Diagnosis of Feline $G_M1$ Gangliosidosis by Enzyme Assay of Cultured Conjunctival Cells

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$G_M1$ gangliosidosis is characterized by a deficiency in the lysosomal hydrolase $\beta$-galactosidase, progressive nervous system disease and ocular lesions. Diagnosis of $G_M1$ gangliosidosis in humans and cats with the analogous disease has been made by measurement of the enzyme activity in various tissues including brain, liver and cultured skin fibroblasts. The authors report the use of cultured conjunctival cells for this purpose derived from cats with feline $G_M1$ gangliosidosis, a model of the human disease (juvenile $G_M1$ gangliosidosis, Derry's disease). Full thickness conjunctival biopsies from three cats with $G_M1$ gangliosidosis and two normal controls were used to initiate cell cultures. Optimal conditions for $\beta$-galactosidase activity were established with an uncultured conjunctival biopsy from a normal cat. The fluorogen, 4-methylumbelliferyl-$\beta$-D-galactopyranoside was used as substrate. After 2 months in culture, and 2 weeks after subculture, cells from cats affected with $G_M1$ gangliosidosis exhibited specific activities for $\beta$-galactosidase of 10, 9 and 12 nmoles 4MU/hr/mg protein, whereas specific activities for normals were 630 and 469 nmoles 4MU/hr/mg. Enzymatic analysis of cultured conjunctival cells may offer an effective alternative for the diagnosis of $G_M1$ gangliosidosis.


$G_M1$ gangliosidosis, a genetic disorder of man and other animals, is characterized by a deficiency in a lysosomal enzyme, acid $\beta$-galactosidase. In humans, three phenotypes exist, infantile, juvenile and adult. They can be distinguished by their age of onset and clinical findings. In the infantile form, the most severe, a cherry-red spot may be seen in the macula in approximately 50% of the cases. Diagnosis of all types is confirmed by the demonstration of a deficiency in the acid $\beta$-galactosidase in various tissues and body fluids. Leukocytes, tears, urine and cultured fibroblasts have been used for enzyme assay. Heterozygote detection and prenatal diagnosis are possible.

Defective catabolism of $G_M1$ ganglioside has also been documented in cats, dogs and cattle and has been reviewed previously.2 Biochemical and ultrastructural studies in cats with $G_M1$ gangliosidosis have shown remarkable similarity to the juvenile (Type II or Derry’s) disease in man.3 The deficiency of acid $\beta$-galactosidase in affected cats has been demonstrated for various tissues including brain, liver, skin and cultured fibroblasts.4

The demonstration of morphological changes in the ultrastructure of conjunctiva has been used in the diagnosis of metabolic diseases.5-9 The present study extends the use of conjunctiva through enzymatic analysis of cells cultured from conjunctival biopsies.

An additional aspect of this investigation was to determine acid $\beta$-galactosidase activity of conjunctival cells from a normal cat at initial biopsy and after successive passages in culture. Previous investigators10,11 have shown that enzyme activities in cell lines from different tissues vary with time in culture. This has not been reported for $\beta$-galactosidase in feline conjunctival cells. Any variation would be important to know since normal controls are used for comparison when an assay is performed to diagnose an enzyme deficiency.

Materials and Methods

Optimal conditions for $\beta$-galactosidase activity using 4-methylumbelliferyl-$\beta$-D-galactopyranoside (Sigma Chemicals, St. Louis, MO) as substrate were established with an uncultured conjunctival biopsy taken from a normal cat under general anesthesia.
The tissue was placed in 1.5 ml of 0.5% n-octyl-β-D-glucoside and homogenized by 20 strokes in a Dounce homogenizer. The homogenizer was rinsed four times with 100 μl/rinse of 0.5% octylglucoside (Sigma). The total, homogenate and washings, was then transferred to a centrifuge tube and centrifuged at 5°C for 30 min at 10,000 g. The supernatant was retained for assay. Determination of enzyme activity was carried out in a total volume of 0.5 ml by modification of a previously reported procedure. The optimized reaction mixture consisted of 0.1 M sodium acetate, pH 4.5, 0.05 M NaCl, 2.5 mM 4-methylumbelliferyl-β-D-galactopyranoside and conjunctival supernatant. After 2 hr at 37°C, the reaction was stopped by the addition of 2.5 ml glycine-NaOH buffer, pH 11. Fluorescence was determined at 365 (excitation) and 450 (emission) nm. Specific activity was expressed as nmoles 4-methylumbellifereone released/hr/mg protein. Protein was determined by the method of Lowry et al.

Full thickness conjunctival biopsies taken under sterile conditions from anesthetized cats with GM1 gangliosidosis and from normal controls were used to initiate cell cultures. Several tissue pieces approximately 1 mm² in size were placed in 25 cm² Corning tissue culture flasks and incubated at 37°C in McCoy’s 5A medium supplemented with 10% fetal bovine serum and 1% antibiotics. Medium was changed twice weekly and cells were subcultured after reaching confluency. Cultures were maintained for 2 months. Two weeks after the last passage, the cells were harvested, sonicated in 0.5% octylglucoside, centrifuged and the supernatant used for the determination of β-galactosidase activity. Cultured cells from three mutant and two normal cats were studied.

A separate conjunctival biopsy from the normal cat used in the optimization study was placed in culture, in the manner indicated above, in order to determine the specific activity at successive passages in culture. At 2 week intervals, some cultures were harvested, sonicated in 0.5% octylglucoside, centrifuged and the supernatant assayed for β-galactosidase activity as indicated above while the remaining cells were subcultured and maintained for the next 2 week interval with twice weekly changes of medium.

All animals used in this study were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Results

The activity of β-galactosidase in uncultured conjunctival tissue from a normal cat, as a function of time exposed to substrate, is shown in Figure 1. The activity did not show a substantial deviation from linearity over 2 hr of incubation. Activity was linear with enzyme concentration to 100 μg/ml (Fig. 2). The optimum pH for substrate hydrolysis was 4.5 (Fig. 3). Stimulation of β-galactosidase hydrolytic activity by NaCl, shown in Figure 4, demonstrates peak activity
The specific activity of β-galactosidase of the cultured conjunctival cells from a normal cat was determined biweekly. Table 1 shows that the specific activity increased with each successive passage. The cells in the initial outgrowth from the biopsy were epithelial-like in appearance. After the first passage, and subsequently, the predominant cell type noted in culture was fibroblast-like, as shown in Figure 5.

The specific activities for the three mutants using the optimized conditions above, were 10, 9 and 12 nmoles 4MU/hr/mg protein and for the two normals were 630 and 469 nmoles 4MU/hr/mg protein after 8 weeks in culture. The average value of the specific activities for the mutants was reduced to approximately 2% of that for the normals. Within each group the relative range was virtually identical with the lowest value being 75% of the highest for the mutant cats and 74.4% for the normal cats.

A mixture of supernatants from a normal and an affected cat containing equal amounts of protein had a specific activity higher than the expected average indicating that an inhibitor was not present (data not shown).

By comparing specific activities of reaction mixtures containing octylglucoside versus water for both a normal and an affected cat it was also demonstrated that the presence of octylglucoside did not adversely affect the specific activity of acid β-galactosidase. In fact, the specific activity was slightly higher in the mixture containing octylglucoside for both the normal and the affected cat (data not shown).

**Discussion**

Cats with G1M gangliosidosis were distinguished clearly from normals on the basis of the specific activity of acid β-galactosidase in cultured conjunctival cells. The increasing specific activity with time in culture implies that a comparison of acid β-galactosidase activity between affected and normal cats could be substantially altered if any change in specific activity dependent on time in culture were not taken into consideration. Enzymatic analysis of cultured cells from a conjunctival biopsy may be an effective alternative means of diagnosis when G1M gangliosidosis is suspected in humans. The procedure is easily per-

**Table 1. Specific activity of acid β-galactosidase in normal cat conjunctival cells with successive passages at 2 week intervals**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Passage</th>
<th>Weeks</th>
<th>Specific activity (nm 4MU released/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncultured</td>
<td>0</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>Cultured</td>
<td>1</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>Cultured</td>
<td>2</td>
<td>4</td>
<td>290</td>
</tr>
<tr>
<td>Cultured</td>
<td>3</td>
<td>6</td>
<td>441</td>
</tr>
</tbody>
</table>
formed with a topical anesthetic. It is safe and, unlike skin biopsies, is nondisfiguring.

Key words: β-galactosidase, cat, GM$_1$ gangliosidosis, tissue culture, conjunctiva

References