Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 41-46

GLOBOID CELL LEUKODYSTROPHY IS A GENERALIZED GALACTOSYL-SPHINGOSINE (PSYCHOSINE) STORAGE DISEASE

Takuro Kobayashi[1], Hisaharu Shinoda[1], Ikuo Goto[1], Tatsuhiro Yamanaka[2] and Yoshiyuki Suzuki[3]

[1]Department of Neurology, Neurological Institute, Faculty of Medicine, Kyushu University 60, Fukuoka 812, Japan

[2]Department of Pediatrics, Yaizu Municipal General Hospital, Yaizu 425, Japan

[3]Division of Inherited Metabolic Disorders, National Center of Neurology and Psychiatry, Kodaira 187, Japan

Received February 4, 1987

SUMMARY: Galactosylsphingosine (psychosine) in somatic organs from a patient with globoid cell leukodystrophy and from the twitcher mouse, an animal model of human globoid cell leukodystrophy was assayed. There was an abnormal accumulation of galactosylsphingosine as in nervous tissues, albeit the concentrations being lower than those in nervous tissues. Galacosylsphingosine accumulation in the kidney of the twitcher mouse increased with age. These findings indicate that globoid cell leukodystrophy is a generalized galactosylsphingosine storage disease.

Globoid cell leukodystrophy (Krabbe's disease, GLD) is a neurological disorder of infancy caused by a genetic deficiency of galactosylceramidase (EC 3.2.1.46) (1). It has long been a mystery as to why galactosylceramide does not accumulate in the GLD brain, despite block of the degrading pathway. In 1980, Svennerholm et al (2) demonstrated an accumulation of galactosylsphingosine , a deacylated form of galactosylceramide, in the GLD brain. These discrepancies have been resolved by the recent observation that there are two enzymes that hydrolyze galactosylceramide and that only one (galactosylceramidase I) is deficient in GLD (3). Galactosylsphingosine, however, is hydrolyzed by only galactosylceramidase I but not galactosylceramidase II. We

0006-291X/87 $1.50

devised a sensitive method for the assay of galactosylsphingosine, and found an abnormal accumulation of this compound in the nervous tissue, particularly in the peripheral nerve, of the twitcher mouse, a murine model of human GLD (4,5). We have now obtained evidence for the accumulation of galactosylsphingosine not only in nervous tissues but also in somatic organs in the twitcher mouse and from a patient with GLD.

MATERIALS AND METHODS

Galactosylsphingosine, glucosylsphingosine, o-phthalaldehyde were purchased from Sigma, St. Louis, MO, and ion exchange resins (AG 50 x 8, 200-400 mesh, hydrogen form and AG 1 x 2, 200-400 mesh, acetate form) were from Bio-Rad, Richmond, CA.

A colony of the twitcher mouse has been maintained in our laboratory. The genetic status of individual mice was determined by assays of galactosylceramidase activity in the clipped tail (6). After decapitation, the tissues were dissected, the wet weights measured and the samples kept at -20 °C until use. Tissues from a patient with GLD were obtained at autopsy and were kept at -80 °C. Just before use, samples were cut and wet weights were measured. Protein content in the homogenate of tissues in a solution of chloroform-methanol (2:1,v/v) was measured by the method of Lowry et al (7) after evaporating the solvent by placing at room temperature for 24 hours.

Assay of galactosylsphingosine in the tissue was performed as described (4). In brief, total lipids were extracted from tissues by chloroform-methanol (2:1,v/v), which was then adsorbed in a column of AG 50 x 8. Cationic lipids were eluted by 0.4 M $CaCl_2$-methanol (1:3,v/v). Hydrophilic materials were then removed by Sep-Pak C18 cartridge. The isolated galactosylsphingosine was coupled with o-phthalaldehyde, which was passed through AG 50 x 8 and AG 1 x 2 columns. The final sample was introduced to a high performance liquid chromatography, which consisted of a silica-gel column (Chemcosorb, 250 x 5.2 mm) and a solvent system of a gradient elution from 0 to 45% ethanol-water (100:2,v/v) in hexane for 30 min with a flow rate of 1.5 ml/min. Fluorescence was detected at an excitation of 335 nm and emission of 420 nm. The amount of galactosylsphingosine was determined by a ratio to the amount of glucosylsphingosine which was included in the initial lipid solution, as the internal standard. With this assay system, galactosylsphingosine in the tissue can be accurately measured in the range of 0.5-750 ng.

RESULTS

Glucosylsphingosine was not detected in any organ from normal, carrier and twitcher mice. Therefore, glucosylsphingosine could serve as the internal standard. Galactosylsphingosine was not detected in liver, spleen, kidney and lung from normal

Table 1. Galactosylsphingosine concentration in various tissues of the 31st postnatal day twitcher mouse

	ng/100 mg tissue	ng/mg protein
Liver	148	0.6
	52	0.3
Spleen	178	11.0
	148	9.5
Lung	42	2.8
	46	3.3
Kidney	124	8.7
	148	11.5
Cerebrum	423	61.5
	538	66.5
Spinal cord	2451	312
Sciatic nerve	3671	367
	3833	391

Tissues were excised from two twitcher mice and galactosylsphingosine was assayed as described in the text.

and carrier mice of up to 12 postnatal weeks. In the twitcher mouse, galactosylsphingosine was detected in all the tissues examined (Table 1). The concentration of galactosylsphingosine was several times higher in the nervous tissues than in the somatic organs. Among the somatic organs, galactosylsphingosine accumulated in larger amounts in the spleen and kidney than in the liver and lung and the accumulation in the kidney increased with age (Table 2). In the kidney and liver from a patient with

Table 2. Galactosylsphingosine concentration in the kidney of twitcher mice of various ages

Days after birth	ng/100 mg tissue	ng/mg protein
11	16	1.9
21	48	3.8
31	124	8.7
	148	11.5
37	242	24.2
	286	37.0

Galactosylsphingosine was assayed in kidneys from one or two twitcher mice of various ages.

Table 3. Galactosylsphingosine concentration in tissues from a patient with globoid cell leukodystrophy

	ng/100 mg tissue	ng/mg protein
Liver	45	3.5
Kidney	77	5.4

Galactosylsphingosine was never detected in the liver and kidney of control subjects.

GLD, there was also an accumulation of galactosylsphingosine (Table 3). In organs from control patients with no metabolic disease, galactosylsphingosine was never detected.

DISCUSSION

Galactosylsphingosine is cytotoxic and when injected into the rat brain, hemorrhagic and necrotic lesions occur (8). This compound induces hemolysis when incubated with red cells (9). These cytotoxicities are partly due to the inhibition of mitochondrial functions of the cell (10,11). In GLD, the pathological changes are mainly ascribed to the accumulation of galactosylsphingosine in the tissue, because the concentration of galactosylsphingosine in the twitcher mouse correlates well with the pathological changes; in the tissue containing higher galactosylsphingosine concentration, severer and earlier pathological changes are observed (4). The lesions are confined to the central and peripheral nervous tissues and somatic organs are morphologically intact, except for the presence of inclusion bodies at the ultrastructural level (12). Therefore, galactosylsphingosine accumulation has been demonstrated only in the nervous tissues (2,4,13). Using a sensitive assay method, we obtained evidence that galactosylsphingosine accumulates not only in nervous tissues but also in somatic organs from the twitcher mouse and from a patient with GLD. The increase of galactosyl-

sphingosine accumulation with age was observed in the kidney, as is the case in the nervous tissue of the twitcher mouse. These findings indicate that GLD is a generalized galactosylsphingosine storage disease. From the present study, galactosylsphingosine is apparently not toxic at the concentration of less than 286 ng/100 mg tissue or 37 ng/mg protein because no destructive changes were observed in somatic organs of the twitcher mouse or from patients with GLD (1). In fact, we found a normal occurrence of galactosylsphingosine in the spinal cord of the mice (less than 37 ng/100 mg tissue at the 30th postnatal day) (4).

It is unknown how galactosylsphingosine is synthesized or from where it is derived, in the tissue in cases of GLD. In somatic organs of the twitcher mouse, the abnormal accumulation of galactosylceramide has been reported (14), and was considered to be originated from nervous tissues (15). Galactosylsphingosine may possibly be converted by deacylation from galactosylceramide but such a metabolic pathway has not been demonstrated or it may be transported from the nervous tissues as is galactosylceramide. The confirmed pathway for the synthesis of galactosylsphingosine is from sphingosine and UDP-galactose (16). This has been shown in the brain of rat and guinea pig. In the same manner as in the brain, galactosylsphingosine may be synthesized from sphingosine in the somatic organ. The abnormal accumulation of galatosylsphingosine in the GLD tissue is presumably the result of a metabolic block by deficiency of its hydrolytic enzyme. The formation in GLD, when elucidated, will lead to a better understanding of the pathogenesis of GLD.

ACKNOWLEDGEMENTS

This work was supported in part by Grant 86-05 from the National Center of Neurology and Psychiatry of the Ministry of Health and Welfare, Japan and by a Grant-in-Aid from Special Research of Selected Intractable Neurological Disorders from the Ministry of Education, Science and Culture, Japan. M.Ohara of Kyushu University provided editorial assistance.

REFERENCES

1. Suzuki,K., and Suzuki,Y. (1983) The Metabolic Basis of Inherited Disease (J.B.Stunbury, J.B.Wyngaarden, D.S. Fredrickson, J.L.Goldstein, and M.S.Brown, ed). pp.857-880, McGraw-Hill, New York.
2. Svennerholm,L., Vanier,M.T., and Mansson,J.E. (1980) J.Lipid Res. 21,53-64.
3. Kobayashi,T., Shinnoh,N., Goto,I., and Kuroiwa,Y. (1985) J. Biol.Chem. 260,14982-14987.
4. Shinoda,H., Kobayashi,T., Katayama,M., Goto,I., and Nagara,H. (1987) J.Neurochem. in press.
5. Kobayashi,T., Yamanaka,T., Jacobs,J.M., Teixeira,F., and Suzuki, K. (1980) Brain Res. 202,479-483.
6. Kobayashi,T., Nagara,H., Suzuki,K., and Suzuki,K. (1982) Biochem. Med.27,8-14.
7. Lowry,O.H., Rosebrough,N.J., Farr,A.L., and Randall,R.J. (1951) J.Biol.Chem. 193,265-275.
8. Suzuki,K., Tanaka,H., and Suzuki,K. (1976) Adv.Exp.Med.Biol. 68,99-114.
9. Taketomi,T., and Nishimura,K. (1964) Jpn.J.Exp.Med. 34,255-265.
10. Strasberg,P. (1986) Biochem.Cell Biol. 64,485-489.
11. Igisu,H., and Nakamura,M. (1986) Biochem.Biophys.Res.Commun. 137,323-327.
12. Takahashi,H., Igisu,H., Suzuki,K., and Suzuki,K. (1983) Am. J.Pathol. 112,147-154.
13. Igisu,H., and Suzuki,K. (1984) Science 224,753-755.
14. Igisu,H., and Suzuki,K. (1984) J.Neuropathol.Exp.Neurol. 43, 22-36.
15. Katayama,M., Siegel,D.S., and Suzuki.K. (1987) Multidisciplinary Approach to Myelin Disease (G.S.Creceuzi, ed). Plenum, New York, In press.
16. Cleland,W.W., and Kennedy E.P. (1960) J.Biol.Chem. 235,45-51.