Concentrations of glycosyl ceramides in plasma and red cells in Fabry's disease, a glycolipid lipidosis

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ABSTRACT Concentrations of four neutral glycosyl ceramides were determined in plasma and erythrocytes from nine hemizygous patients with Fabry's disease (a hereditary glycolipid lipidosis), from the sister of one of the patients, and from the heterozygous mother of another one. The concentration of a trihexosyl ceramide, galactosylgalactosylglucosyl ceramide, was elevated in plasma from the patients about threefold above the normal mean level, and the amount of this lipid was also increased in plasma from the two female relatives. The concentrations of glucosyl ceramide and lactosyl ceramide in plasma were slightly less than normal in the affected males, while globoside or a similar tetrahexosyl ceramide was slightly higher than normal. All but one of the Fabry patients had significantly less globoside in the red cells, as compared with the normal range, although the red cell concentration of trihexosyl ceramide was normal in all of the patients.

Thus the concentration of the lipid that accumulates in the tissues in Fabry's disease is elevated in plasma but not in red cells, whereas in Gaucher's disease the accumulating lipid (glucosyl ceramide) is elevated in both plasma and red cells.

SUPPLEMENTARY KEY WORDS galactosylgalactosylglucosyl ceramide · blood glycolipids · globoside · Gaucher's disease · hereditary

FABRY'S DISEASE is a hereditary glycolipid lipidosis characterized by the deposition of galactosyl-(1→4)-galactosyl-(1→4)-glucosyl-(1→1)-ceramide (GL-3) in the kidney (1) and in other tissues. A companion glycolipid, which accumulates to a lesser extent than GL-3 in the kidneys of these patients but not in other organs that have been examined, has been shown to be galactosyl-(1→4)-galactosyl-(1→1)-ceramide (2). Abnormal amounts of a trihexosyl ceramide, presumed to be identical with GL-3, were found in the urine of patients with Fabry's disease by Urbain, Peremans, and Philippart (3). Krivit and Desnick have also measured this lipid in normal and abnormal urines, and found digalactosyl ceramide in the urine sediments from Fabry patients (unpublished work). The historical background as well as clinical symptoms and biochemical and genetic aspects of Fabry's disease have been reviewed (2).

Recent studies by Opitz et al. (4) have provided further evidence for the X-chromosomal site of inheritance in Fabry's disease. Brady et al. (5) have demonstrated deficiency of a specific ceramide trihexosidase in intestinal tissue from two Fabry patients and diminished activity of the enzyme in tissue from the heterozygous mother of one of the patients.

We have developed a procedure for the isolation and quantitative estimation of neutral glycosyl ceramides from small aliquots of human plasma and erythrocytes and reported the concentrations of these lipids from normal males (6). In the present report we present the results of a similar study of glycosyl ceramides from blood of Fabry patients. They show a consistent, marked elevation in the plasma level of GL-3.

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Abbreviations: GL-1, glucosyl ceramide; GL-2, lactosyl ceramide; GL-3, galactosylgalactosylglucosyl ceramide; GL-4, tetrahexosyl ceramide, assumed but not proven to be globoside.

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MATERIALS AND METHODS

Freshly drawn, heparinized blood was shipped to the laboratory in refrigerated containers. The glycolipids were isolated and the concentrations of each type were determined by procedures previously reported (6). The results summarized in Tables 1–4 were not corrected for manipulative losses during isolation, although the extent of these losses has been evaluated (6) and can be applied to these data. All of the patients were diagnosed as having Fabry's disease from classical clinical symptoms prior to the determination of their glycosyl ceramides.

RESULTS

Values are given in Table 1 for the levels of individual glycosyl ceramides in plasma from nine males with Fabry's disease. Of these patients, Ri.L., Ro.L., D.L., and C.L. are brothers. The other donors are not known to be related. Except for W.G., who lives in West Germany, all of the patients are residents of the United States.

The major constituent of the plasma glycolipid fraction was glucosyl ceramide (GL-1); the concentration of this lipid was lower than the normal range in all but one patient, while concentrations of lactosyl ceramide (GL-2) and a tetrahexosyl ceramide (GL-4), assumed but not proven to be globoside, were not significantly different from normal values given at the bottom of the table. The level of GL-3 in Fabry plasma was, however, significantly higher than normal in all the samples examined; the average was about three times the normal mean concentration. The carbohydrate compositions of the four glycosyl ceramides were the same as those reported for normal plasma (6).

Digalactosyl ceramide was not isolated from plasma of normal subjects or of patients with Fabry's disease. Little or none of this lipid can be present, to judge by results of routine gas chromatographic determinations of galactose/glucose ratios after methanolysis of the GL-2 fractions. Samples from normal plasmas gave an average ratio of 1.03 ± 0.11; the same ratio was obtained with solutions containing equimolar amounts of free glucose and galactose. An average galactose/glucose ratio of 1.09 ± 0.13 was obtained with GL-2 fractions from the 10 Fabry patients listed in Table 1, which suggests that their GL-2 fraction could contain up to 3% digalactosyl ceramide (theoretical ratio = 1.06). The GL-2 from one of the Fabry patients (A.G.) appeared from a single determination to contain a larger but still small proportion of digalactosyl ceramide (ratio = 1.13), but when his plasma GL-2 was analyzed occasionally over a period of 4 months, the average galactose/glucose ratio of the plasma GL-2 fraction was 1.03 ± 0.11 (15 samples).

The levels of glycosyl ceramides in red cells from hemizygous Fabry patients are given in Table 2. Globoside (GL-4) is the predominant glycolipid, as found in normal red cells by Yamakawa and Suzuki (7), by Klenk and Lauenstein (8), and in our study (6), but the concentration of this glycolipid was lower than the normal mean level in all but one of the patients. Levels of the other glycosyl ceramides were within the range of normal values. Digalactosyl ceramide could not be detected among the glycolipids of the red cell (again according to the observed galactose/glucose ratios of GL-2); the observed carbohydrate compositions of each fraction agreed with those of normal red cells.

The concentrations of glycosyl ceramides in red cells and plasma from two normal females (Table 3) were

TABLE 1 Concentrations of Glycosyl Ceramides in Plasma from Hemizygous Patients with Fabry's Disease

Donor	Age	GL-1	GL-2	GL-3	GL-4
			μmoles/100 ml		
C.S.		1.46	0.42	1.46	0.58
W.G.	39	0.72	0.58	0.80	0.26
C.B.	55	0.50	0.42	0.52	0.16
Ri.L.*	7	0.86	0.36	0.82	0.30
C.L.*	12	0.70	0.66	0.56	0.34
D.L.*	15	0.62	0.46	0.98	0.30
Ro.L.*	9	0.78	0.44	0.80	0.44
A.G.	28	0.62	0.34	0.62	0.22
W.R.		0.78	0.44	0.38	0.26
W.W.		0.78	0.60	0.64	0.20
Mean†		0.78 ± 0.15	0.47 ± 0.08	0.76 ± 0.21	0.31 ± 0.09
Normal mean (n = 8–15)		0.98 ± 0.09	0.55 ± 0.09	0.21 ± 0.07	0.28 ± 0.08

GL-1, glucosyl ceramide; GL-2, lactosyl ceramide; GL-3, galactosylgalactosylglucosyl ceramide; GL-4, tetrahexosyl ceramide, assumed but not proven to be globoside.

* Brothers.

† 15 samples from one patient (AG), taken over 4 months during a metabolic study, gave the following mean values: GL-1, 0.73 ± 0.14; GL-2, 0.46 ± 0.05; GL-3, 0.76 ± 0.13; GL-4, 0.30 ± 0.07.

TABLE 2 CONCENTRATIONS OF GLYCOSYL CERAMIDES IN RED CELLS FROM HEMIZYGOUS PATIENTS WITH FABRY'S DISEASE

Donor	Age	GL-1	GL-2	GL-3	GL-4
			μmoles/100 ml packed cells		
C.S.		0.72	2.22	1.48	5.94
W.G.	39	0.48	0.80	1.28	7.80
C.B.	55	0.36	1.26	1.28	5.70
Ri.L.*	7	0.30	1.12	0.64	4.20
C.L.*	12	0.40	1.34	0.62	4.04
D.L.*	15	0.70	1.10	1.38	4.20
Ro.L.*	9	0.52	1.02	0.88	4.00
A.G.	28	0.24	1.32	1.44	4.54
W.W.		0.88	4.84	1.14	6.00
Mean		0.51 ± 0.17	1.67 ± 0.83	1.13 ± 0.27	5.16 ± 1.06
Normal mean(n = 8–15)		0.45 ± 0.08	1.37 ± 0.33	1.13 ± 0.25	6.38 ± 0.63

* Brothers.

TABLE 3 CONCENTRATIONS OF GLYCOSYL CERAMIDES IN PLASMA AND RED CELLS FROM NORMAL FEMALES AND RELATIVES OF PATIENTS WITH FABRY'S DISEASE

		Plasma				Red Cells			
Donor	Age	GL-1	GL-2	GL-3	GL-4	GL-1	GL-2	GL-3	GL-4
		μmoles/100 ml				*μmoles/100 ml packed cells*			
MN (normal)	22	0.54	0.54	0.24	0.12	0.48	1.92	1.28	7.34
SB (normal)	23	0.60	0.38	0.18	0.18	0.56	1.42	1.08	8.60
Male (n = 8–15)		0.98 ± 0.09	0.55 ± 0.09	0.21 ± 0.07	0.28 ± 0.08	0.45 ± 0.08	1.37 ± 0.33	1.13 ± 0.25	6.38 ± 0.63
CL (sister)	14	0.60	0.40	0.54	0.26	0.21	0.40	0.26	2.06
WG (mother)	64	0.94	0.76	0.36	0.16	0.60	1.04	1.02	7.48
Hemizygotes (Table 1)		0.78 ± 0.15	0.47 ± 0.08	0.76 ± 0.21	0.31 ± 0.09	0.51 ± 0.17	1.67 ± 0.83	1.13 ± 0.27	5.16 ± 1.06

within the range of values found with normal male subjects, whereas there was an average twofold increase in the level of GL-3 in plasmas from a heterozygous mother and a sister of one of the Fabry patients. Concentrations of the other plasma glycolipids from heterozygotes were normal.

A comparison was made of the levels of glycolipids in Fabry's disease and a related sphingolipidosis, Gaucher's disease. The results in Table 4 demonstrate a twofold elevation on the average of glucosyl ceramide in Gaucher plasma (as expected), while GL-3 levels were normal in this disease. Both disorders can be characterized, therefore, by the elevation in plasma of that glycosyl ceramide that accumulates in other tissues. In contrast to the results in Fabry's disease, the accumulating lipid (GL-1) in Gaucher's disease also accumulated to some extent in red cells (Table 4). In the sample from L.M., even the concentrations of GL-2 and globoside were increased above the normal mean value. There was a difference in the red blood cell types in these patients that might account for these high levels of glycolipids. A normal population of mature red cells was found in the sample of blood from P.T., whereas L.M. had a high percentage of immature nucleated red cells.[1] Both patients had been splenectomized some time before collection of blood for these analyses.

Thin-layer chromatography of the neutral lipids and phospholipids from Fabry plasma and red cells showed that there were no significant differences from the compositions previously observed in fractions from normal subjects.

A preliminary investigation of the sphingolipid bases from these blood glycolipids, by gas chromatography (9), has indicated that sphingosine is the predominant base in each of the glycolipids from normal and Fabry blood.

DISCUSSION

The major abnormality in the glycosyl ceramides of blood from hemizygous patients with Fabry's disease is a threefold elevation in the concentration of GL-3 in plasma (Table 1). This finding is consistent with previous findings on Fabry's disease, in which GL-3 was reported to occur in abnormally high amounts in kidney, urine, and other non-neural tissues (1,3). An increase in the level of

[1] We are grateful to Doctors Robert Lee and Maxwell Westerman for samples of blood from patients with Gaucher's disease and for clinical data about these patients.

TABLE 4 LEVELS OF GLYCOSYL CERAMIDES IN BLOOD FROM TWO PATIENTS WITH GAUCHER'S DISEASE

Donor	Age	Plasma				Red Cells			
		GL-1	GL-2	GL-3	GL-4	GL-1	GL-2	GL-3	Globoside
		μmoles/100 ml				*μmoles/100 ml packed red cells*			
PT	40	2.00	0.48	0.20	0.52	0.80	1.12	1.40	7.80
LM	64	1.96	0.56	0.20	0.22	1.36	3.92	0.84	14.40
Normal		0.98 ± 0.09	0.55 ± 0.09	0.21 ± 0.07	0.28 ± 0.08	0.45 ± 0.08	1.37 ± 0.33	1.13 ± 0.25	6.38 ± 0.63

this lipid in circulating plasma is also consistent with the reported diminution or complete absence of ceramide trihexosidase activity in biopsied intestinal cells from two hemizygous Fabry patients (5). Although the concentrations of other glycolipids in plasma did not differ appreciably from normal values, slightly lower levels of GL-1 were usually observed.

Glycosyl ceramides from two adult patients with Gaucher's disease were determined primarily to compare plasma GL-3 concentrations in this lipidosis with those found in Fabry's disease. As expected (Table 4), the GL-3 levels in Gaucher plasma and red cells were within normal ranges, while plasma GL-1 increased. It seems likely, therefore, that the increase of GL-3 concentration in plasma will be a characteristic factor in Fabry's disease, and a determination of the plasma level of this lipid might provide a useful diagnosis for the disorder.

An elevation in the concentration of GL-1 in Gaucher plasma is consistent with the findings of abnormal levels of this lipid in spleen (10) and other tissues (11). The data confirm previous results of less specific studies of glycolipids in plasma from splenectomized patients (12), and are compatible with diminished glucocerobrosidase activity in spleen (13).

Glycolipids in the red cells of Fabry patients (Table 2) did not deviate appreciably from normal levels, although an important finding might be the attenuation of globoside concentration found in all but one patient. Since globoside has been presumed to be a major precursor of the GL-3 that accumulates in tissues, a decrease might represent a protective alteration in the biosynthesis of this lipid in the marrow. Since GL-3 does not accumulate in the red cell, it might be concluded that levels of glycolipids observed in the red cell reflect anabolic activity at the bone marrow sites of red cell synthesis, rather than catabolic reactions or exchange of glycolipids into the red cells at a later time. Whatever the explanation, it is interesting that a cell population so rich in glycolipids does not accumulate GL-3 in Fabry's disease.

In the only two female relatives of Fabry patients that have been studied so far, plasma GL-3 levels were higher than in blood from normal women (Table 3). This result agrees with the reported attenuation in the activity of ceramide trihexosidase in the mother of a Fabry patient (5). The intermediate levels of GL-3 in these females might also be compatible with the occasional display of some of the clinical symptoms by heterozygous carriers of the disease, and might be explained by Lyon's hypothesis of inactivation of the locus on one of the X chromosomes in the female (14).

Glycolipids in the red cells of one female relative of a Fabry patient (C.L.) were distinctly different from normal values as well as from those of the heterozygous mother (W.G.). However, the red cell glycolipids in the hemizygous patients in this L family were also different from those of other hemizygotes. Although GL-3 was consistently increased in the plasmas of these patients and their sister, and the clinical symptoms were typical of Fabry's disease, the unusual pattern of red cell glycolipids may indicate that the genetic defect in the L family differs somewhat from that of the W.G. family and others that have been examined. Additional data are needed to show whether genetic variants in this disease will show different profiles of glycolipids in plasma or red cells. A relationship of blood glycolipids to age cannot be ruled out either, and it may be that there are specific disturbances in the intermediary metabolism of red cell glycosyl ceramides at some stages in the disease.

Little or no digalactosyl ceramide could be detected by our analytical procedure in plasma or red cells from any of the Fabry patients or normal subjects, but this substance has been shown by Mårtensson (15) to be a component of normal kidney, and it accumulates in Fabry kidney and urine. It has not yet been found in other non-neural tissues,[2] although small amounts were reported to occur in brain lipids in Tay–Sachs disease by Gatt and Berman (16). These data suggest that digalactosyl ceramide is normally synthesized and metabolized exclusively in the kidney, and that it does not contribute to the plasma pool of glycolipids. This proposal is strengthened by the results in Fabry's disease, where digalactosyl ceramide occurs in abnormal quantities in the kidney, but is absent from plasma. If the behavior

[2] Studies in this laboratory have shown that the major glycolipid of leukocytes is a dihexosyl ceramide with equal amounts of glucose and galactose (Snyder, P., D. E. Vance, and C. C. Sweeley, unpublished). We have not, therefore, been able to confirm the reported occurrence (17) of digalactosyl ceramide in these cells.

of this "marker" glycolipid were typical of the class, then the plasma and kidney pools of all the glycolipids would be separate. If this were true of other tissues as well, the source of increased GL-3 in Fabry plasma might be restricted to liver, where it is presumed to be synthesized for incorporation into lipoproteins of plasma, and to the GL-3 and globoside of red cells. Such possibilities are presently under investigation in a study of the turnover of individual blood glycosyl ceramides.

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