Pediat. Res. *13*: 1294–1297 (1979)

α-L-Iduronidase
feline
Hurler Syndrome

mucopolysaccharidosis
lysomal disorders

Alpha-L-iduronidase Deficiency in a Cat: A Model of Mucopolysaccharidosis I

MARK E. HASKINS, PETER F. JEZYK, ROBERT J. DESNICK, SUSAN K. McDONOUGH, AND DONALD F. PATTERSON

Sections of Pathology (M. E. H.) and Medical Genetics (M. E. H. P.F.J., D. F. P.), University of Pennsylvania, School of Veterinary Medicine, and The University of Pennsylvania Genetics Center, Philadelphia, Pennsylvania, Division of Medical Genetics (R. J. D.), Mt. Sinai School of Medicine, New York, New York. The Cat Hospital of Philadelphia (S. K. M.), Philadelphia, Pennsylvania, USA.

Summary

A 1-yr-old male domestic shorthair cat was referred to the University of Pennsylvania Veterinary Hospital with a history of progressive lameness. The cat had a short, broad face, frontal bossing, a depressed nasal bridge, small ears, bilateral corneal clouding, and thickened skin over the dorsal aspect of his neck. Radiographically, the cervical vertebrae were wide, assymetrical, and appeared nearly fused; there was bilateral coxofemoral subluxation and pectus excavatum. Electrophoresis of glycosaminoglycans (GAG) from the urine revealed an excess of both dermatan sulfate and heparan sulfate. The incorporation of $^{35}SO_4$ into the GAG of fibroblasts revealed an exaggerated accumulation of [^{35}S] -glycosaminoglycans. By light microscopy, neurons swollen with vacuolated cytoplasm were observed. By electron microscopy, the spinal cord neurons contained membrane-bound "zebra bodies". Membrane-bound inclusions containing granular material or an occasional myelin-like figure were present in hepatocytes. The activities of seven lysosomal hydrolases (α-L-iduronidase EC. 3.2.1.76, β-D-glucuronidase EC. 3.2.1.31, arylsulfatase A EC. 3.1.6.1, arylsulfatase B EC. 3.1.6.1, α-D-glucosaminidase EC. 3.2.1.50, β-D-glucosaminidase EC. 3.2.1.30, and β-D-galactosidase EC. 3.2.1.33) were investigated in cells from the affected cat. The activity of α-L-iduronidase was deficient in both cultured fibroblasts and peripheral leucocytes, while the activity of the other enzymes was similar to that in normal cats. It is apparent that the pattern of GAG excretion, evidence of lysosomal storage in various tissues, evidence of defective GAG degradation in cultured fibroblasts, and the specific deficiency in activity of α-L-iduronidase in the affected cat parallel closely the findings in mucopolysaccharidosis (MPS) I of man.

Speculation

This feline model of MPS I (Hurler, Scheie, or Hurler/Scheie Syndrome) should allow advances in the understanding of the pathogenesis and approaches to therapy for this and related genetic storage diseases.

The genetic MPS are a group of diseases caused by inborn errors of GAG metabolism. The advancements in the understanding of the MPS that have occurred since the first descriptions in man in 1917 (13) are the subject of extensive reviews (6, 20, 21). The general characteristics include: dysostosis multiplex, facial dysmorphia, hepatosplenomegaly, mental retardation, lysosomal accumulation of GAG, excretion of urinary GAG, and metachromatic granules in peripheral leukocytes. In each of at least 10 distinct biochemical disorders of GAG degradation in man, there is a relatively characteristic pattern of distribution and severity of lesions that, combined with the presence of a discrete enzyme deficiency, provides for their biochemical and clinical distinction (19). In earlier reports, a genetic MPS was described in Siamese cats closely resembling MPS VI (Maroteaux-Lamy Syndrome) in man (12, 14). Now a second feline genetic MPS is described that is an animal model of MPS I (Hurler, Scheie, or Hurler/Scheie Syndrome).

MATERIALS AND METHODS

Five animals were studied: the affected animal, an obligate heterozygote, and three half-siblings of the affected animal produced from a mating between the obligate heterozygous female and a normal male.

URINE

Spot tests for GAG were performed on fresh urine (5). Electrophoresis of cetylpyridinium chloride-precipitated urine from the affected cat and a human MPS I (Hurler) patient was performed by the method of Wessler (27) on cellulose acetate strips (28) for 90 min at 150 volts. After electrophoresis, the strips were stained with 0.1% toluidine blue in water and washed with a mixture of methanol and acetic acid (200 H_2O:2 methanol:0.1 acetic acid).

SULFATE INCORPORATION

Fibroblasts were incubated in T_{25} flasks with F-12 medium (29) (pH 6.8) containing 1×10^6 counts/ml of $^{35}SO_4$ (30) and 2% fetal calf serum (29). Two cultures were used at 24 hr, four cultures at 48 and 72 hr, and ^{35}S incorporation/mg of protein was determined by the method of Fratantoni *et al.* (7).

PATHOLOGY

Blood samples were taken in EDTA for light microscopy or heparin for electron microscopy. Leukocytes were separated by a modification of the method of Skoog and Beck (12, 24). Leukocytes were fixed in 2½% glutaraldehyde in 0.2 M acodylate buffer with 0.1 M Ca Cl_2 for 30–90 min. Liver was fixed in 5% buffered glutaraldehyde for 24 hr. Spinal cord was fixed in 10% buffered formalin for several wk. All tissues were embedded in Spurr, sectioned on a Sorvall MT2-B microtome to 700 angstroms, stained with lead citrate-uranyl acetate, and examined using a Zeiss EM 9S-2 transmission electron microscope.

ENZYME ASSAYS

Leukocytes were separated from heparinized blood (12, 24), pelleted, and stored at −70°C until assayed. Fibroblasts were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (31) with added glutamine and 10% fetal calf serum (29), and were harvested at confluency using a 1-min wash of 0.25% trypsin. The cells were disrupted in distilled water by repeated freeze-thawing and centrifuged to remove cellular debris. The supernatant was used for enzyme and protein assays.

Arylsulfatase A and B activities were determined using P-nitrocatechol sulfate (32) as substrate; α-L-iduronidase was measured using pheny-α-L-iduronidate (33). Other enzymatic activities were determined fluorometrically using the appropriate 4-methylumbelliferyl-glycoside as substrate (34). Enzyme activities were determined by the methods of Hall and Neufeld (9), Glaser and Sly (8), Beratis *et al.* (4), von Figura and Kresse (26), and Okada and O'Brien (22, 23). Protein was measured by the method of Lowry *et al.* (17). All assays were carried out in duplicate.

RESULTS

CLINICAL FEATURES

The propositus, a 1-yr-old male domestic short-hair cat, was referred to the University of Pennsylvania Veterinary Hospital with a history of progressive lameness over a 4-month period (11). The cat had a short, broad face, frontal bossing, a depressed nasal bridge, small ears, bilateral corneal clouding, and thickened skin over the dorsal aspect of his neck (Fig. 1). He appeared alert but was weak in the rear legs and evidenced pain on manipulation of his head, neck, and rear legs. Radiographically, the cervical vertebrae were wide, assymetrical, and appeared nearly fused; there was bilateral coxofemoral subluxation and pectus excavatum.

URINE

Metabolic screening tests of the urine yielded a positive spot test for GAG. Electrophoresis of cetylpyridinium chloride-precipitated GAG from the urine revealed an excess of both dermatan sulfate and heparan sulfate. Based on densitometry of the mineral oil-cleared electrophoretogram, chondroitin sulfate, dermatan sulfate, and heparan sulfate represented 10, 62, and 28% of total GAG, respectively, for the affected cat and 20, 44, and 36% respectively, for a human MPS I (Hurler) patient.

SULFATE INCORPORATION

The incorporation of $^{35}SO_4$ into the GAG of fibroblasts cultured from skin biopsies of the affected and a normal cat was studied and revealed an exaggerated accumulation of [^{35}S]-glycosaminoglycans in the fibroblasts from the propositus compared to that seen in control fibroblasts. The discrepancy increases with time because the accumulation in normal fibroblasts slows somewhat after the first 24 hr (Fig. 2).

PATHOLOGY

In human MPS I patients, metachromatic inclusions are present in peripheral granulocytes and lymphocytes when stained with toluidine blue (10, 19). By electron microscopy, lymphocytes from such patients contain membrane-bound structures which are electron-lucent or contain granular electron-dense material (3). Toluidine blue-stained peripheral blood smears from the affected cat did not reveal obvious metachromatic granules in lymphocytes or granulocytes, but by electron microscopy small membrane-bound inclusions containing granular electron-dense material were present in about 10% of the granulocytes.

Fig. 1. The facies of the affected cat. Note the frontal bossing, depressed nasal bridge, and small ears.

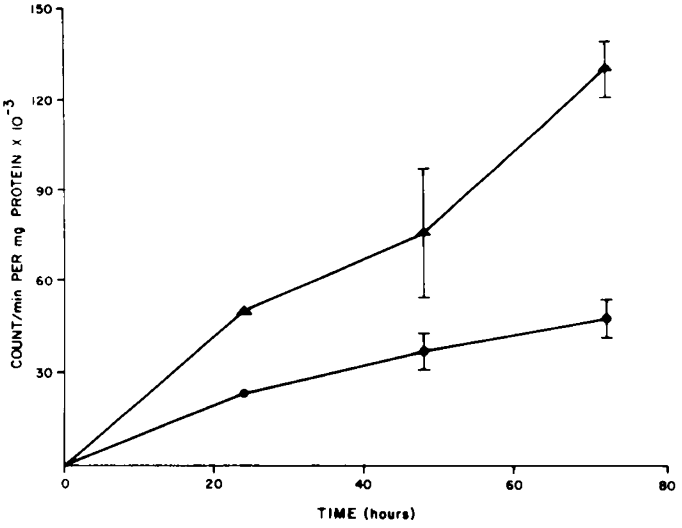

Fig. 2. Incorporation of $^{35}SO_4$ by fibroblasts from a normal cat (●——●) and from the affected (▲——▲) showing the mean ± 2 SD for each time point.

The animal was put to death with a barbiturate overdose at 16 months of age because of progressive lameness and evidence of pain. By light microscopy neurons swollen with vacuolated cytoplasm were observed in the dorsal horn of the cervical spinal cord (Fig. 3a), the reticular formation, cranial nerve nuclei, hypothalamus, hippocampus, and middle layers of the cerebral cortex. By electron microscopy, the spinal cord neurons contained membrane-bound zebra bodies (Fig. 3b). Membrane-bound inclusions containing granular material or an occasional myelin-like figure were present in hepatocytes (Fig. 3c). These inclusions in the central nervous system and liver of the cat are similar to inclusions present in the same tissues from human patients with MPS I (Hurler Syndrome) (1, 16, 25).

The left atrioventricular heart valve leaflets were thickened grossly and were found by electron microscopy to contain cells swollen with membrane-bound cytoplasmic vacuoles. Ultrastructural evidence of storage was also present in the epithelial and endothelial layers of the cornea and in fibroblastic elements of the spleen, liver, and kidney.

ENZYMOLOGY

The enzymatic defect in human MPS I homozygotes has been identified as α-L-iduronidase (2, 18). This enzymatic activity has been shown to be deficient in amniotic cells and leukocytes, as well as in cultured fibroblasts (9, 15). The activities of seven lysosomal hydrolases were investigated in cells from the affected cat (Table 1). The activity of α-L-iduronidase was deficient in both cultured fibroblasts and peripheral leukocytes, while the activities of the other enzymes were similar to those in normal cats. The activity of α-L-iduronidase in peripheral leucocytes from the mother and a male half-sibling of the propositus (from a mating to a normal male) was intermediate between that in the affected cat and normal animals, as would be expected if α-L-iduronidase deficiency were, as in man, inherited as an autosomal recessive trait (Fig. 4).

GENETICS

Pedigree information for the propositus is unavailable. However, the sire of the mother of the affected cat was known to live

Table 1. *Lysosomal enzyme activities in peripheral leukocytes and cultured fibroblasts from the affected cat and from normal cats. Enzyme activities (mean and range) are expressed as nmole/hr/mg of protein. The number of different normal animals (N) studied for each enzyme is indicated. The number of replicate preparations per animal is in parentheses after the range.*

	Affected		Normals		
	Mean	Range	N	Mean	Range
Peripheral Leukocytes					
α-L-iduronidase	2.7	1.3–4.7 (3)	5	48.8	39.9–60.0 (1)
β-D-glucuronidase	248	204–292 (2)	5	481	292–703 (1)
arylsulfatase A	518	(1)	3	959	545–1190 (1)
Arylsulfatase B	90.4	35.6–152 (3)	13	129	35.5–254 (3)
α-D-glucosaminidase	5.5	(1)	4	4.8	3.7–6.3 (1)
β-D-glucosaminidase	2040	(1)	5	2834	2490–3280 (1)
β-D-galactosidase	15	(1)	5	46.2	39–50 (1)
Cultured Fibroblasts					
α-L-iduronidase	5.8	5.4–6.2 (3)	3	93.4	64.5–116 (1)
β-D-glucuronidase	911	790–1030 (3)	3	595	302–913 (1)
Arylsulfatase B	230	(1)	1	299	(1)
α-D-glucosaminidase	25.7	21.2–30.2 (2)	4	26.6	13.1–40.7 (1)
β-D-glucosaminidase	5366	4903–5830 (2)	5	4852	2888–7730 (1)
β-D-galactosidase	249	207–291 (2)	4	318	203–386 (1)

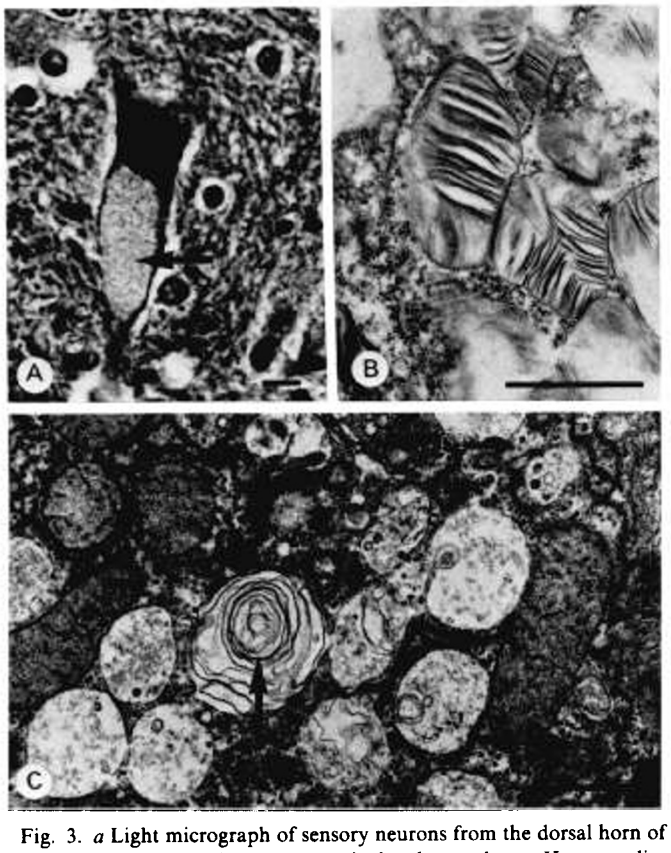

Fig. 3. *a* Light micrograph of sensory neurons from the dorsal horn of the cervical spinal cord showing vesiculated cytoplasm. Hematoxylin, eosin; scale line = 10 μm. *b* Electron micrograph of the vesicular area of a dorsal horn neuron demonstrating zebra bodies. Lead citrate, uranyl acetate; scale line = 1 μm. *c* Electron micrograph of a hepatocyte showing membrane-bound cytoplasmic inclusions containing granular material. One inclusion contains a myelin-like figure (*arrow*). Lead citrate, uranyl acetate; scale line = 1 μm.

in the neighborhood, and the coat color phenotypes of the siblings of the mother and the litter containing the propositus are consistent with the possibility that the affected cat's father was also his maternal grandfather.

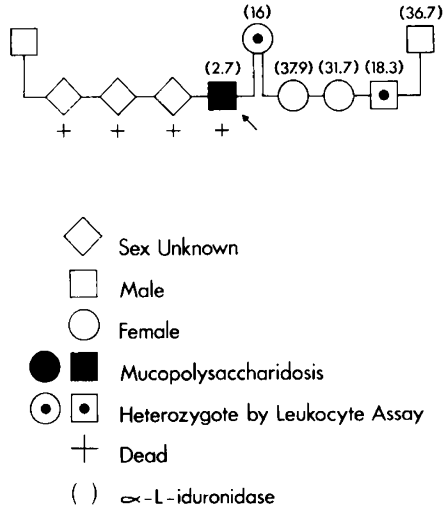

Fig. 4. Pedigree of the cat with mucopolysaccharidosis. α-L-iduronidase activity in peripheral leukocytes is indicated above each animal available for study. Enzyme activities (the mean of two separate preparations, each in duplicate) are expressed as nmoles/hr/mg of protein.

DISCUSSION

MPS I in man is presently subdivided into three separate clinical syndromes that apparently result from three different combinations of two mutant alleles at the same genetic locus. The Hurler Syndrome, the most severe form, is characterized by early corneal clouding, severe dysostosis multiplex, mental retardation, and death usually before age 10; in the Scheie Syndrome, the least severe form, there is joint involvement, corneal clouding, normal intelligence, and possibly normal lifespan; the Hurler-Scheie compound heterozygote has an intermediate phenotype. It is apparent that the pattern of GAG excretion, evidence of lysosomal storage in various tissues, evidence of defective GAG degradation in cultured fibroblasts, and the specific deficiency in activity of α-L-iduronidase in the affected cat parallel closely the findings in MPS I of man. Whereas the clinical features of the feline defect appear to most closely resemble the Hurler Syndrome, it is not clear as yet whether it can be considered an exact counterpart. A breeding colony is presently being established derived from the heterozygous mother to further characterize the condition.

REFERENCES AND NOTES

1. Aleu, F. P., Terry, R. D., and Zellweger, H.: Electron microscopy of two cerebral biopsies in gargolism. J. Neuropath. Exptl. Neurol., *24:* 304 (1965).
2. Bach, G., Friedman, R., Weissmann, B., and Neufeld, E. F.: The defect in Hurler and Scheie syndromes: deficiency of α-L-iduronidase. Proc. Natl. Acad. Sci., *69:* 2048 (1972).
3. Belcher, R.: Ultrastructure and function of eccrine glands in the mucopolysaccharidoses. Arch. Pathol., *96:* 339 (1973).
4. Beratis, N. G., Turner, B. M., Weiss, R., Hirschhorn, K.: Arylsulfatase B deficiency in Maroteaux-Lamy syndrome: Cellular studies and carrier identification. Pediatr. Res., *9:* 475 (1975).
5. Berry, H. K., and Spinanger, J.: A paper spot test useful in the study of Hurler's syndrome. J. Lab. Clin. Med., *55:* 136 (1960).
6. Dorfman, A., and Matalon, R.: The mucopolysaccharidoses (A review). Proc. Natl. Acad. Sci., USA, *73:* 630 (1976).
7. Fratantoni, J. D., Hall, C. W., Neufeld, E. F.: The defect in Hurler's and Hunter's syndromes: faulty degradation of mucopolysaccharide. Proc. Natl. Acad. Sci., USA, *60:* 699 (1968).
8. Glaser, J. H., Sly, W. S.: β-glucuronidase deficiency mucopolysaccharidosis: Methods for enzyme diagnosis. J. Lab. Clin. Med., *82:* 969 (1973).
9. Hall, C. W., and Neufeld, E. F.: α-L-iduronidase activity in cultured skin fibroblasts and amniotic fluid cells. Arch. Biochem. Biophys., *158:* 817 (1973).
10. Hansen, H. G.: Hematologic studies in mucopolysaccharidoses and mucolipidoses, In: Birth Defects: Original Article Series, Vol. 13 pp. 115–128 (Williams and Wilkins, Baltimore, 1972).
11. Haskins, M. E., Jezyk, P. J., Desnick, R. J., McDonough, S. K., Patterson, D. F.: Mucopolysaccharidosis in a domestic short-haired cat: a disease distinct from that seen in the Siamese cat. J. Am. Vet. Med. Assoc., *175:* 384 (1979).
12. Haskins, M. E., Jezyk, P. F., Patterson, D. F.: Mucopolysaccharide storage disease in three families of cats with arylsulfatase B deficiency: leukocyte studies and carrier identification. Pediatr. Res., *13:* 0000 (1979).
13. Hunter, C.: A rare disease in two brothers. Proc. R. Soc. Med., *10:* 104 (1917).
14. Jezyk, P. F., Haskins, M. E., Patterson, D. F., Mellman, W. J., Greenstein, M.: Mucopolysaccharidosis in a cat with arylsulfatase B deficiency: a model of Maroteaux-Lamy syndrome. Science, *198:* 834 (1977).
15. Liem, K. O., and Hooghwinkel, G. J. M.: The use of α-L-iduronidase activity determined in leukocytes for detection of Hurler and Scheie syndromes. Clin. Chem. Acta, *60:* 259 (1975).
16. Loeb, H., Jonniaux, G., Resibois, A., Cremer, N., Dodion, J., Tondeur, M., Gregoire, P. E., Richard, J., and Cieters, P.: Biochemical and ultrastructural studies in Hurler's syndrome. J. Pediatr. *73:* 860 (1968).
17. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem., *193:* 265 (1951).
18. Matalon, R., and Dorfman, A.: Hurler's syndrome, an α-L-iduronidase deficiency. Biochem. Biophys. Res. Commun., *47:* 959 (1972).
19. McKusick, V. A.: The mucopolysaccharidoses, In: Heritable Disorders of Connective Tissue pp. 521–686 (C.V. Mosby, St. Louis, 1972).
20. McKusick, V. A., Neufeld, E. F., Kelly, T. E.: The mucopolysaccharide storage diseases, In: J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson: The Metabolic Basis of Inherited Disease pp. 1282–1307 (McGraw Hill Book Co., New York 1978).
21. Neufeld, E. F., Lim, T. W., Shapiro, L. J.: Inherited disorders of lysosomal metabolism. Annu. Rev. Biochem., *44:* 357 (1975).
22. Okada, S., and O'Brien, J. S.: Generalized gangliosidosis: Beta-galactosidase activity. Science, *160:* 1002 (1968).
23. Okada, S., and O'Brien, J. S.: Tay-Sachs disease: Generalized absence of a β-D-N-acetyl hexosaminidase component. Science, *165:* 698 (1969).
24. Skoog, W. A., and Beck, W. S.: Studies on the fibrinogen, dextran, and phytohemagglutinin methods of isolating leukocytes. Blood, *11:* 436 (1956).
25. Tondeur, M., and Neufeld, E. F.: The mucopolysaccharidoses—Biochemistry and ultrastructure, In: R. A. Good, S. B. Day, and J. J. Yunis: Molecular Pathology pp. 600–621 (Charles C Thomas, Springfield, 1975).
26. von Figura, K., and Kresse, H.: The San filippo B corrective factor: A N-acetyl-α-D-glucosaminidase. Biochem. Biophys. Res. Comm., *48:* 262 (1972).
27. Wessler, E.: Analytical and preparative separation of acidic GAG by electrophoresis of barium acetate. Anal. Biochem., *26:* 439 (1968).
28. Gelman Instrument Co., Ann Arbor, MI.
29. Grand Island Biologicals, Grand Island, NY.
30. New England Nuclear, Boston, MA.
31. North American Biologicals, Inc., Miami, FL.
32. Sigma Chemical Co., St. Louis, MO.
33. A gift from Dr. E. F. Neufeld.
34. Research Products International, Elk Grove, MI.

35. The authors thank D. Buck and L. Hanssens for assistance in growing fibroblasts, R. Reddy for assistance in enzyme studies, J. T. McGrath for the interpretation of central nervous system lesions, and B. Montabana for the preparation of electron microscopic sections.
36. This research was supported by National Institutes of Health Genetics Center grant GM 20138, National Institutes of Health grant GM 25279, and the National Foundation March of Dimes grant 1-273.

37. Requests for reprints should be addressed to: Mark E. Haskins, V. M. D., Ph. D., Section of Pathology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, Pennsylvania 19104, USA.

38. Received for publication June 18, 1979.
39. Accepted for publication July 31, 1979.

0031-3998/79/1311-1294$02.00/0

Printed in U. S. A.