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Involvement of the muscle–tendon junction in skeletal muscle atrophy: an ultrastructural study

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Abstract

Background: The muscle–tendon junction (MTJ) is a physiologically vital tissue interface and a highly specialized region in the muscle–tendon unit. It is the weakest point in the muscle–tendon unit, making it susceptible to strain injuries. Nonetheless, knowledge of the pathological changes affecting this region and of its response to the atrophy process is very limited. The aim of the study was to examine MTJ ultrastructural morphology in patients with different conditions that induce skeletal muscle atrophy and to attempt a grading of the atrophy process. *Materials and Methods*: Fifteen patients undergoing amputation in the distal or proximal third of the lower leg due to chronic or acute conditions were divided into two groups. Specimens of gastrocnemius muscle collected at the time of surgery were analyzed by histology and electron microscopy. The contact between muscle and tendon was measured using a dedicated software that calculated semi-automatically the base (B) and perimeter (P) of muscle cell finger-like processes at the MTJ. *Results*: Electron microscopy. The cells in the atrophic muscle of the chronic group were shallow and bulky. In the acute group, the myotendinous endings differed significantly in their structure from those of the chronic group. In atrophic muscle, the contact between muscle and tendon was reduced by quantitative and qualitative changes in the myotendinous endings. The B/P ratio allowed definition of three grades of myotendinous ending degeneration. *Discussion*: It is unclear whether degenerative changes induced by immobilization in muscle and, specifically, the MTJ are temporary and reversible or permanent. *Conclusions*: This preliminary study suggested a classification of ultrastructural MTJ changes into grade 0, reflecting a quite normal MTJ; grade 1, an intermediate process that might lead to irreversible atrophy or to recovery, spontaneously or with drug therapy; and grade 2, irreversible process with complete structural alteration.

Keywords: muscle skeletal atrophy, TEM, muscle–tendon junction, grading.

Introduction

The muscle–tendon junction (MTJ) is a physiologically vital tissue interface and a highly specialized region in the muscle–tendon unit. Muscle force between the muscle and tendon is transmitted through the MTJ; in fact, in this region the tension generated by muscle fibers is transmitted from intracellular contractile proteins to extracellular connective tissue proteins of the tendon [1, 2]. Morphological studies have demonstrated that at the MTJ the collagen fibrils insert into deep recesses, which are formed between the finger-like processes of muscle cells. Membrane folding increases the contact area between muscle fibers and tendon collagen fibers [3].

The basic definition of skeletal muscle atrophy is a decrease in cell size. Atrophy can also occur as a decrease in muscle mass, protein content, fiber number, or strength, and can be due to a decrease in fiber cross-sectional area, length, or both [4].

Numerous and heterogeneous pathological conditions cause muscle atrophy in humans [5].

Profound atrophy is often a consequence of diseases such as cancer and AIDS. Muscle immobilization, as commonly seen when a limb is placed in a cast after an orthopedic injury, causes rapid muscle loss that may require months of physical therapy to reverse. The effectiveness of glucocorticoids such as dexamethasone is limited by muscle wasting, seen as a side effect of these agents. Even during normal ageing, there is a gradual loss of muscle mass and a diminished capacity to reverse that loss, which results in weakness and morbidity [6–10].

The classical studies of Sweeny PR (1983) [11] and Tidball JG (1984) [12] described the ultrastructural changes of the MTJ in genetically dystrophic chickens and in muscle cell atrophy, respectively.

Immobilization of the muscle-tendon unit is known to be followed by intramuscular fibrosis, muscle cell atrophy and loss of extensibility and strength; in addition, the tensile properties of immobilized muscles are markedly decreased during the disuse period [2]. Although various findings suggest that under loading conditions the MTJ is the weakest element in the muscle–tendon unit, making it susceptible to strain injuries, knowledge of the pathological changes in this region and of its response to the atrophy process is very

limited [13]. Moreover, there is a dearth of scoring systems for rating the grade of ultrastructural MTJ change; such a tool would be very useful towards developing a clinical and experimental model to guide prognosis.

We examined MTJ ultrastructural morphology in muscle tissue from patients with different conditions that induce skeletal muscle atrophy, to attempt a grading of the atrophy process based on a morphometric study of the contact area between muscle and tendon. To do this, we used a dedicated software that calculated semi-automatically the base (B) and perimeter (P) of muscle cell finger-like processes and the B/P ratio.

Materials and Methods

Fifteen patients undergoing amputation in the distal or proximal third of the lower leg due to diverse chronic or acute conditions were divided into two groups.

Group A included 12 elderly subjects (average age 79 years, range 65–85 years, seven right and five left, 10 men and two women), of whom 10 underwent amputation for vascular disease (four from complications of type 2 diabetes, six from complication of chronic vascular disease), one for chronic osteomyelitis four years after sustaining an exposed fracture, and one for squamous cell carcinoma of the skin on the anterior aspect of the leg. Patients with skeletal muscle atrophy suffered from pathology secondary to different conditions, but they have a common history of very long bed rest. The average time from disease onset to amputation was 60 months (range 36–84 months). Group B included three otherwise healthy young subjects involved in car accidents (average age 32 years, range 25–35 years, two right and one left, three men), in whom an exposed fracture (Gustilo IIIC) induced acute arterial insufficiency requiring amputation (average time from the accident 5 hours, range 3–12 hours).

Specimens of the musculo-tendinous junctions were collected at the time of amputation with the patients' informed consent.

Histology

For light microscopy, specimens were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, at 4^0C for 24 hours, embedded in paraffin, cut into longitudinal sections (3 to 5 µm thick), and stained with Hematoxylin–Eosin and Toluidine Blue.

Electron microscopy

Muscle tissue was prepared according to conventional microscopic procedures [14]. Briefly, fresh samples were cut into fragments measuring 1 to 3 mm^3 and fixed by immersion in 0.1 M cacodylate buffer (pH 7.4), 2% formaldehyde and 5% glutaraldehyde for 24 hours. Specimens were post-fixed in 1% osmium tetroxide and stained in 1% uranyl acetate dissolved in 0.05 M sodium maleate (pH 5.2). After dehydration in alcohol, they were embedded in epoxy resin and then stained with lead citrate before examination with a transmission electron microscope (Zeiss EM 900).

Morphometric TEM analysis

Three to five randomly chosen blocks from each subject were examined at standard magnification (5 micrographs/block). Muscle–tendon contact was assessed using a dedicated software (Cotron Ks 300^{tm}) working in semiautomatic mode that measured the base and perimeter of the finger-like processes of muscle cells at the MTJ (Figure 1).

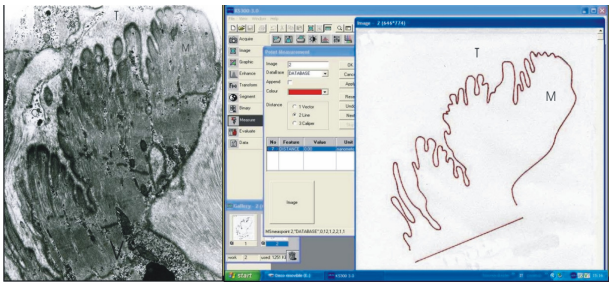

Figure 1 – ***Measurements with Cotron Ks 300^{tm}. Left: specimen from amputation for acute disease (M: muscle cell; T: tendon; arrowhead: base length) (TEM, ×3600). Right: same specimen. Interface of the Cotron Ks 300^{tm} software showing the perimeter and base of the finger-like process of a muscle cell at the MTJ.***

The P/B ratio was taken as a measure of MTJ surface area. Systematic measurement errors were avoided by setting base length in a narrow range (2.45–2.63 µm) (Figure 2).

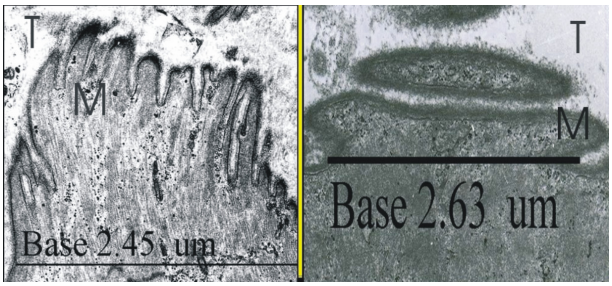

Figure 2 – ***Avoidance of systematic error in measurements. Left: specimen from amputation for acute disease (M: muscle cell; T: tendon) (TEM, ×5800). Right: specimen from a patient with chronic osteomyelitis. (M: muscle cell, T: tendon) (TEM, ×5800). The base length was set in a narrow range 2.45 to 2.63 µm.***

Student's *t*-test was used for statistical analysis. An alpha level <1% (p<0.001) was considered significant.

Results

Histology

The relationships between MTJ, myofascial junctions and myofiber–myofiber junctions were clearly apparent. All group A specimens exhibited split fibers and fibers with centralized nuclei; fiber atrophy and decreased cross-sectional fiber area and length (Figure 3), as well as presence of adipose tissue. Group B muscle fibers had a normal appearance, with centralized nuclei.

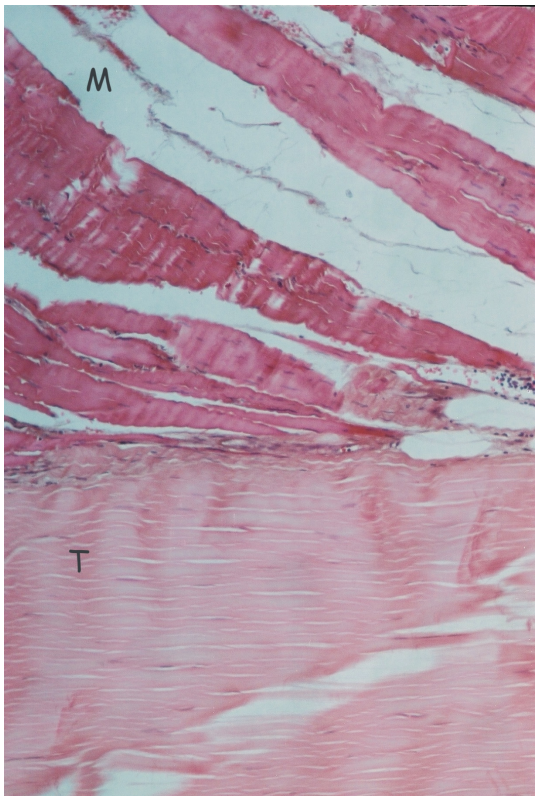

Figure 3 – ***Specimen from a patient with chronic osteomyelitis. Relationships between muscle and tendon cells at the MTJ were clearly apparent. (M: muscle cell, T: tendon) (HE stain, ob. ×10).***

Electron microscopy

Group A: In atrophic muscle cells were shallow and bulky (Figure 4a). In some cells, the processes were completely atrophied or had become cylindrical (Figure 4b). The basal lamina was slightly thickened in both muscle types. Collagen fibril orientation at the tendon end of the junction showed no differences between muscle fiber types, or between immobilized and control limbs. Not all fibrils ran parallel with the stress line of the tendon, as some were oriented transversely or obliquely (Figure 4c). Group B: Structurally, the myotendinous endings of types I and II muscle fibers differed significantly from those observed in the chronic group. In type I muscle cells, the terminal finger-like processes were relatively short and contained several sarcomeres. In type II cells, the processes were longer and further divided into smaller subunits.

Not other pathology-related differences were found.

Morphometric TEM analysis

Group A specimens exhibited a reduction in muscle–tendon contact area, with quantitative and qualitative changes in the myotendinous endings; the average B/P ratio was 2.69 (range: 5.57–1.55). In the group B, considerable folding of the terminal finger-like processes resulted in an average B/P ratio of 10.71 (range: 11.35 to 10.07) (Figure 5). The difference between the two groups was significant (p<0.001).

The B/P ratio was used to rate muscle atrophy at the MTJ on a three-point scale: grade 0: >10; grade 1: from 10 to 5; grade 2: <5.

There is not any differences of B/P ratio in the two study groups, according to the type of muscular fibers (I and II).

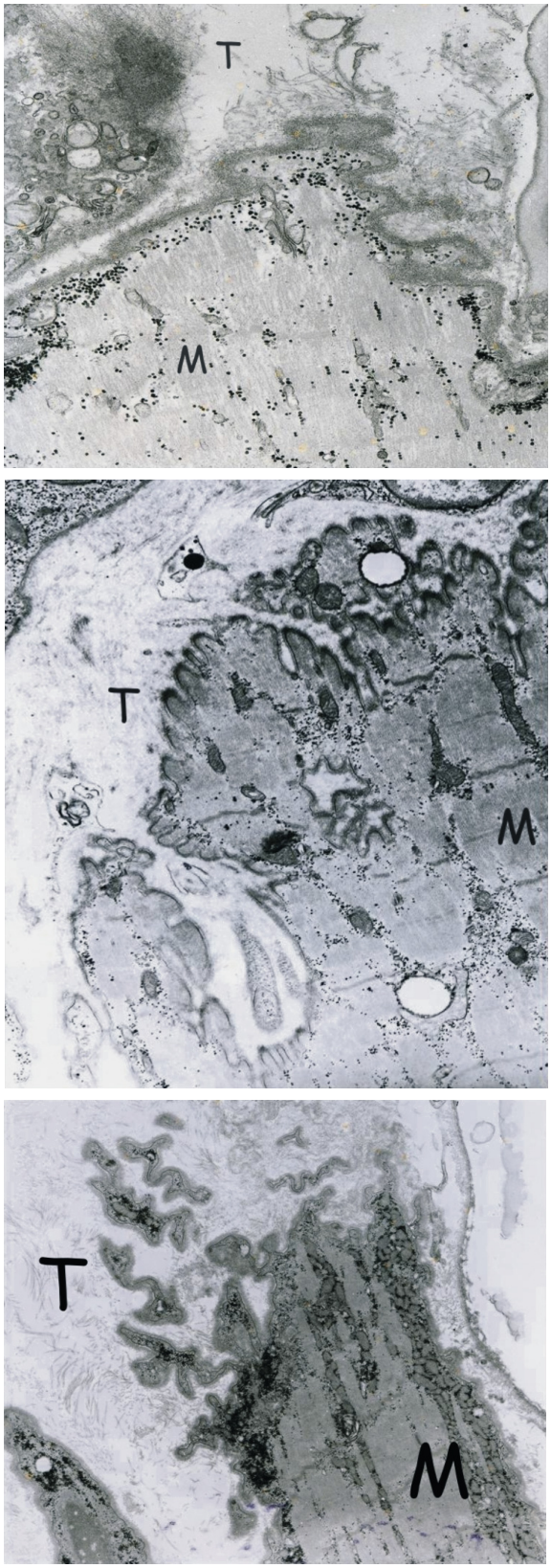

Figure 4 – ***Specimen from a patient with chronic osteomyelitis. Shallow and cylindrical (a) or completely atrophied (b) terminal processes. Some fibrils do not run parallel with the stress line of the tendon but are oriented transversely or obliquely (c). (M: muscle cell, T: tendon) (TEM, ×5800).***

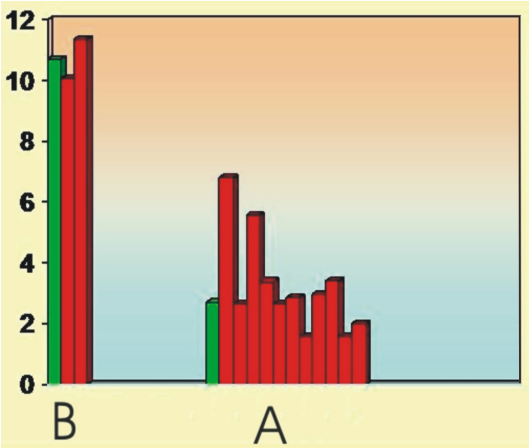

Figure 5 – ***Synopsis of ratio values. Group A: the average B/P ratio of the atrophic muscle was 2.69 (range: 5.57–1.55). Group B: the average B/P ratio of the control group was 10.71 (range 11.35–10.07).***

Discussion

Skeletal muscle atrophy occurs because of ageing, denervation, injury, joint immobilization, bed rest, glucocorticoid treatment, sepsis and cancer. Although, a variety of stimuli induces muscle atrophy, there is a surprising number of similarities in intracellular responses [15–20].

Several authors have described the histochemical adaptation of muscle cells to the atrophy process.

In a recent study, we investigated leg skeletal muscle atrophy caused by a debilitating response to cancer, vascular disease or diabetes. Our elderly patients suffered from different conditions but shared a long history of bed rest and progressive wasting of the lower limb musculature. We found muscle wasting and a strong induction of MuRF1 and MAFbx expression in skeletal muscle in patients with early disease and concluded that the continuing high expression of these proteins over the period when overall proteolysis is accelerated, as in advanced disease, strongly suggested a role for them in both initiation and maintenance of accelerated proteolysis [21].

It is unclear whether degenerative changes to the muscle and, specifically the MTJ, induced by immobilization are temporary and reversible or permanent [22]. Moreover, there is a dearth of scoring systems for rating the grade of ultrastructural MTJ change. Such a tool would be very useful to quantify muscle–tendon unit degeneration and to develop a clinical and experimental model to guide the prognostic process.

Structurally, the MTJ consists of actin filaments that extend from the A-band, actin-binding proteins that bundle the actin filaments together, proteins that link the actin filament bundles to the sarcolemma, transmembrane proteins that link to extracellular components, including components of the external lamina, the external lamina per se, and the proteins that link the external lamina to the collagen-fibril rich matrix outside it [3, 23].

The morphology of the interface between muscle fiber and tendon connective tissue resembles an adhesive joint. The plasmalemma is folded into finger-like extensions and invaginations that increase the interface area by about an order of magnitude over the cross-sectional area of the muscle fiber, and ensure that the stresses applied at the interface are experienced mainly as shear stresses. This is also the case with lateral force transmission, where such morphology seems to create an interface that minimizes the type of stress concentrations that often produce fracture-failure in adhesive joints.

That the MTJ is well designed to transmit force between muscle and tendon is demonstrated by multiple observations that muscle-failure *in situ* is never associated with a separation at the muscle–tendon interface, but rather in the body of the muscle fibers, just proximal to the morphologically defined MTJ [24].

It is established that even during normal ageing there is a gradual loss of muscle mass and a diminished capacity to reverse that loss, which results in weakness and morbidity [5]. In contrast, there is no effective prognostic indicator of the scope for MTJ recovery [25–27]. A practical clinical and experimental tool providing this information would also be useful to gain additional insights into skeletal muscle atrophy.

The present data, obtained from patients with skeletal muscle atrophy secondary to different conditions, suggested to us a rating of MTJ changes into three grades based on the value of the P/B ratio: grade 0, >10; grade 1, from 10 to 5; and grade 2, <5.

The measure chosen here to rate MTJ atrophy, the B/P ratio, could be used as a prognostic indicator of functional skeletal muscle recovery. Grade 0 could reflect a quite normal MTJ; grade 1 an intermediate process that might lead to irreversible atrophy or to recovery, spontaneous or with drug therapy; and grade 2 an irreversible process with complete structural alteration.

Conclusions

This preliminary study suffers from some limitations, mainly the heterogeneous sources of the muscle specimens. In addition, no data are available on the influence of the primary condition and previous level of functioning on the development of atrophy. However, the characteristics of the 12 elderly patients (average age 79 years, range 65–85 years; average time from disease onset to amputation 60 months, range 36–84 months) seem to provide a fairly realistic model of human skeletal muscle atrophy.

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