Apparent Normal Leukocyte Acid Maltase Activity in Glycogen Storage Disease Type II (Pompe's Disease)

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We present a case of glycogen storage disease type II (Pompe's disease) with the classical clinical presentation and characteristic electrocardiographic changes of this disorder. An acid maltase (EC 3.2.1.20) determination in the peripheral leukocytes revealed normal activity; however, acid maltase activity was completely absent in a pre-mortem skeletal muscle biopsy. Post-mortem studies showed acid maltase activity to be absent in all tissues examined, including cultured skin fibroblasts. Massive glycogen deposition corresponded to the localization of the enzymic deficiency, except in the brain, where glycogen content was within the normal range. The acid maltase activity in mixed peripheral leukocytes was due to an isoenzyme of acid maltase in the granulocyte series. Antenatal diagnosis was accurate in a subsequent pregnancy, but discordance between enzyme activity in different cell lines in an individual with a genetic disease is a conceivable source of error in both prenatal and postnatal diagnoses.

Additional Keyphrases: enzyme activity · heritable disorders · isoenzymes · prenatal diagnosis

Leukocyte enzymic assay is a well-recognized and widely utilized technique for diagnosis of many lysosomal storage diseases. We report a case of glycogen storage disease type II (Pompe's disease) that presented a diagnostic problem because the mixed peripheral leukocytes showed a normal acid maltase (EC 3.2.1.20; α-D-glucoside glucohydrolase) activity in a patient who had the classical clinical features of the disorder.

Case Report

The patient was an eight-month-old white girl who had recurrent earaches, rhinorrhea, and cough since the age of five months. Three weeks before admission, she showed decreased appetite and became lethargic. On admission, she was pale and in mild respiratory distress. Radiologic examination revealed marked cardiomegaly, hyperaeration of the left upper lobe, and atelectasis of the left lower lobe. An electrocardiogram showed a short PR interval (0.07), left axis deviation for age (45°), abnormal primary ST-T wave changes, and left ventricular hypertrophy. At this time a clinical diagnosis of Pompe's disease was made. However, leukocyte acid maltase activity was normal. A specimen from biopsy of the gastrocnemius muscle was entirely devoid of acid maltase activity. The patient showed a transient response to digitalization, but remained cyanotic when removed from the supplemental oxygen. She refused oral feedings, her overall condition deteriorated rapidly, and she succumbed in cardiopulmonary failure. An autopsy was carried out 2 h after death.

Materials and Methods

Leukocytes were isolated by the method of Percy and Brady (1), as modified (2). Tissues were prepared for enzymic analysis by homogenization in water at 5 °C as a 100 g/L suspension, with use of a Waring Blender. The homogenate was centrifuged at 15 000 rpm for 30 min, and the clear supernatant was used for analysis. Acid maltase activity was assayed at pH 4.0, with maltose as the substrate according to the method of Nitowsky and Grunfeld (3). Acid maltase activity in urine and in cultured skin fibroblasts was measured according to the method of Salafsky and Nadler, with use of 4-methylumbelliferyl-α-D-glucoside (4). Protein was determined by the method of Lowry et al. (5). Glycogen was isolated from tissues of the patient and control tissues by the method of Bergstrom et al. (6) and quantitated by the orcinol/sulfuric acid method (7). The absorption spectra of the isolated glycogens were determined as the iodine complexes.

Results and Discussion

Table 1 summarizes the results of analyses for total glycogen in the various tissues of the patient. The absorption spectra of the glycogen–iodine complexes were characteristic for normal glycogen. Acid maltase activity was absent from all the tissues listed, and glycogen accumulation correlated with enzymic deficiency in these tissues, except for cerebellum tissue. No acid maltase activity could be detected in cultured skin fibroblasts from the patient. Acid maltase activity in leukocytes was 4.3 μmol of maltose split per gram of protein per minute (normal 3.5–5.5). Acid maltase activity was measured in urine with 4-methylumbelliferyl-α-D-glucoside as substrate. The ratio of the enzyme's activity at pH 4 to that at pH 6 was 0.9 for the patient, compared with an average of 2.5 for five control urines. These results are similar to those reported by Salafsky and Nadler (4).

Pompe's disease is an autosomal recessive disorder characterized by widespread deposition of membrane-bound glycogen of apparently normal structure (8). In the infantile form of the disorder, profound hypotonia, cardiomegaly, and an abnormal electrocardiogram are early findings. A progressive downhill course then ensues, dominated by muscular weakness, respiratory tract infections, and poor growth. Death usually occurs within the first year of life, by cardio-respiratory failure; no treatment is known. The accumulation of glycogen is thought to be related to the deficiency of a lysosomal acid maltase (9). The findings in most reported cases support this concept of the disorder, particularly in heart, skeletal muscle, and liver. The occurrence of extralysosomal glycogen in the tissues of at least some patients has raised the question of a second enzymic defect (10). Furthermore, the discovery of the age-related, relatively mild acid maltase-deficient variants, with a more restricted tissue distribution but without massive glycogen accumulation, reinforces the fact that a completely coherent picture of Pompe's disease has not yet emerged (11, 12).

Apart from the very apparent diagnostic considerations, our findings for mixed leukocytes represent a departure from the initial report that in the infantile form of the disorder

leukocyte acid maltase was deficient (13). Since that time, variable results have been found with the use of leukocytes, prepared in various ways, for the diagnosis of the infantile form of Pompe's disease (14–18).

The apparent discrepancies now seem to have been resolved by the finding that an isoenzyme of acid maltase is normally present in kidney cells and in the granulocytes of the peripheral leukocytes (19). Both the acid maltase isoenzyme and the renal maltase isoenzyme are present in the granulocytes, whereas in the lymphocytes only acid maltase isoenzyme is found. Deficient acid maltase activity will thus be found in the lymphocytes in patients with Pompe's disease, while a residual activity of the renal maltase will be present in their granulocytes. Therefore, the diagnosis of Pompe's disease can be made from an analysis of lymphocytes, cultured skin fibroblasts, or liver or skeletal muscle biopsy material.

From the diagnostic point of view, this case illustrates the hazard of extrapolation from the results of mixed leukocyte analyses to the whole patient. This caveat extends to prenatal diagnosis, where the basic assumption is that the enzymic constitution of cultured amniotic fluid cells precisely reflects the metabolic aberration causing or associated with the disorder in question. The fact that the fibroblasts were devoid of acid maltase activity strongly suggested that accurate antenatal diagnosis was possible in this family.

In a subsequent pregnancy in this family, amniocentesis values for acid maltase activity were one-half that of the control values, indicating a nonaffected fetus. Electron microscopy of the amniotic fluid cells revealed no evidence of lysosomal accumulations of glycogen.\(^4\) The prediction that the child would be phenotypically normal was confirmed upon delivery. The case illustrates the importance of mobilizing as much information as possible, not only for diagnostic purposes, but also for a more complete understanding of the molecular basis and pathophysiology of genetic disorders.

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References