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Review

A review of the histopathological findings in myasthenia gravis: Clues to the pathogenesis of treatment-resistance in extraocular muscles

Tarin A. Europa[a], Melissa Nel[a], Jeannine M. Heckmann[a,b,*]

[a] *Neurology Research Group, Division of Neurology, Department of Medicine, University of Cape Town, Cape Town, South Africa*
[b] *Neurology Division, Department of Medicine, University of Cape Town, Cape Town, South Africa*

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Abstract

In myasthenia gravis autoantibodies target components of the neuromuscular junction causing variable degrees of weakness. In most cases, autoantibodies trigger complement-mediated endplate damage and extraocular muscles may be most susceptible. A proportion of MG cases develop treatment-resistant ophthalmoplegia. We reviewed publications spanning 65 years reporting the histopathological findings in the muscles and extraocular muscles of myasthenic patients to determine whether pathological changes in extraocular muscles differ from non-ocular muscles. As extraocular muscles represent a unique muscle allotype we also compared their histopathology in myasthenia to those in strabismus. We found that in myasthenia gravis, the non-ocular muscles frequently demonstrate neurogenic changes regardless of myasthenic serotype. Mitochondrial stress/damage was also frequent in myasthenic muscles and possibly more evident in muscle-specific kinase antibody-positive MG. Although myasthenia-associated paralysed extraocular muscles demonstrated prominent fibro-fatty replacement and mitochondrial alterations, these features appeared commonly in paralysed extraocular muscles of any cause. We postulate that extraocular muscles may be more susceptible than limb muscles to poor contractility as a consequence of myasthenia, resulting in a cascade of atrophy signaling pathways and altered mitochondrial homeostasis which contribute to the tipping point in developing treatment-resistant myasthenic ophthalmoplegia. Early strategies to improve force generation in extraocular muscles are critical.

Keywords: Extraocular muscles; Ophthalmoplegia; Strabismus; Denervation; Contractility; Mitochondria.

1. Introduction

Pathogenic autoantibodies in myasthenia gravis (MG) target the nicotinic acetylcholine receptor (AChR) or other muscle endplate proteins such as muscle-specific kinase (MuSK) resulting in AChR loss or reduced clustering of AChRs at the endplate, respectively [1]. The first clinical manifestation of MG is often fatigable weakness of the extraocular muscles (EOMs) and/or levator palpebrae superioris resulting in fatigable diplopia and/or ptosis [2]. The disease may progress to involve the limb, bulbar and respiratory muscles resulting in variable weakness, which usually responds to cholinesterase inhibitors (CHEI) and/or immune therapies in cases where patients remain symptomatic on CHEIs. However, we and others have reported that some individuals with otherwise characteristic MG, develop treatment-resistant ophthalmoplegia while their non-ocular muscles respond to treatment [3,4,5,6,7,8]. Although most MG patients with EOM weakness experience resolution within twelve months of immune therapy, approximately 20% develop treatment-resistant ophthalmoplegia [7,9]. These individuals frequently have AChR-antibody positive MG (AChR-MG) although this subphenotype has also been described in individuals with MuSK-antibody positive MG (MuSK-MG) and triple seronegative MG (no serum antibodies detected against AChR, MuSK and lipoprotein-related protein 4 (LRP4)) [10,11]. At present, the underlying pathogenetic mechanisms of treatment-resistance remain unknown. Here

* Correspondence to: Neurology E8-74, Groote Schuur Hospital, Observatory, 7925 Cape Town, South Africa.
E-mail address: jeanine.heckmann@uct.ac.za (J.M. Heckmann).

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we reviewed reports on muscle histopathology in MG over the past 65 years, to discover clues to the pathogenic processes that underpin the development of treatment-resistant ophthalmoplegia in MG.

The first aim of this review was to summarise and compare the histopathology in EOMs and non-ocular muscles in myasthenic patients. Secondly, we aimed to compare the histopathology of EOMs between MG cases and non-MG strabismus cases to assess which pathological changes are unique to ophthalmoplegia in MG. Although we appreciate that the pathological changes in humans with MG may be the consequence of more chronic disease than in experimental autoimmune MG (EAMG) models where the animals are sacrificed soon after developing weakness, we have also included histopathological reports in EAMG as it may provide clues to the early changes in the muscle during antibody-mediated MG.

2. Methods

We conducted a search of all articles on structural changes in the muscles of patients with myasthenia gravis found on the NCBI Pubmed database. Studies on MG as well as experimental autoimmune MG (EAMG) models in rodents were included. Search terms included “extraocular”, “ophthalmoplegia”, “muscle” and “myasthenia” in addition to “microscopy”, “histology”, “ultrastructure”, “mitochondria”, “mitochondrial respiration”, “oxidative phosphorylation”, “mitochondrial dysfunction”, “COX negative fibres”, “ragged red fibres”, “citrate synthase”, “swollen mitochondria” and “oxidative stress”. Secondary manual searches were conducted on citations of articles in our primary search results.

We focused specifically on the pathological features of the muscle and not on the neuromuscular junction. We grouped these reports according to those described prior to radio-immunoassays and those after radio-immunoassays by pathogenic serotype. One report [12] included a subset of patients in whom AChR antibody testing had been performed, but we have grouped these with the pre-availability of radio-immunoassays as most of the cohort were not serologically tested for AChR antibodies and their treatment program resembled the pre-assay era.

3. Results

In this review, we have included 25 histopathological reports on MG-associated changes in human muscles, of which 15 preceded serotyping of MG by pathogenic antibodies. We excluded the reports of 4 groups in Japanese [1968–1971] and [2011], 2 in Polish [1969–1970], and 1 each in Portuguese [1984] and French [1956] as there was no accompanying English text. The results are summarized in Table 1.

3.1. Histopathological features of non-ocular muscles in MG prior to the era of antibody assays

These reports were published between 1953 and 1993. Most of the muscle biopsies were taken from patients aged 25–50 years, and between 3 months and 34 years after the onset of MG symptoms (supplementary Table 1). The severity of weakness of the muscle at the time of biopsy was not always stated, although where possible an approximation of the Osserman severity grade (in [5]) was applied. While the cases from this period may have included patients with congenital myasthenic syndromes who had shown CHEI responsiveness, but in whom MG-associated antibodies had not been assayed and the use/response to immune therapies was not recorded, a substantial proportion of the cases had thymic abnormalities which is expected in 75% of MG cases (thymic hyperplasia and thymoma) [5,13,14,15,16,17]. The most recent series in this group (1993) included 12 (of 30) who had undergone AChR-antibody assays (10/12 were AChR-antibody positive [12]. Three series [5,13,14] included biopsies which were taken post-mortem although not all cases died of MG-related complications.

Several non-specific light microscopic changes were described in between half [18] to all [19] of the MG patients undergoing limb muscles biopsies. Firstly, neurogenic atrophic changes were prominent in a proportion of patients with grouped fibre atrophy and small angulated fibres which were suspected to be secondary to functional denervation at the motor endplate [5,13,14,19,20,21]. Fenichel [22] suggested atrophic changes with fatty replacement were more frequently associated with biopsies after prolonged disease duration. Muscle fibre atrophy occurred in up to half of the cases [19] and involved type II fibres [13,19,21] and less frequently type I fibres. Architectural disruption of the myofibrillary structures and Z-line streaming were frequently observed. Individuals who underwent biopsies in two different muscles did not show the same histopathological features in the two specimens [19,23].

Isolated inflammatory infiltrates with a lymphocytic predominance were described surrounding small blood vessels or rarely, degenerating muscle fibres (termed ‘necrosis’) [12,13,19,20,21]. These infiltrates, termed “lymphorrhages”, reported in between 23% [19] and 32% [21] of biopsies, were suggested to be more likely in those biopsies taken early in the disease process and/or prior to steroid therapy [12,22]. The Dutch group associated the presence of lymphorrhages in muscle biopsies with thymoma-MG [5], but this was not found by others [12]. The presence of inflammatory cells did not appear to correlate with the disease severity grade [12].

Brownell [15] described two cases with particularly severe neurogenic atrophy of the tongue with fatty replacement of atrophic fibres after >5 years of MG symptoms. In retrospect, these cases may have had MuSK-MG, as they had severe bulbar weakness with triple furrowing of the tongue and neostigmine-resistant weakness [15]; features now recognized as strongly suggestive of MuSK-MG [24].

Table 1
Summarising the histopathological changes in the non-ocular and extraocular muscles in myasthenia gravis (MG) and comparisons with strabismus from other (non-myasthenic) causes.

Muscle type	Light microscopy (*n*)	Electron microsopy (EM)	
		EM general (*n*)	EM mitochondria (*n*)
MG limb before serotyping ($n=13$)*	AF type II >> type I (6), N-atrophy (6), MFD (4), LI (4), FCMR (2), lymphorrages (2), cores/targets (1), necrosis (2)	ZBS (2), IMCL (2)	Enlarged (2), SSA (2), abn. cristae (2)
MG limb AChR+ ($n=5$)**	N-atrophy (3), AF type II >> type I (5), MFD (1), rims (2), cores (2)	ZBS (1), IMCL (1)	Enlarged (1), SSA (2), abn. cristae (2)
MG limb MuSK+($n=5$)**	N-atrophy (2), AF (4), MFD (4), rims (2), cores (1)	ZBS (2), IMCL (2)	Enlarged (1), SSA (1), abn. cristae (1)
MG EOM ($n=6$)#	N-atrophy (2), AF (3), MFD (1), FCMR (3), lymphorrhages (1), LI (1), degenerative fibres (1)	ZBS (1), IMCL (1)	Enlarged (1), SSA (1)
Strabismus EOM ($n=9$)	AF (3), MFD (5), FCMR (3), rims (2), degenerative fibres (1), LI (1)	ZBS (5), IMCL (4)	Enlarged (3), SSA (3), abn. cristae (6), degenerated (1)

(*n*) refers to the number of reports (see detailed supplementary tables); myasthenia gravis (MG); "N-atrophy" refers to neurogenic atrophy; AF to atrophic fibers; MFD to myofibrillary disarray; FCMR to fibrocellular ± fatty muscle replacement; LI to lymphocytic infiltrates; ZBS to Z-band streaming; IMCL to intramyocellular lipid; SSA to subsarcolemmal infiltrates; abnormal (abn.) cristae refers to fragmented, disrupted or abnormally arranged cristae; AChR+ refers to acetylcholine receptor antibody positive MG; "rims" to peripherally located mitochondrial aggregates on Gömori Trichrome stain; cores (or minocore/target fibres) refers to central areas devoid of mitochondria; MuSK+ refers to muscle specific kinase antibody positive MG; EOM to extraocular muscle. Note that * refers to only 2 reports on ultrastructure before serotyping; ** 1 report on ultrastructure in MuSK+ MG and 2 in AChR+ MG; #1 report on ultrastructure in EOMs in MG.

Ultrastructural studies were few but showed disintegration of the myofibrillar structures and abnormal mitochondria (some with crystalline inclusions) that appeared enlarged and collecting as subsarcolemmal aggregates [18,20].

From these early reports on muscle biopsies from myasthenic individuals who were infrequently treated with adrenocorticotropic hormone (ACTH) or prednisone, we can conclude that limb (and/or diaphragm) muscle showed more neurogenic compared to myopathic features. The pathological consequences of chronic denervation observed in myasthenia were postulated to be the consequence of functional denervation and/or absence of acetylcholine as trophic factor at the muscle endplate [5,12,18,19,25,26].

3.2. Comparison of histopathology of non-ocular muscles in AChR- and MuSK-MG

Subsequent to the identification of pathogenic antibodies by radio-immunoassays, a few case series and case reports [27,28,29,30] compared or described the histopathological features in limb muscles of AChR-MG and MuSK-MG (Supplementary Table 2). Rostedt-Punga et al. compared limb muscle biopsies of AChR-MG and MuSK-MG cases taken between 17 and 43 years after symptom onset at which stage the cases had either recovered or only had moderately affected myasthenic weakness [31]. Although most of the patients were older than 50 years, and the MuSK-MG cases were on average 10 years older than the AChR-MG cases, they reported similar mitochondrial abnormalities in both the MuSK- and AChR-MG groups [31]. In a smaller middle-aged cohort, in which the AChR-MG cases were older than MuSK-MG cases, and in whom limb muscle biopsies were performed prior to immune therapy in most, although the biopsied muscles were also frequently noted to have normal strength, a higher frequency of atrophic fibres were reported among AChR-MG compared to MuSK-MG cases. Nevertheless, similar features and frequency of mild mitochondrial stress (minicores and subsarcolemmal mitochondrial aggregates) were reported [32]. Although a few patients had COX negative fibres (<2%) almost all were older than 40 years [32]. Others reported more prominent neurogenic changes in AChR-MG and all the samples showed evidence of mitochondrial stress [33]. Although there were a few more individuals in the MuSK-MG group who showed more severe mitochondrial ultrastructural damage, the mitochondrial enzyme immunohistochemistry quantification showed greater abnormalities in the AChR-MG group [32,33].

Given the few samples and often in older individuals, the exposure to chronic immune therapies and the inclusion of patients in remission, it is difficult to determine whether there are clear differences in the pathological findings between AChR-MG and MuSK-MG muscles. However, neurogenic changes and mitochondrial damage were recurring themes [31,32,33]. With the exception of lymphocytic infiltrates reported in earlier literature, the light microscopy findings were similar to those reported prior to 1994.

3.3. Histopathological findings in myasthenic extraocular muscles

Two early reports on EOM histopathology in MG cases at autopsy, showed lymphocytic infiltrates in the EOMs (disease duration <3 years on average) (Supplementary Table 3) [5,13]. Two patients were recorded specifically as having total external ophthalmoplegia and the EOMs were "almost completely replaced with fat" [5]. More recent case reports describe similar fibrofatty changes in EOMs in MG patients with treatment-resistant ophthalmoplegia in AChR-

MG (after 1.2 years), and triple seronegative MG (after 3.5 years), but without lymphocytic infiltrates [34,35] similarly to Hoogenraad et al. [36]. The ultrastructural evidence of mitochondrial stress was similar to the reports in non-ocular muscles in MG [35]. Although based on only a few reports, the histopathology of EOMs, which were also very weak, were reported to show more fibrocellular and fatty replacement of the atrophic fibres compared to the descriptions in limb muscles.

While Sakimoto and Cheng-Minoda [37] focused on the endplate junctions of the EOMs of myasthenic patients who underwent biopsies within months of showing non-responsiveness to cholinesterase inhibitors, they reported increased post-junctional folds in some cases despite normal myofibre and mitochondrial ultrastructure, which was interpreted as a compensatory response to denervation.

3.4. Comparisons with muscle pathology in experimental MG models

Although the animals in experimental autoimmune MG (EAMG) models are often sacrificed soon after EAMG induction, histopathological evidence for muscle mitochondrial stress was observed in both MuSK- and AChR-MG induced EAMG (Supplementary Table 4). Ultrastructural mitochondrial changes were noted within 24 h of passive transfer of AChR-antibodies in rodents [38] and one week after active AChR-EAMG induction [39]. However, after 8 weeks from the active induction of AChR-EAMG and MuSK-EAMG the latter showed 2-fold more frequent ragged red fibres [40]. Of interest, Zhou et al. (2014) compared EOMs with non-ocular muscle histology at 48 h after passive AChR-EAMG and noted more lymphocytic infiltrates in the EOMs compared to the limb and diaphragm muscles [41].

3.5. Comparing the EOMs histopathological findings in strabismus with those in MG

As the most distinguishing feature of the EOM biopsies in MG was fatty replacement of the extraocular muscle fibres, we were interested in the histopathological features of paresed or paralysed EOMs without the influence of MG conditions (Supplementary Table 5). Surprisingly, the histopathological reports in EOMs of patients with strabismus were similar to the descriptions in MG (Table 1). The most severe cases with EOM and levator muscle paralysis showed similar light microscopic [42,43,44,45,46,47,48,49] and ultrastructural mitochondrial abnormalities ranging from mild [50] to pronounced alterations [46,48,51]. The severity of the pathological changes appears to correlate somewhat with the degree of paralysis; those cases who could still generate muscle force despite ocular misalignment (e.g. sensory esotropia in the blind eye or pendular nystagmus), showed normal histology including ultrastructure [35,45]. Taken together we conclude that the structural mitochondrial changes noted in the EOMs may in part be a secondary consequence of severe muscle weakness [52].

4. Discussion

Although this review, which summarizes the histopathology in muscle biopsies of myasthenic individuals, is based on relatively few reports, two predominant themes emerge; there is a consistent picture in non-ocular muscles, irrespective of the pathogenic antibody subtype, of substantial structural change to myofibres consequent to functional denervation and mainly type II fibre atrophy. Secondly, varying degrees of mitochondrial stress and damage are described in both AChR-MG and MuSK-MG. Interestingly type II fibres also contain less mitochondria than type I fibres [53] and may explain their increased susceptibility to the effects of mitochondrial stress. An important aim of the review was to focus on the extraocular muscle changes in MG; EOMs compared to limb muscles showed similar, albeit more severe, pathological features, but the few reports which exist are based on severely weak EOMs in contrast to the muscle biopsies sampled from limbs which were either mildly weak or not weak at all. Moreover, the pathology in the EOMs was not specific to MG, but also found in paralysed EOMs due to other causes. Indeed, limb muscle biopsies from patients with chronic neurogenic weakness, such as spinal muscular atrophy, showed similar light microscopic and mitochondrial degenerative changes [33,54] suggesting that at least some of the features observed in the myasthenic muscle biopsies are non-specific and related to denervation and poor contractility.

It is known that a major effector mechanism of AChR-MG is a complement-mediated attack, which is activated by the pathogenic immunoglobulin (Ig)G1 and IgG3 antibodies, resulting in AChR loss and thereby functionally denervating the muscle endplate [2,12,55]. Although the IgG4 antibodies in MuSK-MG are not complement activating, there was nevertheless a reduction in the density of AChRs at the muscle endplate [1,30] and evidence of neurogenic atrophy [33]. It is therefore not surprising that features of functional denervation are described in both AChR-MG and MuSK-MG. The mitochondrial myopathic changes described in MG may be more frequent or more pronounced in MuSK-MG, although inconsistent [56], and even in cases without substantial weakness [33]. Moreover, in AChR-EAMG models the muscle transcript changes in EOMs and limb muscles were most prominent in the gene pathways related to metabolism, and particularly a shift to oxidative metabolism [57]. Due to the EOMs' unique metabolic characteristics such as high firing rates and energy requirements, they may be particularly susceptible to the "MG-induced" shift to oxidative metabolism and increased muscle proteolysis and thereby a reduction in the generation of muscle force [57]. In EAMG rodent models and in vitro muscle preparations treated with MG sera, a prominent effect was noted on muscle contractility which was over and above the neuromuscular junction transmission defect [58,59,60]. The poor muscle force generation would further impact on mitochondrial biogenesis [61] and subsequent muscle atrophy [62,63] (see Fig. 1).

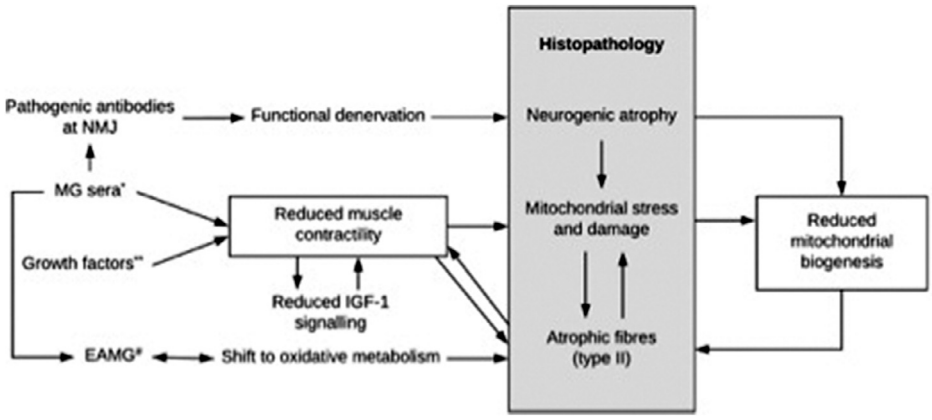

Fig. 1. Histopathological changes in myasthenic muscles inform hypothesis of pathogenetic mechanisms underpinning treatment-resistant ophthalmoplegia in myasthenia gravis.
MG refers to myasthenia gravis; NMJ refers to neuromuscular junction; EAMG refers to experimental autoimmune myasthenia gravis. *[60, 71]; **[67]; # [57].

The EOMs also differ from limb skeletal muscle in their response to botulinum toxin (Botox) and certain growth factors [64]. As an example, the pre-synaptic 'denervation' with Botox in chick embryos showed severe limb muscle atrophy with almost complete fatty replacement within 7 days [65] whereas EOMs injected with Botox did not develop muscle atrophy despite transient paralysis, a phenomena which is thought to be explained by continued EOM satellite cell activation and the potential for remodeling [64,66]. However, a single injection of certain muscle growth factors (BMP4, TGFB1, Wnt3A) into mouse EOMs had a profound reduction of force generation and muscle fibre diameter when examined after 7 days [67]. In addition, several lines of evidence from animal models and in vitro C2C12 myotube cycle stretch experiments [68] have shown that preventing muscle/myotubes from generating a force for ≥48 h (vs continued stretching), resulted in downstream transcriptional signaling changes and myofiber atrophy. EOMs may be more susceptible to these factors.

At a molecular level, a substantial reduction in muscle force promotes muscle atrophy via downregulation of IGF1/Akt signaling, which normally has an anabolic effect in muscle by inhibiting protein degradation and enhancing protein synthesis [in 69]. In addition, knock-down of critical genes in the IGF1/Akt/mTOR signaling pathway had a negative effect on muscle contractility and mitochondrial biogenesis [69]. This pathway has been implicated in the pathogenesis of MG [70]. Furthermore, gene expression profiling of transdifferentiated myocytes stimulated with MG sera showed that the expression of *IGF1R, AKT1* and *AKT2* genes was highly correlated in myocytes from patients with treatment-resistant ophthalmoplegia, compared with myocytes from MG controls (with treatment-responsive EOMs) [71]. This suggests that altered IGF1 signaling may be an effector pathway in the pathogenesis of EOM paralysis in MG though validation in EOMs is required.

The muscle histology in MG suggests that mitochondrial homeostasis is impacted even in mildly weak myasthenic muscles. Although chronic denervation has been shown to impact mitochondrial biogenesis [72], substantial changes in the muscle transcriptome was evident within 7 days of denervation together with a shift in mitochondrial homeostasis from fusion to fission and fragmentation [72,73]. In turn, dysfunctional mitochondria further trigger atrophy signaling pathways by excessive production of reactive oxygen species.

We postulate that extraocular muscles may be at greater risk of mitochondrial damage secondary to reduced myofiber contractility and functional denervation, resulting in a cascade of atrophy signaling pathways which may contribute to the tipping point in the development of treatment-resistant ophthalmoplegia in MG (Fig. 1). Strategies to improve contractility in weak EOMs are critical early in MG. Preliminary studies in MG suggest earlier and possibly a more aggressive approach to immune therapies in the context of extraocular muscle paralysis, is required [9]. Nevertheless, controlled trials are required to definitively formulate treatment strategies.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.03.009.

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