Histopathological Findings in the Biopsied Muscle of a Juvenile Type III Glycogenosis

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Abstract: We report a case of juvenile type III glycogenosis that was confirmed by histopathological and biochemical studies. The histopathological findings consisted of vacuoles, periodic acid-Schiff positive materials, type 2B fiber deficiency, mildly positive acid phosphatase reaction and intensely positive non-specific esterase reaction. It is suggested that the enzyme reactions may be related to membrane-bound sacs containing glycogen.

Key words: type III glycogenosis, debranching enzyme deficiency, acid phosphatase, non-specific esterase, type 2B fiber deficiency.

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Introduction

Type III glycogenosis has sometimes been called “Forbes’ disease”, as the clinical description of the first patient recognized to have an abnormal polysaccharide in the liver and skeletal muscle was by Forbes (Illyingworth et al., 1956; Forbes, 1953; Brown, 1986). The symptoms and signs of type III glycogenosis, which have a debranching enzyme deficiency, are hepatomegaly, growth retardation, tendency to hypoglycemia, failure to thrive, and elevated liver enzymes in serum (Brown, 1986). Murase et al. (1973), Hokezu et al. (1983), Ugawa et al. (1983), Yamane et al. (1984), and Watanabe et al. (1986) reported several cases of type III glycogenosis in Japan.

Although the enzyme defect is expressed in muscle tissue, clinical myopathy is frequently overlooked. DiMauro et al. (1979) reported a neuromuscular disorder in five adult type III glycogenosis. The clinical picture of these five patients was characterized by muscle weakness, that probably was present since childhood but not recognized as a problem until adult life.

We report histopathological findings in the biopsied muscle of a case of juvenile type III glycogenosis with an elevated creatine kinase value and subtle symptoms of myopathy.
Case

A 15-year-old boy who began to feel tired quite easily in the fall of 1988, came to the clinic of the Third Department of Internal Medicine, University of Occupational and Environmental Health, for a medical check up. As a blood chemistry study revealed an elevated creatine kinase value with abnormal serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels, he was referred and admitted to the Department of Neurology for further evaluation on January 20, 1989. There was no family history of neuromuscular diseases.

He was 151 cm tall, and weighed 33 kg. There was no hepatosplenomegaly. His muscles were slightly weak, deep tendon reflexes were normal, and sensation was intact.

Blood laboratory findings on admission included creatine kinase 1,366 units, serum glutamic oxaloacetic transaminase 359 units, serum glutamic pyruvic transaminase 315 units, lactate dehydrogenase 734 and aldolase 6.9 units. An electromyogram included a few low amplitude potentials, and results of nerve conduction studies were normal. For histochemical, electron microscopic and biochemical studies, a left deltoid muscle biopsy was performed and one portion of the specimen was submitted to our laboratory for histological and histochemical stains and reactions.

Methods

The portion of the specimen was frozen in isopentane, cooled with liquid nitrogen, stored at −80°C, and 10 μm thick frozen sections were sliced with a cryostat. The following histological and histochemical stains and reactions were carried out as described by Dubowitz (1985): hematoxylin and eosin (HE), modified Gomori's trichrome reaction, periodic acid-Schiff (PAS), oil red O, nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR), menadione linked α-glycerophosphate dehydrogenase (MAG), acid phosphatase, alkaline phosphatase, non-specific esterase, ATPase, phosphorylase, phosphofructokinase, AMP deaminase and cytochrome c oxidase.

Three hundred and eighty-six muscle fibers in ATPase reactions were classified into four fiber types: type 1, 2A, 2B, and 2C (Dubowitz 1985), and muscle fiber diameters of each fiber type were measured in photographs of 150 magnifications with an image analyzer (Avio Excel, Nippon Avionics Co.).

For electron microscopic studies, another portion of the specimen was processed at the Department of Neurology, and for biochemical analyses of glycolytic enzymes, the remaining portion of the specimen was shipped in dry ice to the Department of Pediatrics, Hamamatsu Medical College.
Type III Glycogenosis

Results

There was no prominent variation in muscle fiber size measuring from 20 to 60 μm in caliber on HE, and neither degenerated nor regenerated fibers were found. From 5 to 10% of the muscle fibers contained vacuoles and small basophilic droplets. A few vacuoles (Fig. 1) were located beneath the sarcolemma and the other vacuoles (Fig. 2A) were found in the central portion of the sarcoplasm. The number of internal nuclei was not increased and no inflammatory cell infiltration was found. No ragged-red fibers or nemaline bodies were recognized on modified Gomori's trichrome reaction.

Most vacuoles seen on HE were stained with PAS, and serial sections on PAS and ATPase showed that type 2 fibers contained more PAS positive materials than type 1 fibers (Fig. 2). The PAS positive materials became negatively stained with PAS after α-amylase preincubation, suggesting the presence of polysaccharide. Some muscle fibers had several foci of mild acid phosphatase reactions (Fig. 3) and high enzyme activity by non-specific esterase (Fig. 4).

Type 2B fiber deficiency was found in fiber type distribution on ATPase: type 1; 62.3%, type 2A; 36.3%, and type 2B; 1.4% (Fig. 5).

Other histochemical reactions including oil red O, NADH-TR, MAG, alkaline phosphatase, phosphorylase, phosphofructokinase, AMP deaminase and cytochrome c oxidase did not show any abnormal findings.

Electron micrographs revealed subsarcolemmal and intermyofibrillar glycogen accumulations and autophagic vacuoles (Fig. 6).

Biochemical studies disclosed that the debrancher activity was 40% of normal value and that the activities of the other glycolytic enzymes were normal (Table 1).

![Fig. 1](image-url). A transverse section of the left deltoid muscle stained with HE. Two muscle fibers have vacuoles (arrows) in the subsarcolemmal regions. The bar is 20 μm.
Fig. 2. Serial sections stained with PAS (A) and reacting on ATPase (B; routine, C; with pH 4.50 preincubation, and D; with pH 4.25 preincubation). Type 1 fibers and type 2A and 2B fibers have a weak and strong stain on PAS, respectively, and small PAS positive spots (arrow) are scattered in type 2A and 2B fibers (A). The bar is 20 μm.

Fig. 3. A section reacted for acid phosphatase. The bar is 20 μm.
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Fig. 4. A section reacted for non-specific esterase. The three muscle fibers have small circles or round stains. The bar is 20 μm.

Fig. 5. Muscle fiber diameters. The histogram of the diameters of each fiber type shows type 2B deficiency and slight fiber atrophy. Mean diameter: type 1 = 35.1 ± 5.2 μm, type 2A = 34.9 ± 4.2 μm, type 2B = 36.1 ± 2.2 μm.
Type 1: ●●●, Type 2A: ●●●, Type 2B: ○○○

Discussion

Glycogenoses involving muscles are type II (Pompe’s disease, acid maltase deficiency), type III (Forbes’s disease, debranching enzyme deficiency), type IV (Andersen’s disease, branching enzyme deficiency), type V (McArdle’s disease, myophosphorylase deficiency), and type VI (Tarui’s disease, phosphofructokinase deficiency).

The histological and histochemical stains and reactions that we performed on the case
Fig. 6. Ultrastructural findings of a longitudinal section of the muscle. A lot of glycogen particles are seen between myofibrils, and an autophagic vacuole contains glycogen, vesicles and amorphous material. The bar is 0.5 μm.

Table 1. Biochemical analyses of the biopsied muscle

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylase (−AMP)</td>
<td>2.1</td>
<td>3.10±1.6</td>
</tr>
<tr>
<td>Phosphorylase (+AMP)</td>
<td>95.5</td>
<td>44.7±19.6</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>307.5</td>
<td>261.1±70.1</td>
</tr>
<tr>
<td>Phosphohexoisomerase</td>
<td>1229.9</td>
<td>838.9±221.1</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>18.1</td>
<td>19.9±11.1</td>
</tr>
<tr>
<td>Aldolase</td>
<td>419.3</td>
<td>380.6±112.0</td>
</tr>
<tr>
<td>Glyceraldehyde-3P-dehydrogenase</td>
<td>1789.1</td>
<td>1708.1±536.6</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>1006.4</td>
<td>770.6±172.8</td>
</tr>
<tr>
<td>Phosphoglycerate mutase</td>
<td>1900.9</td>
<td>820.4±191.3</td>
</tr>
<tr>
<td>Enolase</td>
<td>475.2</td>
<td>331.3±64.3</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>1667.3</td>
<td>991.2±292.2</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1302.4</td>
<td>1789.1±517.5</td>
</tr>
<tr>
<td>Acid maltase*</td>
<td>8.4</td>
<td>7.3±2.2</td>
</tr>
<tr>
<td>Neutral maltase*</td>
<td>11.7</td>
<td>18.1±5.1</td>
</tr>
<tr>
<td>Debranching enzyme**</td>
<td>8.9</td>
<td>21.7±7.7</td>
</tr>
</tbody>
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Enzyme activities without asterisk(s) are expressed as nmoles of substrate utilized/min/mg protein (Mean±SD), activity * as nmoles 4MU/mg/30min, and activity ** as nmoles glucose incorporated/min/100mg NCP.

In this report excluded the possibility of type IV, V and VI glycogenoses. Though the abnormal polysaccharide in type IV glycogenosis stains positively with PAS but resists α-
Type II Glycogenosis

amylase preincubation (Dubowitz, 1985), the PAS positive materials in our case was digested by α-amylase. Phosphorylase is negative in type V glycogenosis and phosphofructokinase is negative in type VII glycogenosis, but both reactions were positive in our case.

Studies of involved muscle in type II glycogenosis demonstrated a highly positive acid phosphatase reaction (Dubowitz, 1985), and ultrastructurally revealed glycogen-engorged lysosomes (Engel et al., 1973). Minimally affected muscles in the adult form of type II glycogenosis showed multiple small foci of intense acid phosphatase reactivity (Engel et al., 1973). Though our case was similar to the minimally affected muscles of type II glycogenosis, it appeared to be type III glycogenosis because the acid phosphatase reaction in our case was less prominent than type II glycogenosis. The diagnosis of type III glycogenosis was confirmed by the biochemical analysis of the debrancher activity.

The histopathological features of the muscle in our case are type 2B fiber deficiency, small foci of mild acid phosphatase reaction and non-specific esterase positive materials.

Pellissier et al. (1979) reported a case of an infantile type III glycogenosis with multicore structures and type I fiber predominance.

No increased acid phosphatase reaction in type III glycogenosis is found by light microscopic observation (Dubowitz, 1985; Nonaka, 1987), but our case had small foci of mild acid phosphatase reaction. Multiple small regions of increased acid phosphatase activity in type II glycogenosis are considered to be autophagic vacuoles, that contain a limited amount of glycogen and cyttoplasmic degradation products (Engel, 1986). As this type of autophagic vacuoles can also appear in debranching enzyme deficiency or other myopathies (Engel, 1986; Pellissier et al. 1979), the small foci of mild acid phosphatase reaction in our case may be autophagic vacuoles.

The origin of the non-specific esterase positive materials remains unexplained, but the Golgi system or T-tubule system may play a role in producing the non-specific esterase positive materials. The limiting membranes of some types of the spaces consisting of glycogen in type II glycogenosis are generated by proliferating transverse tubular system networks and by the Golgi system (Engel, 1986). If autophagic vacuoles in type III glycogenosis derive from the same mechanism as type II glycogenosis, the non-specific esterase positive materials may be in or adjacent to the limiting membranes confining glycogen or degradation products.

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References


少年期に発症した糖原病Ⅲ型の筋生検所見

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要 旨：筋症状を呈する糖原病Ⅲ型の症例は比較的稀である。少年期に発症し、軽度であるが確実な筋症状を示した糖原病Ⅲ型の1症例を経験し、筋生検を施行したので報告する。Hematoxyline and eosinにて筋胞体中に中等量の空胞を認め、タイプ2筋線維はperiodic acid-Schiffにて濃染し、ATPaseにてtype 2B deficiencyを認めた。さらに、軽度のacid phosphatase活性の増加、小円形または輪状のnon-specific esterase反応像を認めた。これら酵素反応は、glycogenを取り囲む膜構造との関連が予想される。

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