients (Table I). In I-cell disease, where patients also display neurologic complications, lysosomes have many defects including storage of both cystine (6) and cholesterol (7). The primary genetic lesion of this disease is a deficiency in the Golgi enzyme N-acetylglucosaminephosphotransferase which is required for the formation of terminal 6-phosphomannosyl residues needed to target enzymes to lysosomes (16).

The NPC mutant mouse, like human I-cell disease, sequesters both cystine and cholesterol in lysosomes. The above findings suggest that the NPC mutant mouse may serve as an experimental animal for the study of lysosomal accumulation and transport of cystine, thus providing an opportunity to examine some aspects of the lysosomal cystine transport deficiency seen in cystinosis.

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