GLOBOID CELL LEUKODYSTROPHY: ADDITIONAL DEFICIENCY OF PSYCHOSINE GALACTOSIDASE

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Summary; Activities of psychosine galactosidase (galactosylsphingosine galactosyl hydrolase) were extremely deficient in the brain, liver and kidney of patients with globoid cell leukodystrophy. The enzyme was similarly deficient in tissues of a fetus affected with this disease. This is the second enzymatic block demonstrated in this disease, since genetic deficiency of galactocerebroside β-galactosidase has previously been established. This finding may provide a clue to the understanding of the pathogenesis of this disorder.

Globoid cell leukodystrophy (Krabbe's disease) is a genetically determined, rapidly fatal neurological disorder of infants. We have previously demonstrated profound lack of galactocerebroside β-galactosidase activities in various tissues, serum, leukocytes and cultured fibroblasts of patients with this disease (1 - 3). Intrauterine diagnosis of an affected fetus was accomplished based on the deficient activity of galactocerebrosidase in cultured amniotic fluid cells (4). Serum, leukocytes and fibroblasts of parents of patients show intermediate activities of the enzyme, as expected for a primary enzymatic defect of the autosomal recessive nature (5).

While the deficient galactocerebroside β-galactosidase appears to explain most of the clinical and morphological features of the disease, it does not readily explain the rapid and almost total disappearance of oligodendroglial cells. Insertion of solid

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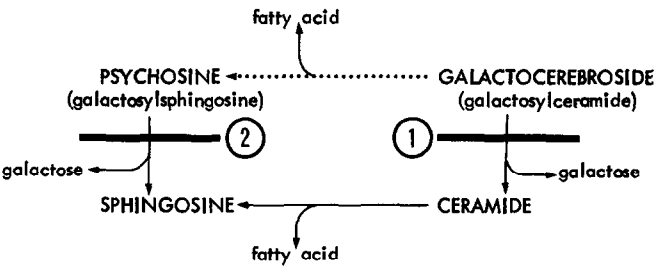

Figure 1. Degradative pathway of galactocerebroside. We have previously demonstrated the genetic block at Step 1 in globoid cell leukodystrophy. The present report describes an additional enzymatic block at Step 2. The dotted arrow indicates the still hypothetical initial de-acylation of galactocerebroside.

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galactocerebroside into rat brain produces a globoid cell reaction essentially identical with that in affected human patients (6), but oligodendroglia appear unaffected in such experimental conditions. Attempts to clarify the pathogenesis of the massive oligodendroglial cell destruction led us to the present investigation. Galactocerebroside is normally degraded first to ceramide by galactocerebroside β-galactosidase (7), and then to sphingosine and fatty acid by ceramidase (8) (Fig. 1). There is, however, a logically possible alternate degradative pathway for galactocerebroside, involving initial de-acylation to psychosine (galactosylsphingosine) followed by cleavage of galactose from psychosine. Psychosine, with its free amino group, is known to be cytotoxic (9). If such a hypothetical degradative pathway exists, it will be the only available route for cerebroside degradation in patients with globoid cell leukodystrophy because of the block at the step of galactocerebroside β-galactosidase. It would result in constant formation of psychosine which could exert injurious effects to the tissue. Furthermore, if there is an additional block to cleave galactose from psychosine, such toxic effects would be even more prominent, because there might then be an accumulation of psychosine in the tissue.

Materials and Methods: For this investigation, postmortem specimens of the brains, livers and kidneys of two patients with globoid cell leukodystrophy

were used. Postmortem specimens of age-matched normal individuals were included as controls. In addition, brain specimens of patients with varieties of hereditary or demyelinating neurological disorders were examined as pathological controls. Also included were the brain and liver of the fetus with globoid cell leukodystrophy and those of two control fetuses. All specimens had been stored frozen at -30°C until the assay. Previous enzymatic assays had shown that galactocerebroside β-galactosidase was extremely deficient in all globoid cell leukodystrophy specimens included in this investigation. Activities of psychosine galactosidase were determined according to the standard procedure we described previously for the rat brain enzyme (10). In order to avoid the loss of activity during purification, a 10% aqueous homogenate of tissues was used as the enzyme source, rather than the partially purified enzyme. The homogenate was disrupted ultrasonically and frozen-thawed four times. The amount of the labelled substrate was increased to 200μg/tube, from 20μg/tube in the standard system, to offset the effect of endogenous galactocerebroside which is a competitive inhibitor of psychosine galactosidase (10).

Results: The results clearly indicated that psychosine galactosidase activities were markedly deficient in the brain, liver and kidney of patients with globoid cell leukodystrophy, while high activities were observed in all normal and pathological controls (Table 1). The brain and liver of the fetus, aborted on the basis of deficient galactocerebroside β-galactosidase (4), also showed essentially non-detectable psychosine galactosidase activity. The presence of inhibitors in the pathological specimens was excluded by mixing experiments. Equally deficient activities in histologically normal liver and kidney, as well as in the fetal tissues, indicated that the deficient activities are unrelated to histological alterations. The high activities in varieties of pathological controls assured that the deficiency is specific for globoid cell leukodystrophy.

Table 1. Psychosine Galactosidase in Globoid Cell Leukodystrophy

Specimen	Activity (nmoles/hr/g tissue)
Gray Matter	
Globoid cell leukodystrophy (n = 2)	0, 2.7
Pathological controls (n = 5)*	58.9 - 170 (mean = 88.1)
Normal controls (n = 3)	109, 43.5, 45.8
White Matter	
Globoid cell leukodystrophy (n = 2)	0, 1.4
Pathological controls (n = 5)*	52.5 - 181 (mean = 129)
Normal controls (n = 3)	129, 62.2, 46.9
Liver	
Globoid cell leukodystrophy (n = 2)	7.1, 9.2
Normal controls (n = 2)	65.8, 40.2
Kidney	
Globoid cell leukodystrophy (n = 2)	6.0, 5.6
Normal controls (n = 2)	72.2, 76.0
Fetal Brain	
Globoid cell leukodystrophy (n = 1)	0.48
Controls (n = 2)	104, 92.3
Fetal Liver	
Globoid cell leukodystrophy (n = 1)	0.96
Controls (n = 2)	108, 131

* Pathological controls included tissues of patients with Schilder's disease, G_{M1}-gangliosidosis, Tay-Sachs disease, Hurler syndrome and metachromatic leukodystrophy. All specimens of globoid cell leukodystrophy patients had previously shown marked deficiency of galactocerebroside β-galactosidase.

Discussion: These observations establish that there is an additional enzymatic block in globoid cell leukodystrophy at the step of psychosine degradation. This finding is consistent with the above hypothesis that psychosine might be the compound responsible for the extensive cellular destruction. The postulated initial de-acylation of galactocerebroside, however, has not been demonstrated. Furthermore,

a preliminary analysis of brain tissue of a patient failed to detect psychosine in an extractable free form. This finding, however, does not yet exclude our hypothesis, because psychosine is highly reactive with the free amino group, and if it is formed in situ, it is likely to form complexes with other compounds, which may not be easily detectable unless a suitable analytical procedure is devised. On the other hand, we have obtained further evidence for the highly toxic nature of psychosine. When approximately 1 mg of psychosine was injected into the cerebral hemisphere of young rats, majority of them died of severe hemorrhagic necrosis and edema within several hours, while we had never experienced any mortality when rats were similarly injected with other lipids, such as galactocerebroside. Psychosine was also extremely toxic to cultured rat cerebellar explants. At concentrations of 1 mM and 0.1 mM, all explants underwent complete degeneration within two days, while galactocerebroside did not show such toxicity at these concentrations (Silberberg, Miyatake and Suzuki, preliminary observation). More detailed studies are in progress in this direction.

The genetic implications of the deficient psychosine galactosidase cannot be properly assessed at present, because the possibility of a single β-galactosidase catalyzing both galactocerebroside and psychosine degradation has not been excluded (10). If the same enzyme catalyzes the two reactions, then our findings are to be expected. The significance of psychosine galactosidase deficiency in relation to the pathogenesis of globoid cell leukodystrophy, however, still need to be explored. On the other hand, if these two reactions involve closely related but different enzymes, we have an enzymatically and genetically intriguing problem similar to that in other apparent multiple enzyme deficiencies, such as total hexosaminidase deficiency (11) or multiple sulfatase deficiency (12).

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