CONGENITAL MYOPATHY WITH CYTOPLASMIC BODIES

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Goebel, H. H., Schloon, H. and Lenard, H. G.: Congenital myopathy with cytoplasmic bodies. Neuropediatrics 12: 166-180 (1981). Since early infancy, a 15-year-old girl had suffered from an apparently static neuromuscular disorder that chiefly afflicted her proximal muscles but did not spare her distal ones. Her CPK values had repeatedly been mildly elevated and her electromyogram had been considered "myopathic". There were no similar neuromuscular disorders in the family. Quadriceps muscle biopsy showed a type I myofiber predominance of 96%, type I myofiber atrophy and numerous cytoplasmic bodies within myofibers suggesting that this girl's muscle disease represented "congenital myopathy with cytoplasmic bodies" as cytoplasmic bodies were recently reported in other sporadic and hereditary neuromuscular disorders of unknown origin.

Congenital myopathy cytoplasmic bodies type I fiber predominance filamentous bodies ultrastructure

Introduction

Since the nosological delineation of the congenital myopathies, several such conditions have been identified by light and electron microscopic criteria. In infancy, the diagnosis of an individual "congenital myopathy" is chiefly based on morphological abnormalities (Dubowitz 1978), although admittedly similar or identical structural features have been encountered in neuromuscular disorders others than congenital myopathies.

Certain inclusions within myofibers as the filamentous body (Mair and Tomé 1972) and the cytoplasmic body (Macdonald and Engel 1969) have largely been considered non-specific findings.

In spite of previous criticism of the entire concept of classifying individual congenital myopathies due to structural changes (Fardeau et al. 1978, 1979, Brooke et al. 1979), other reports recently focussed on a myopathy morphologically marked by the presence of cytoplasmic bodies (Kinoshita et al. 1975, Clark et al. 1978, Jerusalem et al. 1979). The latter authors concluded that neuromuscular conditions which

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showed numerous cytoplasmic bodies may be heterogeneous rather than a single entity.

The study of a 15-year-old girl with a questionably progressive proximally accentuated myopathy morphologically characterized by numerous cytoplasmic bodies, gives further support to the concept of a cytoplasmic body myopathy.

Clinical data

The patient is the third child of non-consanguineous healthy parents; both her brother and her sister are healthy. The girl was first brought to the hospital at the age of five days because of feeding difficulties and vomiting that had developed during the first days of life following a normal delivery. Neurological examination revealed mild muscle hypotonia of the extremities, a high threshold of newborn reflexes and a marked hypotonia of the soft palate, corresponding to impaired swallowing. An electroencephalogram showed an undifferentiated monomorphic pattern alternating with periods of almost no activity. At that time, the symptoms were interpreted as residual perinatal brainstem damage.

Feeding difficulties, muscle hypotonia and superextendable joints had persisted for the first months of her life. Development of gross motor functions was delayed: She did not sit before 12 months of age; when 13 months old, she could not stand without support; she walked at the age of two years.

During infancy, choreoathetoid movements had occurred. Her subsequent motor development was characterized by persisting muscle weakness associated with atrophy of proximal muscles, but there was no obvious progression of her symptoms and no cessation of development. An electromyogram was considered "myopathic" when she was 12 years old. By that time, a tenotomy of the Achilles tendon was done because of a marked footdrop on the left side. Her intellectual development was unimpaired. She was sent to school with one year delay, but thereafter she was a good student. At the age of 12 years, her IQ was 104. At age 15 years, she presented with a myopathic face and muscle atrophy, most apparent in both limb girdles, but more in her upper than in her lower one. Her muscle weakness corresponded to the pattern of muscle atrophy, chiefly affecting proximal but also distal muscles. She was unable to close her eyelids completely, but her speech was not affected. Getting up from the floor was not possible without Gowers' phenomenon or other supporting manoeuvres. She could sit upright from a recumbent position only by rolling first to the side. She stood and walked unbalanced and tended to fall when pushed. Besides these motor handicaps she could cope with her daily activities. Because of marked weakness in her arms, she had to use both hands when drinking from a cup. She was able to dress herself and to do her hair. Mild progression of her motor function impairment was suspected in recent years, but it could not
be properly documented. Mild contractures of both elbows and radiocarpal joints were present. Footdrop was more pronounced on the left side, where her heel did not come down to the floor when standing. Tendon reflexes were absent in her biceps muscles, whereas her quadriceps and gastrocnemius muscles showed weak reflex responses. Her CPK was 251 U/l and 79 U/l (normal below 50 U/l). Her electrocardiogram was normal. Electromyography was not repeated.

Material and methods

Under local anesthesia, a muscle biopsy was excised from her quadriceps muscle at the age of 15 years and prepared according to routine procedures (Dubowitz and Brooke 1973, Goebel et al. 1979) for histological, enzyme histochemical, and electron microscopic examinations. An unfixed piece of tissue was flash frozen in isopentane in liquid nitrogen, and 10 μm thick sections were submitted to the following battery of stains and enzyme histochemical preparations: modified trichome, H&E, oil-red O, PAS, amylophosphorylase, NADH-TR reductase, menadione-linked α-glycerophosphate dehydrogenase (MAG), adenosine-triphosphatases (ATPases) after alkaline (pH 10.4) and acid (pH 4.5 and pH 4.3) preincubations, acid phosphatase, alkaline phosphatase and non-specific esterase. A histogram of muscle fibers was plotted from an ATPase preparation (pH 10.4) according to standard techniques which also provided normal control data (Dubowitz and Brooke 1973).

A second specimen was clamped in situ, excised while still in the clamp and immediately fixed in cacodylat buffered glutaraldehyde, washed in the same buffer, osmicated, dehydrated in increasing concentrations of ethanol and embedded in araldite. 1 μm thick toluidine-blue stained sections served to cut ultrathin sections from suitable areas which then were contrasted with uranyl-acetate and lead citrate.

Morphology

Light microscopy

The quadriceps muscle revealed an increased spectrum of myofiber diameters ranging between 10 and 120 μm and rounding of cross-sectioned myofibers (Fig. 1). There was considerable numerical increase of internally located nuclei. Muscle fibers of larger caliber contained single or multiple inclusions, cytoplasmic bodies (Fig. 2a). There was no evidence of necrosis, phagocytosis or regeneration of myofibers, but mild broadening of the endomysium. There were many ringbinden within small myofibers. The amount of glycogen and lipids appeared within normal limits. Amylophosphorylase activity was present in each fiber. NADH and MAG preparations gave good typability, atrophy and marked predominance of type I fibers which also harbored the cytoplasmic bodies. ATPase preparations confirmed type I fiber predominance and atrophy (Fig. 1) and a type II B fiber deficiency. Hist-
Fig. 1 Type I fiber predominance and type I fiber atrophy are apparent, as only a few dark type II fibers are present in the quadriceps muscle. ATPase, pH 10.4; X 200

Fig. 2 a) A darkly stained irregular shaped inclusion (I) body is present in the center of a muscle fiber, surrounded by a light halo. Modified trichome; X 500
grams (Fig. 2 b) substantiated a type I fiber predominance of 96.4% and histographic abnormalities confined to type I fibers, a variability coefficient of 535 (normal below 250). Mean fiber diameters for type I and type II fibers were 46 μm for type I fibers and 43 μm for type II fibers. In araldite-embedded thick sections, small inclusions (Fig. 2 a) were frequently surrounded by a lighter halo. They were more numerous in thick sections than in cryostat sections.

Electron microscopy

Cytoplasmic bodies (Figs. 3, 4 a) and filamentous bodies (Fig. 4 b) were numerous, the former marked by distinct electron-dense bodies which were entered by many filaments in a radial fashion. Filaments were also found within the center of such cytoplasmic bodies (Fig. 5 a, b) which themselves were composed of a finely granular matrix. At high magnification, granularity consisted of irregularly arranged minute filaments (Fig. 4 a). The filaments making up the halo of the cytoplasmic body measured 7 to 10 nm, the filaments of the filamentous bodies measured 8–15 nm. Filaments encountered within the center of the cytoplasmic body measured 10 nm. Both types of bodies were present in the peripheral and the central parts of muscle fibers (Figs. 3, 4 a, b). Transitional stages between filamentous and cytoplasmic bodies (Fig. 5 b) were also seen. Filamentous and cytoplasmic bodies were never encountered in other cells but muscle fibers. In various
Fig. 3  Two cytoplasmic bodies (CB) are located in the center of a muscle fiber. Their halo is very small. X 9720.
Fig. 4  a) Two cytoplasmic bodies surrounded by a wide halo of radially oriented filaments (F) are located in the periphery of a muscle fiber. X 9700. Inset: At higher magnification, fine filaments (F) may also be recognized in the electron-dense center of the cytoplasmic body. X 61,250. b) A filamentous body (FB) is sharply demarcated from surrounding sarcomeres. X 9700
Fig. 5  

a) Numerous central nuclei adjacent to a cytoplasmic body (CB) whose light center contains irregularly arranged filaments. X 6630.  
b) Along the long axis of a myofiber, a large inclusion represents a transitional stage between the cytoplasmic body (CB) and a filamentous body (FB). X 4800. Inset: Another peripheral inclusion chiefly consists of filaments (F) and a small rim of electron-dense material, another transitional stage between filamentous and cytoplasmic bodies. X 5820.
Fig. 6  a) A well circumscribed area of two successive sarcomeres consists of myofilaments (M) replacing the regular sarcomeric arrangement of thick and thin filaments. X 16,320.  b) Another circumscribed region within the center of a muscle fiber contains haphazardly arranged filaments (F) extending over several sarcomeres. X 10,670.
Fig. 7  a) A small muscle fiber is largely devoid of myofibrils but contains a cytoplasmic body (CB) and abnormal triad (T) complexes. X 14,220. b) There are three circumscribed areas (A) where the sarcomeric pattern is replaced by fine filaments and light granular material resembling minicores. X 10,670
Fig. 8 Several membrane-bound vacuoles (V), filled with a finely granular material, are embedded in a matrix of fine filaments vicinal to electron-dense structures that resemble Z-discs (Z). X 27,500. Inset: At higher magnification, one such vacuole (V) appears as a dilated terminal sac attached to a T-tubule (T) and another terminal sac (S). X 62,500
regions, the regular pattern of sarcomeres was replaced by filaments parallel to the long axis of the sarcomeres (Fig. 6 a) or arranged in a haphazard fashion (Fig. 6 b), not surrounded by any separating membrane. Glycogen granules were scantily disseminated inside certain inclusions but often clustered markedly around cytoplasmic bodies (Figs. 3, 5 a). Several myofibers were almost entirely devoid of myofilaments (Fig. 7 a). Focal lesions associated with a numerical decrease in myofilaments and absence of mitochondria resembled minicores (Fig. 7 b). Regionally, the preexisting sarcomeric pattern had been replaced by irregularly arranged remnants of filaments and Z-discs (Fig. 8). Small muscle fibers largely devoid of myofibrils and myofilaments though containing cytoplasmic bodies (Fig. 7 a) often showed proliferation of the sarcotubular network associated with formation of pentads and other abnormalities of the triad complexes (Fig. 7 a). Large membrane-bound sacs (Fig. 8) filled with a finely granular amorphous material possibly corresponded to widened sarcotubular cisterns. Central clustering of nuclei was also present (Fig. 5 a). Mitochondria and other cytoplasmic organelles appeared unchanged. Membrane-bound granular bodies were occasionally seen a few of which resembled lipofuscin.

Discussion
This girl's neuromuscular disorder has been marked since early infancy by proximal muscle weakness and muscle atrophy, a progression of which could not be unequivocally documented, moderate elevation of CPK activity, a "myopathic" EMG, and morphologically type I muscle fiber predominance and change in fiber size associated with numerous cytoplasmic bodies and, therefore, thought to represent a congenital myopathy which is frequently characterized by type I fiber predominance and type I fiber atrophy (Fardeau et al. 1978, 1979, Brooke et al. 1979). The actual frequency of these cytoplasmic bodies was difficult to discern since lesions were much more obvious at the ultrastructural level and transitional stages between small sarcomeric lesions and full fledged cytoplasmic bodies were encountered. These nosological criteria may identify our patient's neuromuscular condition as a congenital myopathy (Dubowitz 1978) further characterized by structural abnormalities, cytoplasmic bodies. The fine structure of the cytoplasmic bodies conformed to those previously described (Macdonald and Engel 1969). The cytoplasmic bodies were unlike rods because they lacked the specific Z-disc lattice, but they were more similar to spheroid bodies previously observed in an autosomal dominant neuromuscular disorder (Goebel et al. 1978). Ultrastructural differences between spheroid bodies and cytoplasmic bodies were previously outlined (Goebel et al. 1978). Our patient's biopsy lacked fully developed spheroid bodies, both by electron microscopy and in enzyme histochemical preparations.
Filamentous bodies represent a random finding in muscle fibers not especially related to a particular neuromuscular disorder (Mair and Tomé 1972). They are usually located in the periphery of the muscle fiber where they occur singly or in small numbers. In our patient’s muscle biopsy, they were rather numerous, located in peripheral and central parts of myofibers. There were also transitional stages between filamentous and cytoplasmic bodies attesting to the assumption that the filamentous bodies were not incidental findings but rather an integral part of the population of myofiber inclusions of this muscle biopsy inasmuch as cytoplasmic bodies also consist of filaments.

While cytoplasmic bodies were previously noted in various neuromuscular disorders (Engel 1962, Mair and Tomé 1972, Kinoshita et al. 1975), they represented a consistent finding in several members of a kindred afflicted with an autosomal dominant myopathy (Clark et al. 1978). They were also the most striking abnormality in the muscle biopsy of a 31-year-old female who died of a neuropathy associated with a type I fiber predominance of 72% in her deltoid muscle and respiratory failure (Jerusalem et al. 1979) and in several weak and non-weak muscles of a 53-year-old man whose neuromuscular disorder was equivocally regarded as of neurogenic origin (Nakashima et al. 1970). This latter patient’s intramuscular abnormalities also comprised aggregates of filaments and focal loss of cross striation (Nakashima et al. 1970). Another woman who died at the age of 23 years of respiratory insufficiency had a similar myopathy which was chiefly present in proximal muscles, but was also found in the anterior tibial muscles, as in our patient, and who displayed cytoplasmic bodies as a characteristic feature in her muscles (Kinoshita et al. 1975). Cytoplasmic bodies associated with core-targetoid fibers were experimentally produced in organophosphate neuropathy (Fukuhara et al. 1977), an association of ultrastructural phenomena which was also seen in our patient’s muscle biopsy.

A recent exhaustive review (Brooke et al. 1979) emphasizes the non-specificity of morphological findings encountered in congenital myopathies and the need to synthesize all available data for precise nosological diagnosis. Our patient’s findings conform to this concept of “congenital non-progressive myopathies” (Brooke et al. 1979). Further separation of these entities into individual diseases based on pure morphological criteria has yielded inconsistencies in and overlap of individual neuromuscular conditions including different patterns of inheritance within the same morphologically defined congenital myopathy. Therefore, a term “congenital myopathy with rods” might be more appropriate than “congenital rod myopathy” indicating that rods may also occur in congenital myopathies – and the same semantic attitude may pertain to other congenital myopathies as the core diseases or congenital fiber type disproportion.
Hence, it is also appropriate for our patient's neuromuscular disorder to be designated as "congenital non-progressive myopathy with cytoplasmic bodies". The autosomal dominant pattern of inheritance of a congenital myopathy associated with cytoplasmic bodies (Clark et al. 1978) further substantiates the observation that the group of congenital myopathies may be enlarged by those non-progressive congenital neuromuscular diseases which are morphologically marked by numerous cytoplasmic bodies and related structures as filamentous bodies.

Zusammenfassung


References


