THE GAUCHER MOUSE

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Summary

A model of Gauchers disease was produced through the administration of conduritol B-expoxide. Tissue levels of glucosylceramide were elevated in the experimental animals. The activity of β-glucosidase in homogenates of brain, liver, and spleen was reduced 93% in the treated animals. Six other lysosomal hydrolases measured were uneffected.

Introduction

Gaucher's disease is classified as one of the group of sphingolipidoses and is transmitted as an autosomal recessive trait. There are two distinct clinical types which have been well documented. The infantile or acute type is uniformly fatal and has central nervous system damage. The adult type, in which there is no central nervous system problem, is relatively benign and is characterized by a variety of hematological changes primarily due to splenic enlargement. The currently accepted biochemical definition of the disease is an elevation of tissue levels of glucosylceramide (1) in concert with a decreased activity of its associated hydrolytic enzyme activity. This decreased β-glucosidase activity is detectable when either glycosylceramide (2), 4MU- or pNP-β-D-glucoside (3) or glucosyl sphingosine (4) are utilized as the substrates.

During studies on the effect of various compounds on the purified β-glucosidase from calf spleen (5) and brain (6) it was found that conduritol B-expoxide was an extremely effective inhibitor. We wish to report the pharmacological production of a "Gaucher mouse" by the *in vivo* administration of this material.

Materials and Methods

Conduritol B expoxide was synthesized as previously described (7),

dissolved in water, and administered to experimental animals at 100 mg/kg body weight dosage while control animals received only water. In one experiment 90 day old C57/Bl mice were injected intraperitonaly daily for a three week period. Another group of mice received the drug subcutaneously one day after birth until 4 weeks of age, at the same dosage level.

The glucosylceramide was extracted from the tissue and purified by a published procedure (1). The sphingosine base was quantitated fluorimetrically after acid hydrolysis according to a published technique (8). Aliquots of a water homogenate were employed to quantitate the level of several lysosomal acid hydrolases according to published procedures (9) using the 4-methylumbelliferyl glycosides as substrates.

Results

Initial experiments were undertaken to establish the toxicity of the conduritol B-expoxide and there were no adverse effects at doses as high as 100 mg/kg body weight.Therefore, a routine daily injection at 100 mg/kg was adopted inorder to conserve this synthetic compound.

Three month old mice

There were no weight differences seen between the experimental and control animals during the course of these studies nor were there any behavioral differences evident. The level of glucosylceramide present in spleen, liver, and brain tissue of these animals is shown in Table 1, part A. It is evident that there is an approximately 50% increase in the quantity of glucosylceramide present in the peripheral tissues from the experimental animals. There was an approximately five fold increase in the brain samples from the treated animals.

It was of interest to examine the levels of several hydrolytic enzymes from these animals and this information is presented in Table 2 part A. There is a very drastic reduction in detectable β-glucosidase activity. Mixing aliquots of control and experimental homogenates gave expected values suggesting that the reduced activity is not caused by the presence of an inhibitor. Free conduritol B expoxide is apparently not accumulated in the tissues but possibly excreted or converted to inactive products. The tissue concentration from the previous injection would be too low to affect the control homogenates. The level of several other hydrolytic enzymes were not significantly different from that of controls.

Table 1. Tissue levels of glucosylceramide in experimental and control mice.*

			Glucosylceramide (nmoles/gr wet wt)
A.	3 month animals	Tissue	
	Experimental	Spleen	138
	Control	Spleen	74
	Experimental	Liver	127
	Control	Liver	75
	Experimental	Brain	33
	Control	Brain	6.9
B.	Infant Animals		
	Experimental	Brain	60
	Control	Brain	21
	Experimental	Liver	49.3
	Control	Liver	32.2

*Each value is the average of analysis of at least 3 tissue samples from separate animals.

Table 2. A comparison of tissue levels of several hydrolytic enzyme activities from experimental and control animals*

A. 3 month animals	α-mannoside	β-glucoside	β-hexosaminide	β-galactoside	α-glucoside	β-glucuronide
Experimental liver	17.6	2.24	278	5.84	2.57	5.49
Control liver	20.1	67.8	287	6.6	3.22	8.24
Experimental brain	13.11	0.1	2016	17.8	4.45	5.4
Control brain	8.66	9.49	966	16.47	4.12	3.09
B. Infant Mice'						
Experimental spleen	86.2	1.49	1483	53.8	2.24	56.6
Control spleen	60.13	21.0	1858	55.3	5.79	80.2
Experimental brain	24.4	1.25	1896	22.26	1.52	15.18
Control brain	11.4	17.84	781	28.1	1.79	5.11

* All values expressed as nmoles substrate hydrolyzed /mg protein/hr. Tissues from 3 different 3 month old animals, 8 experimental infant mice or 16 control mice were assayed.

Infant animals

There was a significant difference in the growth of the experimental animals as compared to the controls. The various organs were removed and weighed. Expressed as percentages total body weight there was no difference for brain, liver, and kidney, however, the spleen weight was found to be reduced 40% as compared to controls.

The experimental animals were found to show signs of tail arching, potentiated high amplitude action tremor, minimal startle response, normal swimming and righting response. This suggests a central nervous system involvement, presumable gray matter, without changes in the peripheral nervous system.

The quantity of glucosylceramide in the brain and other tissues from these animals is listed in Table 1 part B. There is a 3 fold elevation of the level of this sphingolipid in the brain samples from experimental animals and only a 50% increase in liver tissue.

The spleen tissue and brain tissue were assayed for their level of acid hydrolases and these results are presented in Table 2 part B. It is quite apparent that there is a drastic reduction of β-glucosidase activity in the experimental animals to a level of only 7% of control animals. Mixing aliquots of these homogenates did not suggest the presence of inhibitory substances. Elevated levels were seen with hexosaminidase and mannosidase, while α-glucosidase and β-galactosidase were close to that of normal brain tissue. The only other activity showing any significant decrease was that of splenic α-glucosidase, of these young animals.

Samples of the sphingolipid-containing silicic acid column fraction were subjected to borate impregnated TLC. Material comigrating with glycosylceramide was found present only in the experimental animal's brain.

Discussion

Several compounds have been found to inhibit purified calf brain and spleen β-glucosidase (5,6). These included conduritol B expoxide which had

been shown to be a site-specific irreversible competitive inhibitor of non-mammalian β-glucosidase (10). This is a small molecular weight (162), water soluble material structurally related to inositol. It was, therefore, decided to test the ability of this compound to cause β-glucosidase inhibition in vivo, in an attempt to produce an animal model of Gauchers disease. The data presented in Tables 1 and 2 strongly indicate that from the biochemical definition of this disease this has been successful. The apparent specificity of the inhibitor is evident from the data provided for all the experimental samples. Of 6 hydrolases assayed for, only β-glucosidase activity was severely decreased. This was not due to the presence of free conduritol B expoxide in the tissue homogenates of the experimentals as evident from mixing experiments. This suggests a high degree of specificity for this compound in its ability to irreversibly inhibit only the β-glucosidase in vivo.

The ability of conduritol B expoxide to enter the brain as judged by its biochemical consequences, is probably due to its structural similiarity to glucose. The clinical manifestations of the treated animals corroborate the biochemical changes seen in brain tissues.

This is the first report of the creation of a model for a sphingolipidosis by a pharmocological agent. This should provide the opportunity to study the pathogenesis of a lysosomal storage disease. In humans the occurance is Mendelian with 25% of the offspring affected. In the injected mice it is 100%. This animal also allows for examining central nervous system alterations since the brain is also effected. The animal probably will not be useful for studying enzyme replacement therapy or the genetics of the disease.

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