ULTRASTRUCTURAL FINDINGS OF URAEMIC MUSCULAR DAMAGE: FUNCTIONAL IMPLICATIONS

G M Savazzi, E Govoni*, M M Bragaglia*, V Cambi, L Migone

University of Parma, and *University of Bologna, Italy

Summary

The ultrastructure (US) of the skeletal muscle from 10 patients on conservative treatment (PCT) and 10 haemodialysed patients (HP) was studied. The two groups exhibited no qualitative differences but quantitative alterations of the mitochondrial apparatus and capillary vessels were more impressive in the HP group. Disarray and loss of the myofibrillar sarcoplasm was the most obvious change in the uraemic muscle. The US findings are consistent with a neurogenic atrophy of the uraemic skeletal muscle but other features indicate a primary myopathic damage probably caused by a deficit of muscle microcirculation and by changes in the mitochondrial respiratory chain.

Introduction

The aim of this study was to determine whether ultrastructural investigation of the skeletal muscle from uraemic patients undergoing conservative treatment or chronic haemodialysis treatment could:

1. Enhance information provided by light microscopy concerning the pathogenesis of muscle damage in uraemia.

2. Explain the common neuromuscular symptoms on the basis of ultrastructural changes.

3. Establish whether the reduction or disappearance of neurological and muscular symptoms of chronic haemodialysis patients really corresponds to reduced neuromuscular damage.

Material and methods

This report is based on an electron-microscopic study of 10 patients on conservative treatment (serum creatinine ≥14mg%) and 10 haemodialysed patients (treated
for 71 to 158 months) with electromyographic neurogenic changes [1] and histological and histochemical evidence on muscle biopsy corresponding to the classic pattern of neurogenic atrophy.

In each case a biopsy sample was taken from both the rectus femoris and gastrocnemius muscle; standard methods employed have been described elsewhere [2].

Results

Muscle biopsy submicroscopic findings in the two groups of patients examined presented no qualitative differences, and they are therefore described together.

*Myofibrillar sarcoplasm changes* involving the diameter of myofibrils and their spatial filamentary organisation:

i) Irregular widening and dense streaming of the Z-line material into the I-band part or into the entire length of the sarcomere. The details of the underlying thin and thick myofilaments are obscured by Z-line electron-dense, granular-appearing material.

ii) Disorganisation and formation of abnormal aggregates of myofilaments, non segregated in myofibrils, forming the ‘cores’ of the so-called ‘target’ fibres (Figure 1).

![Figure 1. Gastrocnemius muscle biopsy (GMB), conventional electron microscopy (CEM). Longitudinal section of a target fibre showing three concentric zones, normal outer zone, intermediate zone containing Z-line fragments, electron-dense central zone with ‘pig-tail’ formation (7160 X, Reduced for publication)](image)
iii) Degeneration and loss of myofilaments, initially restricted to the peripherally located myofibrils. As the myofibre damage progresses, sparse patches of degeneration and loss of myofilaments are found in the internal myofibrils. The final step corresponds to a loss of intact myofibrils, more marked at the fibre periphery. The myofilaments are replaced by an amorphous-granular matrix containing variable quantities of beta-glycogen, lipid vacuoles, lipofuscin granules together with sparse clustered mitochondria. Randomly disposed sarcomeres with no definite spatial orientation (straw-truss appearance) and sparse fragments of sarcomeres radially disposed around an electrondense, finely granular ovoid mass, forming the so-called 'cytoplasmic body' (Figure 2) are often seen.

![Figure 2. GMB, CEM. A sharply circumscribed 'cytoplasmic body' resulting from a homogeneous osmophilic mass and a peripheral halo of radially oriented filaments (9070 x, reduced for publication)](image)

*Extrafibrillar sarcoplasm changes* as qualitative and quantitative alterations of mitochondria and accumulation of intrasarcoplasmic inclusions:

i) Transverse reorientated hypertrophic intermyofibrillar mitochondria (Figure 3).

ii) Hypertrophied longitudinal mitochondria, or 'sentinel mitochondria' (Figure 3, inset).

iii) Subsarcolemmal clusters of hyperplastic small electrondense mitochondria (Figure 4).
Figure 3. GMB, CEM. A large area of a myofibre containing many hypertrophic transverse re-oriented mitochondria (7460 x, reduced for publication. Inset: a sentinel mitochondria: its axis coincides with the long axis of the myofibrils (17200 x, reduced for publication)

Figure 4. GMB, CEM. Sarcolemmal diverticulum containing a large cluster of small electron-dense hyperplastic mitochondria. The adjacent sarcolemma shows a 'saw-toothed' appearance (4500 x, reduced for publication)
Figure 5. GMB, CEM. Large subsarcolemmal groups of cylindrical structures (concentric laminated bodies) formed by a variable number of lamellae. Enclosed within these bodies is a cytoplasmic area containing glycogen granules (6680 X, reduced for publication)

Figure 6. GMB, CEM. Two intramuscular capillaries showing a thickened and reduplicated amorphous-appearing basal lamina (5250 X, reduced for publication). Inset: thickened basal lamina of an intramuscular capillary containing a caleospherite (28870 X, reduced for publication)
iv) Mitochondria showing ‘hypercristae’, intracristae, paracrystalline inclusions.

v) Subsarcolemmal large groups of ‘concentric laminated bodies’ (Figure 5).

vi) Increase in sarcotubular profiles.

**Sarcolemmal changes** such as an earlier regular ‘saw-toothed’ profile of the sarcolemma, whereas in the more atrophic myofibres, a more irregular and tortuous appearance of the plasma membrane is found.

**Myonuclear changes** such as deep infoldings of the nucleolemma.

**Intramuscular capillary changes** thickening of the basal lamina showing a normal amorphous appearance (Figure 6) or containing scattered/clustersed spherical crystalline-like inclusions arising from concentric deposition of calcific material, called ‘calciospherites’ (Figure 6 inset).

**Discussion**

The earliest submicroscopic changes in muscle fibres involve the contractile matrix of skeletal myofibres, the myofibrillar sarcoplasm. As the degeneration proceeds and the myofilaments gradually disappear, the myofibril size become smaller until they break down completely and lysis of the myofilaments extends to increasingly large areas of fibre and only a finely granular non-contractile sarcoplasmic matrix remains in the background.

Rarely, intermyofibrillar cross-overs are detected, such a sign possibly suggesting a limited attempt of morpho-functional compensation.

Findings indicative of reinnervation in the form of target and/or targetoid fibres (Figure 1) are more frequent. It is worth recalling, however, that in terms of mechanical performance the motor neurones reinnervating these fibres make up a wider but less efficient motor unit [3].

The early reduction of muscle strength in uraemic patients may not be immediately detectable without ergometric methods, but the patient himself becomes aware of it once the loss of contractile material has become widespread through the musculature. As the phenomenon progresses there is also reduction and atrophy of the muscle mass. Therefore disarray and loss of the contractile matrix easily explain in terms of a strict morphological profile some of the symptoms of uraemic patients: easy fatiguability, reduced muscular strength and muscle atrophy.

In the extramembranous sarcoplasm, the earliest changes affect the mitochondria near the Z-band; normally small and round these mitochondria account for real hypertrophy and become oval, then lengthen transversely, so that they lie perpendicular to the main axis of the myofibrils. This re-orientation depends on ex vacuo phenomena caused by the progressive loss of the contractile matrix because reduction or loss of whole myofibrils removes the mechanical support for the mitochondria normally lying between them.

Another qualitative change of mitochondria is represented by an increased
number of cristae, which increases the respiratory surface for each organelle; at this stage an evident hyperplasia becomes the paramount finding.

The changes are all probably an expression of an attempt to increase the metabolic respiratory capacity in order to supply the needs of the contractile muscle apparatus, but the same progressive hyperplasia and a further loss of the contractile structure indicates the incapacity of such changes to keep up with the greater metabolic requirement of the remaining myofibres.

In addition, the endomysial capillaries constantly show a thickening of the basement membrane. This finding was commonly observed in both conservatively treated and haemodialysed patients. On the contrary, deposition of electron dense material resulting from salt precipitation with formation of intra-luminal occlusions (calcospherites) was found in one patient undergoing conservative treatment and in four haemodialysed patients. Such calcified intramuscular microvessels were more frequently encountered in the haemodialysed population.

Therefore our ultrastructural results are not in keeping with the general hopeful assumption that the diminished subjective symptomatology of the haemodialysed patient in comparison with the conservatively treated patient means reduced damage of the muscular parenchymal structures.

From the pathogenetic point of view, the histological and histochemical findings observed in the uraemic skeletal muscle are consistent with neurogenic atrophy [1], and the ultrastructural findings confirm these previous light microscopic observations; nevertheless many submicroscopic features also indicate the existence of primary myopathic damage probably caused by a deficit in the muscle microcirculation and by the changes of the mitochondrial respiratory chain.

References

1 Savazzi GM, Cambi V, Migone L et al. Proc EDTA 1980; 17: 312
3 Leuman JAR. J Neurol Neurosurg Psychiatry 1959; 22: 306

Address for correspondence: V Cambi, Department of Internal Medicine and Nephrology, University of Parma, Italy