The contemporary role of electron microscopy in neuromuscular pathology

Giovanna Cenacchi, Lucia Tarantino, Arianna Corbu, Lucilla Badiali De Giorgi, Marina Fanin (1), Elena Pegoraro (1), Luigi Fulizio (1), Corrado Angelini (1), Giuseppe N Martinelli

Clinical Department of Radiological and Histopathological Sciences University of Bologna; (1) Department of Neurological Sciences, University of Padova and Venetian Institute of Molecular Medicine ,Italy

Abstract

Electron microscopy has a strategic position in the diagnosis of neuromuscular disorders. In muscular fibers the main abnormalities includes vacuoles, vesicles, inclusion bodies, sarcoplasmic reticulum modifications and mitochondrial alterations. The differential diagnosis includes polymyositis, inclusion body myositis, some sarcolemmal and non-sarcolemmal dystrophies, lysosomal storage diseases. Degenerative alterations of mitochondria, causing swelling and cristae fragmentation, may be considered also as a precocious feature of drug-induced muscle damage guiding molecular genetic analysis. The ultrastructural approach seems to improve the diagnostic accuracy in muscle disorders, making possible the identification of changes and structures related to new hypothesized pathogenetic mechanisms.

Key words: muscle pathology, electron microscopy, vacuoles, autophagic, pathogenetic mechanism.

Basic Applied Myology 17(3&4): 167-171, 2007

Muscle biopsy is an essential component of the investigation of patient with neuromuscular disorder, and is the deciding factor for diagnosis in most instances. Electron microscopy, EM, has a strategic position improving the diagnostic accuracy of numerous muscular diseases, some not revealed by light microscopy [16, 22]. Moreover, it gives insight into pathophysiologic mechanisms and can guide molecular genetic analysis. Muscle disorders can be divided into three groups [22]. The first concerns diseases of the interstitial tissue: inflammatory disorders as well as abnormal deposits such amyloidosis. For the first group, histological and immunohistochemical techniques are more effective than EM. The second group refers to diseases associated with defects of protein components of sarcolemma, extracellular matrix and nuclear membrane: with these diseases EM can be considered a valuable tool confirming diagnosis suggested by immunohistochemical and molecular analysis [16, 22]. Finally, ultrastructural study provides the best results for sarcoplasmic abnormalities including vacuoles, inclusion bodies, and some changes not revealed by light microscopy [16, 22].

Materials and Methods

To define the relationship between molecular mechanisms and submicroscopic alterations we examined at ultrastructural level 45 muscle biopsies: 4 inclusion body myositis, IBM, 1 polymyositis, 2 dermatomyositis, DM, 2 HIV-related myopathy; 6 LGMD1C, 10 LGMD2B, 10 LGMD2I previously diagnosed by IHC, WB and DNA molecular assay; 4 acid maltase deficiency, AMD, 1 Danon disease, 1 ceroid lipofuscinosis, CL, and 4 drug-induced myopathy. Immunocytochemical analysis was performed by immunogold technique in LGMD1C as previously described [33]. Briefly, the specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer 0.1M, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in ethanol and embedded in Araldite. Thin sections, stained with uranyl acetate and lead citrate were observed under a transmission electron microscope Philips 410.

Results

Electron microscopy of IBM patients reveals cytoplasmic accumulation of 15-21 nm tubulo-

filamentous inclusions. Abnormal nuclei with intranuclear filaments are also detected (Fig. 1).

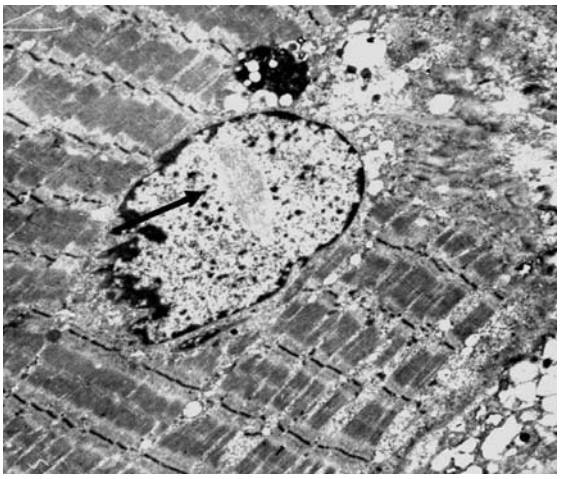

***Fig. 1**: IBM muscle biopsy showing nucleus containing a filamentous inclusion (arrow). x 6.400.*

Sarcoplasmic reticulum cisternae appear dilated and associated with mitochondria characterized by unspecific degenerative changes. Many vacuoles containing some electrondense myelin figures and amorphous material are also visible. Similar likely autophagic vacuoles are clearly detectable in polymyositis specimens in which they are closely related to areas featuring myofibrillar loss. In the subsarcolemmal region myofibers show few filamentous cytoplasmic bodies. The same ultrastructural characteristics are found in patient with clinically diagnosed dermatomyositis, but in this latter some alterations of endothelial cells are detectable as the presence of tubuloreticular inclusions in the perinuclear area. These inclusions were considered deriving from a virus infection, as well as unspecific features of activated cell: in fact tubuloreticular structures are clearly visible also in the capillary endothelial cells of HIV-related myositis. Finally, among lysosomal storage diseases, the ultrastructural diagnosis of AMD is based on the presence of secondary lysosomal vacuoles containing many glycogen particles (Fig. 2).

Sometimes, glycogen aggregates are seen freely accumulated in the sarcoplasm probably due to the lysosomal membrane rupture. In Danon disease EM reveals scattered clusters of autophagic vacuoles containing cytoplasmic debris, electron-dense materials and myeloid bodies: some of these autophagic vacuoles have basal lamina on the luminal side (Fig. 3), while other clusters are not limited by a membrane. Exceptionally, EM examination shows the lysosomes of ceroid lipofuscinosis with curvilinear profiles of CLN2 (Fig. 4).

In all CAV3-deficient patients muscle fibers show an almost complete loss of caveolae at the sarcolemma together with the presence of vacuoles generally appearing empty or containing membrane rearrangements. Swollen Golgi, dilated T-tubules are also visible. Caveolin 3 appears localized as aggregates of gold particles along the sarcolemma in the control biopsies while it disappears in CAV3-deficient patients. Inflammatory response was observed in the majority of muscle biopsies from LGMD2B. Non necrotic fibers show alterations at different submicroscopic levels: sarcolemma and basal lamina, subsarcolemmal region and sarcoplasmic compartment. In the subsarcolemmal region there is a prominent aggregation of small vesicles likely deriving from the Golgi apparatus which appeared constituted by empty, swollen cisternae. In the sarcolemma we find many gaps, microvilli-like projections, while basal lamina is multilayered. At the ultrastructural level muscle fibers from LGMD2I show focally thinning of basal lamina, swollen and reduplication of ER cisternae with membrane rearrangement and numerous lipid vacuoles both in the intermyofibrillar and subsarcolemmal sarcoplasm.

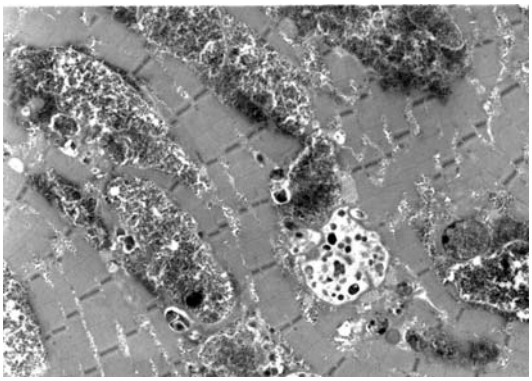

***Fig. 2**: AMD: sarcoplasmic vacuoles containing aggregates of glycogen particles. x 5.000.*

Discussion

Inflammatory myopathies can be subdivided in two main groups: infectious myositis and immunogenic myositis [2, 3, 8, 10, 25, 33]. The autoimmune myopathies include polymyositis (PM), dermatomyositis (DM), overlap syndromes, and inclusion body myositis (IBM). Recent findings have confirmed that PM is an uncommon, but frequently misdiagnosed disorder: PM mimics many other myopathies and remains a diagnosis of exclusion. Definite diagnosis requires muscle biopsy: ultrastructural studies which are necessary in many cases, especially in IBM, by screening other myopathies with inflammation, such as dystrophies, toxic and metabolic myopathies [1, 9, 21]. The diagnosis of IBM rests on morphological criteria including inflammatory infiltrates with mononuclear cell invasion of non-necrotic muscle fibers, rimmed vacuoles and either intracellular amyloid or cytoplasmic /nuclear inclusions consisting of 15-21 nm filaments at the ultrastructural level. Membrane-bound and free

glycogen accumulation allows the diagnosis of severe vacuolar myopathy such as the adult-onset variant of acid maltase disease, AMD [29]. Among the autophagic vacuolar myopathies, a subgroup is characterized by unusual autophagic vacuoles with sarcolemmal features and includes Danon disease [29]: autophagic vacuoles appear to be accumulations of lysosomes. Some autolysosomes were surrounded by membranes with an inner basal lamina, reminiscent of surface sarcolemma. Recently, EM helped to define the genetic spectrum on ceroid lipofuscinosis, mainly in very unusual localization as muscle [18]. The identification of gene and protein defects in an increasing number of neuromuscular diseases has induced an enhanced expectation by clinicians and patients of a firm diagnosis of muscle disease even if specific management and treatment are still lacking for most myopathies: such expectations have stimulated the need of accurate definition of pathogenetic mechanism by EM of many sarcolemmal dystrophies [16]. Dysferlin gene has been recently identified to be involved in limb-girdle muscular dystrophy type 2B and its allelic disease, Miyoshi myopathy which are characterized by an active muscle degeneration and regeneration process. Dysferlin is well known to have an essential role in skeletal muscle fiber repair, but the process underlying the pathogenetic mechanism of dysferlinopathy is not completely understood even if EM contributed to define the morphological features of the molecular-induced alteration of muscle fibers which can be considered a useful tool in the differential diagnosis [5, 6, 7, 13, 15, 28].

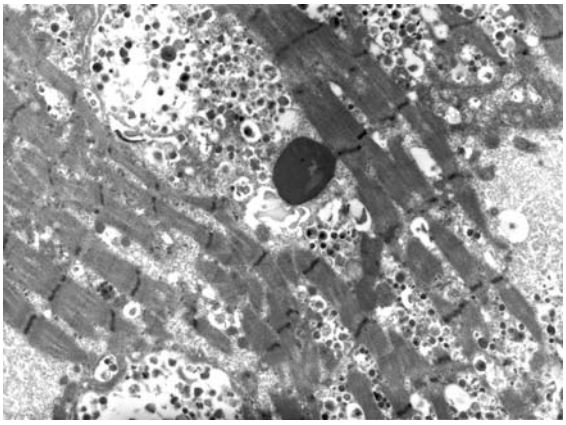

***Fig. 3**: Danon disease: the autophagic vacuole shows a basal lamina circumscribing the vacuolar lumen. x 5.000.*

Disruption of CAV3 gene underlies "caveolinopathies" [17, 22, 31]. Caveolin 3 is a specific striated muscle protein and it is thought to function as building block in the construction of caveolae and T-tubules [17, 22, 31]. Caveolins are scaffolding proteins that organize specific caveolin-interacting lipids and proteins within caveolae microdomains. Caveolae, small membrane invaginations, are implicated in signal transduction and

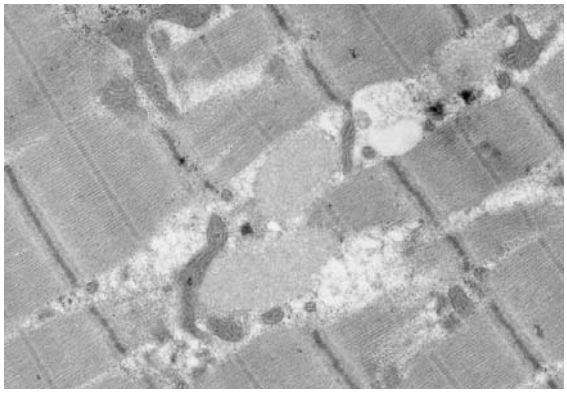

***Fig. 4**: Ceroid lipofuscinosis characterized by vacuoles containing many curvilinear profiles. x 5000.*

ion channels regulating pathways. Our results likely represent the morphological basis of the hypothesized role of caveolin in muscle degenerative and proliferative/regenerative mechanisms with its involvement in vesicles trafficking and voltage-gate ion channel regulation.

Limb girdle muscular dystrophy type 2I (LGMD2I) is due to mutations in the fukutin-related protein gene (*FKRP*), encoding a putative glycosyltransferase involved in α-dystroglycan processing [20, 27, 30]. The retention of mutant FKRP in the endoplasmic reticulum along with a reduced enzymatic function of the protein has recently been suggested as a possible mechanism of disease in LGMD2I. Defects in the dystroglycan-dystrophin complex play a crucial role in both basement membrane organization and link with intracellular actin [11, 20, 30]. Our data suggest that the observed ultrastructural alterations may be related to both sarcoplasmic structures involved in FKRP metabolism and malfunctioning. In fact EM analysis evidences rough endoplasmic reticulum modifications as dilated cisternae and triad reduplication suggesting a possible retention and accumulation of protein before its proteasome degradation as previously described; moreover, the hypoglycosylated form of α-dystroglycan fails to properly bind laminin to basal membrane which appears focally interrupted and thinner compromising the functional network with extracellular matrix [20, 27, 30]. Glycosylated proteins are largely represented in human tissue resulting in a huge diversity of protein-bound glycans: mitochondria aggregates founded in the subsarcolemmal areas associated with an increase of lipid vacuoles can be thus considered secondary alterations likely derived from metabolism defects closely related to glycoprotein dysfunction [11].

Finally, mitochondrial disorders are a heterogeneous group of multisystem disorders with an extreme variability in clinical presentation [14, 19, 24]. Light microscopy and EM of skeletal muscle, measurement of electron transport complex and search for specific defects in mtDNA in muscle biopsy specimen are

widely used. The key diagnostic features include ragged red fibers, COX-deficient myofibers and abnormal ultrastructure of mitochondria [19]. Thus, the decline of mitochondrial energy production resulting in increased oxidative stress and apoptosis does play a significant role in degenerative diseases and ageing. Statins were originally developed to lower plasma cholesterol in patients with hypercholesterolemia [4, 12, 26]. Myopathy is the most common side effect with symptoms ranging from fatigue, weakness, and pain to symptoms associated with rhabdomyolysis which is a life-threatening condition. The development of statin-induced rhabdomyolysis is rare occurring in approximately 0.1% of patients; however, the occurrence of less severe symptoms is underreported and may be 1-5% or more [4]. The mechanisms causing statin-induced myopathy have not been elucidated; however, research efforts suggest that apoptosis of myofibers may contribute [4, 12]. The mitochondrion is considered a regulatory center of apoptosis, and therefore its role in the induction of apoptosis will be discussed as well as the mechanism of statin-induced apoptosis and myopathy [12].

In conclusion, electron microscopy remains an interesting tool for studying muscle pathologies. Used diagnostically, its main indication is myopathies with autophagic vacuoles. Moreover, it can help researchers and pathologists to understand pathological mechanisms of various muscular diseases and even guide genetic analysis.

Acknowledgments

This work has been supported by CARISBO Foundation grant.

Address correspondence to:

Giovanna Cenacchi, MD, Dipartimento Clinico di Scienze Radiologiche e Istocitopatologiche, Via Massarenti, 9, 40138 Bologna, Italy, Tel +39-051 6364514, fax +39-051 6364571.

E-mail: giovanna.cenacchi@unibo.it

References

[1] Askanas V, Engel VK. Inclusion-body myositis. Newest concept of pathogenesis and relation toag aging to to aging and Alzheimer disease. J Neuropathol Exp Neurol. 2001; 60: 1-14.

[2] Authier FJ, Gherardi RK. Muscular complications of human immunodeficiency virus (HIV) infection in the era of effective anti-retroviral therapy] Rev Neurol (Paris). 2006; 162: 71-81.

[3] Baer AN, Differential diagnosis of idiopathic inflammatory myopathies.Curr Rheumatol Rep. 2006; 8: 178-87.

[4] Baer AN, Wortmann RL. Myotoxicity associated with lipid-lowerin drugs. Curr Op Rheum 2007; 19: 67-73.

[5] Bansal D, Miyake K, Vogel SS, et al. Defective membrane repair in dysferlin deficient muscular dystrophy. Nature 2003; 423: 168-72.

[6] Boito C, Melacini P, Vianello A, Prandini P, Gavassini BF, Bagattin A, Siciliano G, Angelini C, Pegoraro E. Clinical and molecular characterization of patients with limb-girdle muscular dystrophy type 2I. Arch Neurol 2005; 62: 1894-1899.

[7] Cenacchi G, Fanin M, De Giorgi LB, Angelini C. Ultrastructural changes in dysferlinopathy support defective membrane repair mechanism. J Clin Pathol. 2005; 58: 190-5.

[8] Dalakas MC. Autoimmune muscular pathologies. Neurol Sci 2004; 25: S7-S8.

[9] Dalakas MC. Sporadic inclusion body myositis-diagnosis, pathogenesis and therapeutic strategies. Nature Clin Pract 2006; 2: 437-447.

[10] Dalakas MC, Hohlfeld. Polymyositis and dermatomyositis. Lancet 2003; 362: 971-982.

[11] Damen g, De Klerk H, Huijmans J, Den Hollander J, Sinaasappel M. Gastrointestinal and other clinical manifestations in 17 children with congenital disorders of glycosylation type Ia, Ib, Ic.J Ped Gastroenterol Nutr 2004, 38: 282-287.

[12] Dirks AJ, Jones KM. Statin-induced apoptosis and skeletal myopathy. Am J Cell Physiol 2006; 291: C1208-C1212.

[13] Doherty KR, McNally EM. Repairing the tears: dysferlin in muscle membrane repair. Trends Mol Med 2003; 9: 327-30.

[14] Enns GM, Hoppel CL, DeArmond SJ, Schelley S, Bass N, Weisiger K, Horoupian D, Packman S. Relationship of primary mitochondrial respiratory chain dysfunction to fiber type abnormalities in skeletal muscle.Clin Genet. 2005; 68: 337-48.

[15] Fanin M, Angelini C. Muscle pathology in dysferlin deficiency. Neuropathol Appl Neurobiol 2002; 28: 461-70.

[16] Fernandez C, Figarella-Branger D, Meyronet D, Cassote E, Tong S, Pellissier JF. Electron microscopy in neuromuscular disorders. Ultrastruct Pathol 2005; 29: 437-450.

[17] Galbiati F, Razani B, Lisanti M. Emerging themes in lipid rafts and caveolae. Cell 2001; 106: 403-411.

[18] Goebel HH, Wisniewski KE. Current state of clinical and morphological features in human NCL. Brain Pathol 2004; 14: 61-69.

[19] Kyriacou K, Hadjisavvas A, Zenios A, Papacharalambous R, Kyriakides T Morpho-

logical methods in the diagnosis of mitochondrial encephalomyopathies: the role of electron microscopy. Ultrastruct Pathol. 2005; 29: 169-74.

[20] Lamperti C, Cagliani R, Ciscato P, Moroni I, Viri M, Romeo A, Fagiolari G, Prelle A, Comi GP, Bresolin N, Moggio M.Congenital muscular dystrophy with muscle inflammation alpha dystroglycan glycosylation defect and no mutation in FKRP gene. J. Neurol Sci 2006; 243: 47-51.

[21] Li J, Yin C, Okamoto H, Jaffe H, Oldfield EH, Zhuang Z, Vortmeyer AO, Rushing EJ. Proteomic analysis of inclusion body myositis. J Neuropathol Exp neurol 2006; 65: 826-833.

[22] Matsuda C, Hayashi YK, Ogawa M, Aoki M, Murayama K, Nishino I, Nonaka I, Arahata K, brown R. The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle. Hum Mol 2001; 10: 1761-1766.

[23] Meola G. Advanced microscopic and histochemical technique: diagnostic molecular era of myology. Eur J Histochem 2005; 49: 93-96.

[24] 24.Miles L, Wong BL, Dinopoulos A, Morehart PJ, Hofmann IA, Bove KE Investigation of children for mitochondriopathy confirms need for strict patient selection, improved morphological criteria,and better laboratory methods. Hum Pathol. 2006; 37: 173-84.

[25] Miller FW, Rider LG, Plotz PH, Isemberg DA, Oddis CV. Diagnostic criteria for polymyositis and dermatomyositis. Lancet 2003; 362: 1762-1763.

[26] Oh J, Ban MR, Miskie BA, Pollex RL, Hegele RA. Genetic determinants of statin intolerance. Lipids Health Dis. 2007; 21: 6-7.

[27] Schachter H, Vajsar J, Zhang W. The role of defective glycosylation in congenital muscular dystrophy. Gliconj J 2004, 20: 291-300.

[28] Selcen D, Stilling G, Engel AG. The earliest pathologic alterations in dysferlinopathy. Neurology 2001; 56: 1472-81.

[29] Sugie K, Noguchi S, Kozuka Y, Arikawa-Hirasawa E, Tanaka M, Yan C, Saftig P, 29.Von Figura K, Hirano M, Ueno S, Nonaka I, Nishino I. Autophagic vacuoles with sarcolemmal features delineate Danon disease and related myopathies. J Neuropathol Exp Neurol 2005, 64: 513-522.

[30] Von der Hagen M, Kaindl AM, Koehler k, Mitzscherling P, Hauster HJ, Stolyenburg-Didinger G, Huebner A. L Limb girdle muscular dystrophy type 2I caused by a novel missense mutation in the FKRP gene presenting as acute virus-associated myositis in infancy. Eur J Pediatr 2006; 165: 62-63.

[31] Woodman SE, Sotgia, F, Galbiati F, Minetti C, Lisanti MP.Caveolinopathies mutations in caveolin-3 cause four distinct autosomal dominant muscle diseases. Neurology 2004; 62: 538-543.

[32] Wortmann RL, Di Mauro S. Differentiating idiopathic inflammatory myopathies from metabolic myopathies. Rheum Dis Clin North Am. 2002; 28: 759-78.

[33] Zuber C, Fan J, Guhl B, Roth Applications of immunogold labeling in ultrastructural pathology. Ultrastruct Pathol. 2005; 29: 319-30