Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1978 Pages 589-595

MACULAR CHERRY-RED SPOTS AND MYOCLONUS WITH DEMENTIA: COEXISTENT NEURAMINIDASE AND β-GALACTOSIDASE DEFICIENCIES

David A. Wenger, Theodore J. Tarby and Candace Wharton

The B. F. Stolinsky Research Laboratories, Department of Pediatrics, Box C233 University of Colorado Medical Center, Denver, Colorado 80262, USA

Received April 7, 1978

SUMMARY

A patient was previously characterized as having a variant form of G_{M1} gangliosidosis based on severe deficiencies in β-galactosidase activity in both leukocytes and fibroblasts using 4-methylumbelliferyl-β-D-galactoside and G_{M1} ganglioside. Reexamination of her cultured fibroblasts revealed a severe deficiency in neuraminidase activity using neuramin lactose, fetuin and 2-(3'-methoxyphenyl)-N-acetyl-D-neuraminic acid as substrates, but normal neuraminidase activity using G_{M3} ganglioside as a substrate. The presence of normal levels of β-galactosidase activity in leukocytes from the mother of the patient indicates that the β-galactosidase deficiency is not the primary enzyme defect in this type of patient.

O'Brien (1) has recently reviewed nine patients representing unusual β-galactosidase variants. Suzuki et al. (2) have similarly reviewed nine and presented six additional juvenile and adult patients with β-galactosidase deficiency. Both reviews have delimited a subgroup of these patients that characteristically presents during the second decade of life with mild to moderate intellectual impairment, progressive ataxia, myoclonus, macular cherry-red spots, coarse facial features and foam cells in bone marrow. There is no visceromegaly in these patients. Beta-galactosidase activities in the parents of these patients have not often been reported, but, in the parents of two of these patients, β-galactosidase activities were reported as normal.

Two recent papers (3,4) have reported neuraminidase deficiencies in cultured fibroblasts from patients with myoclonus and macular cherry-red spots but without dementia. Thomas et al. (4) have demonstrated carrier levels of neur-

---

G_{M1}, G_{M1} ganglioside; G_{M3}, G_{M3} ganglioside; MPN, 2-(3'-methoxyphenyl)-N-acetyl-D-neuraminic acid; 4MU-α-L-fuc, 4-methylumbelliferyl-α-L-fucoside; 4MU-β-gal, 4-methylumbelliferyl-β-D-galactoside; 4MU-β-glcUA, 4-methylumbelliferyl-β-D-glucuronide; 4MU-α-man, 4-methylumbelliferyl-α-D-mannoside; NANA, N-acetyl-neuraminic acid and Neur-lac, Neuramin lactose.

0006-291X/78/0822-0589$01.00/0

aminidase activity in the parents and children of their patient. A neuraminidase deficiency has also been reported in patients with Mucolipidoses I (5), II and III (6). An infantile form of isolated neuraminidase deficiency has also been described (7).

In light of these reports we have re-examined the cultured fibroblasts of a 12 year-old black female with intellectual impairment, myoclonus, macular cherry-red spots and a severe β-galactosidase deficiency in both leukocytes and cultured fibroblasts. This patient has been described previously as having a variant form of G_{M1} gangliosidosis (8).

MATERIALS AND METHODS

Leukocytes were prepared from heparinized blood using 3% dextran and fibroblasts were grown and harvested as previously described (9). The pellet of cells was frozen at -20°C until assayed. The harvested fibroblasts were not frozen if neuraminidase activity was to be measured as this activity was lost on freezing. On the day of assay, leukocyte and fibroblast pellets were homogenized in distilled water in a Duall homogenizer to a protein concentration of 0.8-2.0 mg/ml. The protein concentration was determined by the method of Lowry et al. (10) using bovine serum albumin as the standard. The total homogenate was used for all assays.

The hydrolysis of G_{M1}, 4MU-β-gal, 4MU-α-L-fuc, 4MU-α-man, 4MU-β-glcUA and sphingomyelin were assayed as described previously (11). The hydrolysis of Neur-lac (Sigma Type I, approximately 85% N-acetylneuraminosyl-α-(2-3) lactose and 15% N-acetylneuraminosyl-α-(2-6) lactose, lot #27C-00881), fetuin (Grand Island Biological Co., lot #R7172Q) and G_{M3} ganglioside purified from the spleen of a patient with Gaucher's disease was measured as follows. Neur-lac (500 µg) and fetuin (1 mg) were incubated in acetate buffer (0.05 M, pH 4.2) and G_{M3} ganglioside in acetate buffer (0.1 M, pH 4.6) containing 200 µg Triton X-100. Fibroblast homogenate (25-75 µg of protein) and distilled water were added to make a total incubation volume of 0.2 ml. After incubation for 2 hours (Neur-lac), 4 hours (fetuin) or 16 hours (G_{M3}) the free NANA was measured by the method of Warren (12) with appropriate corrections for substrate and tissue blanks. MPN neuraminidase activity was measured using the method of Thomas et al. (4). MPN (Boehringer Mannheim, lot #1357101) was incubated in acetate buffer (0.2 M, pH 4.6) in a total incubation volume of 0.1 ml. Enzyme determinations on the proband and her family have been done a minimum of four times with no significant change in the observed values.

RESULTS

The proband in this family was thought to have some type of storage disease because of the presence of foam cells in the bone marrow. The fibroblast culture was received, subcultured and assayed for a number of lysosomal hydrolase activities. As shown in Table I, fibroblasts from the proband had high normal activity for sphingomyelinase and α-L-fucosidase and normal activity for α-manno-

Table I. Lysosomal hydrolase activities* in cultured skin fibroblasts and leukocytes

Tissue / Subject		Substrates: 4MU-β-gal	G_{M1}	4MU-α-L-fuc	4MU-α-man	4MU-β-glcUA	Sphingomyelin
Fibroblasts							
Controls	N	38	6	32	38	38	60
	S.D.	130	61.1	33.0	29.1	41.8	22.7
	Mean	389	228	47.9	83.0	94.4	92.2
Proband		44.0	9.5	84.8	75.9	75.0	153
G_{M1} gangliosidosis, Type 1		1.0	0	50.9	134	125	63.2
Leukocytes							
Controls	N	46	14	38	38	38	-
	S.D.	20.4	10.3	21.1	69.9	81.4	-
	Mean	96.2	29.1	61.5	224	338	-
Proband		8.3	1.8	86.2	250	281	-
Mother		81.0	40.7	-	-	-	-
Brother 1		87.0	52.1	-	-	-	-
Brother 2		75.2	39.5	-	-	-	-

* nmoles/mg protein/hr

sidase and β-glucuronidase. Acid β-galactosidase activities measured with 4MU-β-gal and G_{M1} were about 10 and 4 percent of control activities respectively. A diagnosis of some variant form of G_{M1} gangliosidosis was entertained. This residual β-galactosidase activity was higher than that found in the laboratory for fibroblasts from other patients with infantile or adult G_{M1} gangliosidosis.

To confirm this diagnosis, heparinized blood was obtained from the proband, her mother and two unaffected brothers. Studies on the leukocytes from the proband confirmed the β-galactosidase deficiency (less than 10% of control values using 4MU-β-gal and G_{M1} as substrates). Other enzymes assayed were within normal limits (Table I). Beta-galactosidase determinations on leuko-

cytes from the mother of the proband failed to show the half normal activity for this enzyme as would be expected in an obligate heterozygote, nor did either of the brothers of the proband show any β-galactosidase deficiency. Neither blood nor tissue specimens were obtainable from the father, nor was a skin biopsy obtainable from the mother.

Since the mother did not appear to be a carrier of β-galactosidase deficiency and the proband resembled clinically patients recently described with neuraminidase deficiency, her cultured fibroblasts were retrieved from liquid nitrogen storage and sub-cultured. These cultured fibroblasts from the skin of the proband had no ability to catalyze the hydrolysis of NANA from Neur-lac, fetuin or MPN (Table II). By contrast, the proband's fibroblasts could catalyze the hydrolysis of NANA from G_{M3} normally. The previously observed β-galactosidase deficiency was confirmed. Cultured fibroblasts from a patient with primary β-galactosidase deficiency have normal neuraminidase activities (Table II).

Initial attempts to determine the basis underlying the apparent deficiency in two enzyme activities in the proband have been unsuccessful. In vitro mixing experiments using fibroblast homogenates from the proband and controls resulted in no deviation from computed enzyme activities for either β-galactosidase or neuraminidase. Overnight dialysis of the fibroblast homogenate from the proband against 0.1 M NaCl in 0.1 M, pH 5.0 acetate buffer did not alter the residual β-galactosidase activity differently from similarly treated control fibroblast homogenates. The pH optimum of the residual β-galactosidase activity in the proband's fibroblasts was identical to that in control fibroblasts. Treatment of the fibroblast homogenate with neuraminidase from Cl. perfringens did not alter the residual β-galactosidase activity.

DISCUSSION

The results presented here bear on several aspects of the study of storage diseases. The most obvious is the question of accurate diagnosis. The proband, and presumably other patients discussed in the literature do not have primary deficiencies in β-galactosidase activity. The finding of normal levels

Table II. Neuraminidase activities in cultured skin fibroblasts

Subject		Substrates			
		Neur-lac*	Fetuin*	MPN*	G_{M3}**
Controls	N	24	9	10	15
	S.D.	0.13	0.06	0.08	25.4
	Mean	0.40	0.14	0.40	67.1
Proband		0	0	0	92.8
G_{M1} gangliosidosis, Type 1		0.51	0.12	0.39	70.6

* nmoles/mg protein/min; ** nmoles/mg protein/16 hrs

of this enzyme activity in obligate carriers is strong evidence against this being the primary biochemical defect in this disorder. This is particularly true when coupled with the previously demonstrated complementation between fibroblasts of this type patient and fibroblasts from patients with typical G_{M1} gangliosidosis (13,14). Since patients with all four types of β-galactosidase deficiency (as defined by Koster et al.(14)) produce normal quantities of catalytically defective but immunologically cross-reactive protein (15) and the majority (85%) of this activity resides in a monomeric protein of molecular weight 72,000 (16), the mechanism of the observed complementation has remained unexplained. The simplest explanation for the observed complementation, a β-galactosidase deficiency in this type patient that is secondary to some other biochemical defect, now seems most likely.

The nature of the primary biochemical lesion in these patients remains to be elucidated. Neither deficiency is the obligate result of the other primary deficiency since patients with neuraminidase deficiency but normal β-galactosidase activity are known (3,7) and our patient with infantile G_{M1} gangliosidosis has normal neuraminidase activities. Neuraminidase activity determinations on fibroblasts from obligate carriers of this disorder would help to

establish the primary or secondary character of this deficiency, as would cell-fusion experiments with fibroblasts from a patient with a solitary deficiency in neuraminidase activity.

The mechanism underlying the secondary β-galactosidase deficiency also remains to be determined. The results of the mixing and dialysis experiments reported here are evidence against the presence of a small molecular weight inhibitor being present in great excess. It should be noted, however, that mixing experiments involving liver homogenates from patients with a secondary β-galactosidase deficiency due to inhibition by the storage products of muco-polysaccharidoses also failed to show the presence of an inhibitor (17). Inhibition of β-galactosidase by chondroitin sulfate was later demonstrated (18). The absence of any specific effect of neuraminidase from Cl. perfringens on the residual β-galactosidase activity in fibroblast homogenates from the proband weighs against an obligate simple activation of β-galactosidase by the neuraminidase in which the proband is deficient. This observation also suggests that no NANA-containing storage product is responsible for the β-galactosidase deficiency.

Finally, it should be noted that G_{M3} neuraminidase activity was normal in cultured fibroblasts from the proband. O'Brien (3) has suggested that ganglioside neuraminidase activity should be normal in patients with the myoclonus-cherry-red spot/neuraminidase-deficiency syndrome. These observations point to the need for detailed study of the substrate specificities of human neuraminidases in both normal and neuraminidase-deficient individuals.

ACKNOWLEDGEMENTS

This research was supported in part by grants from The National Foundation-March of Dimes and from NIH (HD 10494, HD 07773, NS 10698). D. A. W. is a recipient of a Research Career Development Award (1 K04 NS 00108). The authors thank Dr. Parvin Justice for providing blood and tissue specimens from this family.

REFERENCES

1. O'Brien, J.S. in Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S. (1978) The Metabolic Basis of Inherited Disease, pp. 841-865, McGraw-Hill, New York.

2. Suzuki, Y., Nakamura, N., Fukuoka, K., Shimada, Y. and Uono, M. (1977) Hum. Genet. 36, 219-229.
3. O'Brien, J.S. (1977) Biochem. Biophys. Res. Commun. 79, 1136-1141.
4. Thomas, G.H., Tipton, R.E., Ch'ien, L.T., Reynolds, L.W. and Miller, C.S. (1978) Clin. Genet. 13, In Press.
5. Cantz, M., Gehler, J. and Spranger, J. (1977) Biochem. Biophys. Res. Commun. 74, 732-738.
6. Strecker, G. and Michalski, J.C. (1978) FEBS Letters 85, 20-24.
7. Kelly, T.E. and Graetz, G. (1977) Am. J. Med. Genet. 1, 31-46.
8. Justice, P.M., Wenger, D.A., Naidu, S., and Rosenthal, I.M. (1977) Pediat. Res. 11, 407.
9. Wenger, D.A., Sattler, M., Clark, C. and McKelvey, H. (1974) Clin. Chim. Acta 56, 199-206.
10. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
11. Wenger, D.A., Sattler, M., Clark, C. and Wharton, C. (1976) Life Sci. 19, 413-420.
12. Warren, L. (1959) J. Biol. Chem. 234, 1971-1975.
13. Galjaard, H., Hoogeveen, A., Keijzer, W., de Wit-Verbeek, H.A., Reuser, A.J.J., Ho, M.W., and Robinson, D. (1975) Nature 257, 60-62.
14. Koster, J.F., Niermeijer, M.F., Loonen, M.C.B., and Galjaard, H. (1976) Clin. Genet. 9, 427-432.
15. O'Brien, J.S. and Norden, A.G.W. (1977) Am. J. Hum. Genet. 29, 184-190.
16. Norden, A.G.W., Tennant, L.L. and O'Brien, J.S. (1974) J. Biol. Chem. 249, 7969-7976.
17. Öckerman, P.A. (1968) Scand. J. Clin. Lab. Invest. 22, 142-146.
18. Kint, J.A., Dacremont, G., Carton, D., Orye, E. and Hooft, C. (1973) Science 181, 352-354.