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Ultrastructural features of supraspinal muscles in rabbits after long-term transcutaneous lateral electrical surface stimulation (LESS)

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Abstract: Lateral electrical surface stimulation is one of methods used in the therapy of the progressive form of idiopathic scoliosis (IS) in children and youth. However, there are data suggesting that this method may lead to serious adverse side effects, when used for a too long period of time per day. To clarify this issue, the present study was aimed at disclosing possible changes in the ultrastructural appearance of rabbit supraspinal muscles undergoing long-term stimulation (9 h per day, 3 months), an animal model successfully used to mimic the situation in humans. In comparison to the control animals, muscles of "overstimulated" rabbits exhibited clear signs of microscopical lesions, including depletion and disintegration of myofilaments, proliferation, dilatation and, sometimes, swelling of sarcoplasmic reticulum and/or mitochondria, as well as signs of destruction of the Z line. The above-mentioned abnormalities, especially the signs of degenerative processes associated with the Z line and the observed microlesions strongly suggest that the failure of the long-term LESS therapy of the IS may be attributable to these ultrastructural lesions.

Key words: Idiopathic scoliosis - Lateral electrical surface stimulation (LESS) - Muscle - Ultrastructure - Rabbit

Introduction

Lateral electrical surface stimulation (LESS) has been thought to be one of the very promising techniques in the therapy of progressive idiopathic scoliosis (IS) in children [3, 4, 11, 27, 28]. However, there is a growing body of evidence that in its "classical" form, (*i.e.*, 9 hours a day, rectangular impulse; duration of single impulse: 0.1-0.2 ms, duration of impulse series: 4.0 s; pause between series: 8 s; impulsation frequency: 40 Hz; based on [1, 4, 28]), LESS is able to cause adverse side-effects that drastically diminish its tolerance by the patient. Thus, dissomnia, different forms of contact dermatitis as well as changes in muscles, are the most often observed side-effects of this therapy [1, 2, 4, 11, 12, 22, 26]. In our previous paper, concerning the light-microscopical evaluation of LESS-induced changes in the rabbit supraspinal muscles, we found that this procedure led to an increase in the muscle congestion, granular atrophy and, in approximately 20% of the affected muscle fibers, their wave-like course [13]. Thus, the above-mentioned effects raised a debate as to whether this approach should be used as a therapeutic tool in its present form [11, 14, 23, 24]. Furthermore, the method appears also too expensive to be widely used in the clinic. As the most pronounced adverse side-effect appear to be changes in the stimulated muscles, the present study was aimed at disclosing possible changes in the ultrastructural appearance of rabbit supraspinal muscles undergoing long-term (*i.e.*, "classical") electrostimulation (9 h per day, 3 months), an animal model successfully used to mimic the situation in humans.

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Materials and methods

Ten male, pure-breed (New Zealand White), age-matched (born within 5 days) and clinically healthy rabbits were used in the present investigation. The animals were adapted for 30 days to the new environment. They were kept indoors in a room with controlled

temperature (18˚C) and humidity (70%). Each animal was placed in a separate metal cage (50 × 50 × 50 cm) and received dry chow and water *ad libitum.* At the beginning of the investigation, the rabbits were aged approximately 3.5 months and weighed 2000-2200 g. All experimental and surgical procedures were approved by the Local Ethics Commission of the Warmia and Mazury University in Olsztyn (32/N, 2002).

After the period of acclimatization, the animals were randomly divided into two groups: control (C; n=5), comprising animals not undergoing electrostimulation but wearing the backpack with the device and the experimental one (E; n=5) where the animals were subjected to LESS for 9 h/day, during 3 months. Localization of the stimulatory electrodes, preparation of the skin surface and all technical parameters of the electrostimulator (SCOL-2, Elmech, Warsaw, PL) have previously been described in detail [11]. Briefly, skin rectangle (15 × 6 cm) located on the right side of the animal above the *musculus longissimus thoracis* (MLT; a part of *musculus longissimus dorsi*, the most important muscle entity stabilizing the spine) has been carefully shaved and then, in order to remove hair rests, treated with a dermatologically neutral depilatory cream. Two flat, circular electrodes have been mounted on skin surface by double-sided adhesive tape, approximately 2-3 cm laterally from the median line. The distance between both electrodes was set to approximately 10 cm. Electrodes were then connected with the SCOL-2 device, running the "classical" LESS protocol: 9 hours per day, rectangular impulses, duration of single impulse: 0.1-0.2 ms, duration of impulse series: 4.0 s; pause between series: 8 s; impulse frequency: 40 Hz.

The ultrastructural examination was done on samples collected both from places subjected to LESS (on the right side) and from non-electrostimulated ones (on the left side of the animal). Blocks (1.3 × 0.4 × 0.3 cm) of the muscle were gently stretched and pinned to balsa wood plates, fixed by immersion in 1.5% glutaraldehyde in phosphate buffer (pH 7.2), postfixed in 2% osmium tetroxide (2 h, room temperature) and then embedded in Epon 812. Semi-thin sections were stained according to the method of Levis and Knight [15]; the appropriate place for preparing ultrathin sections was established under a light microscope. The ultrathin sections were then contrasted with uranyl acetate [25], followed by lead citrate [21]. Ultrastructural examination was conducted using an Opton 900 PC transmission electron microscope (Opton, Germany).

Results

Data concerning changes evoked by LESS in stimulated MLTd, found at the light-microscopic level, have previously been published elsewhere [13].

Electron-microscopic evaluation of muscle sections taken from bilateral MLT of animals belonging to the control group did not reveal any signs of abnormalities (Fig. 1).

Electron micrographs obtained from sections prepared from the left MLT (*i.e.*, contralateral to the side of stimulation) showed no pathology in the vast majority of striated myocytes (Fig. 2). Mitochondria were usually rod-shaped (Figs. 2, 4). Sporadically, intracellular spaces were observed, which were either empty (Fig. 5) or contained lamellar structures (Fig. 6). Clusters of glycogen particles of various sizes were visible in the sarcoplasm (Figs. 2, 6). The spaces between myofibrils usually had correct dimensions (Figs. 2, 6). Only sporadically the sarcoplasm occupied a larger area (Fig. 4). The Z line frequently showed an irregular course and sometimes it was invisible (Figs. 4, 6).

On the other hand, electron microscopic examination revealed lesions in the ultrastructure of approx. 50% of muscle fibers in the right MLT of rabbits subjected to long-term LESS (Fig. 3). Thus, from several to several dozen of muscle fibers with depletion of myofilaments and dilatation of intermyofibrillar space were observed within each analyzed section (Fig. 7). The myofibril system was loose, and sometimes resembled combed hair. In about 30% of such fibers, the continuity of myofibrils was disrupted in various parts of the sarcomere (Fig. 7). Sporadically, entire sarcomeres fell apart (Fig. 8). Vesicular dilatation of the sarcoplasmic reticulum and its proliferation were also observed (Fig. 8), however, the intensity of such changes varied significantly. In some cases, granular and fibrous remnants of muscle fibers were observed, with macrophages located nearby (not shown).

Clusters of normal or enlarged/swollen mitochondria were observed relatively frequently (Fig. 7). Furthermore, various-sized empty spaces, often filled with disintegrated lamellar structures, were often observed (Figs. 9, 10). Relatively frequently, a thickening of the Z line was seen, often close to the lamellae-filled spaces (Fig. 9). Sporadically, the Z line showed an irregular, zig-zagging course (Fig. 3).

Discussion

The present study revealed that the failure of the "classical" LESS (with the curative time-frame set for 9 hours a day) in patients suffering from IS may be attributable to pronounced ultrastructural lesions in muscles undergoing a kind of "overstimulation". Thus, the experimental design allowed us to provide a clear-cut evidence that too long electrostimulation of skeletal muscle is responsible for both the atrophic (depletion of myofilaments, disorganization of the Z line) and degenerative changes (various-sized and -shaped intracellular empty spaces, dilatation and swelling of sarcoplasmic reticulum and/or mitochondria, together with the destruction of the Z line) that, taken together, may be the reason for the low compliance of patients, what, in turn, results in therapy failure.

Interestingly, similar but definitely less pronounced changes observed in a subset of myocytes of the contralateral (*i.e.*, unstimulated *musculus longissimus thoracis*), may be explained by the assumption that the muscle entity became injured due to the unproportional load of work as it tried to counteract the LESS-forced scoliosis [29]. However, this hypothesis need to be verified in detail.

It should be stressed, however, that changes observed in both the muscular and connective tissues are, most probably, secondary to lesions of the afferent limb of the

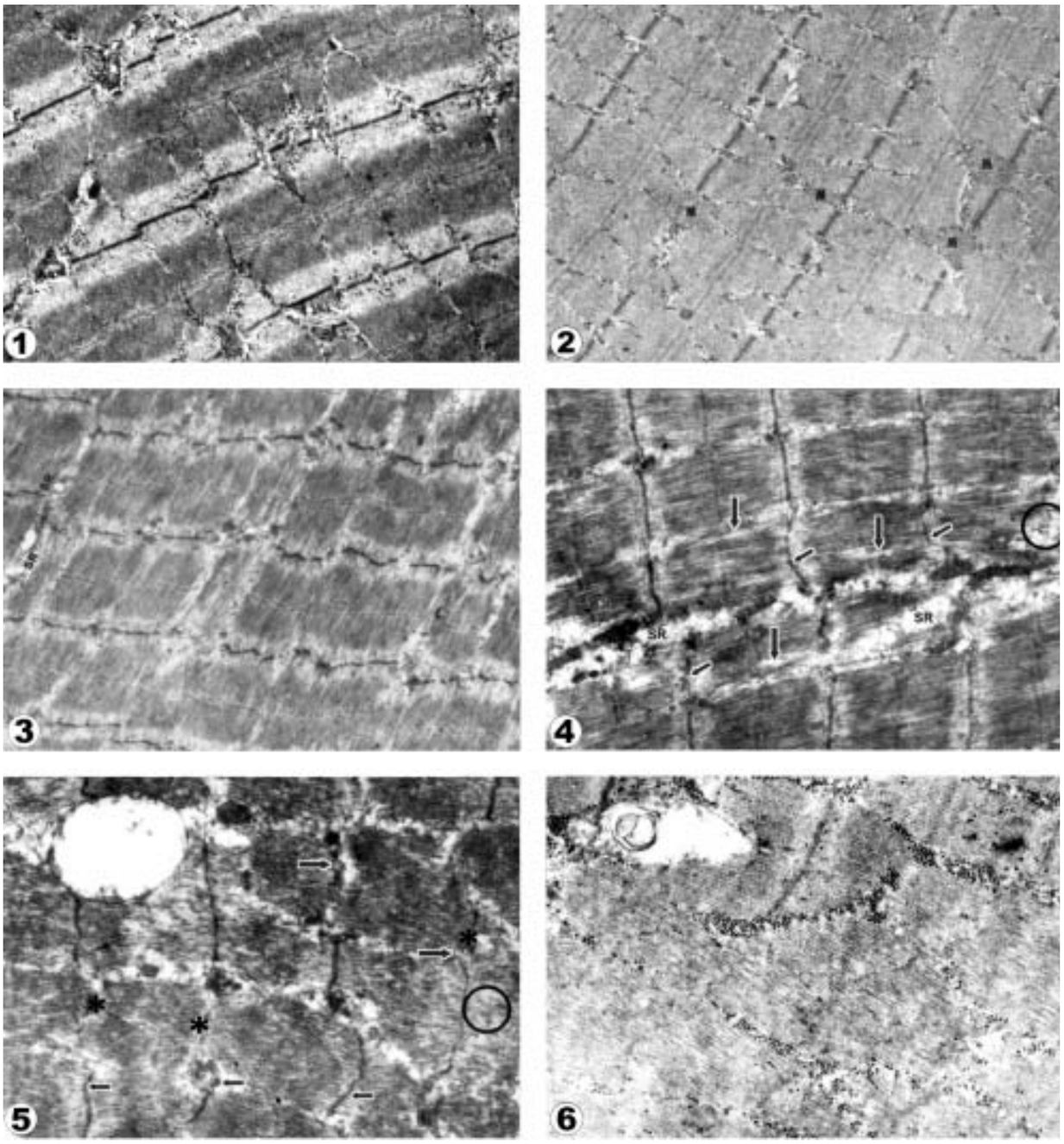

Fig. 1. Control group, *musculus longissimus thoracis dexter* (MLTd). Correct ultrastructure of muscle fibers. × 15 500. **Fig. 2.** Electrostimulated (E) group, left MLT (MLTs). The structure of the muscle is devoid of any visible lesions that may be attributed to the electrostimulation of the contralateral muscle. Note the presence of rod-shaped mitochondria (M) and clusters of glycogen. × 15 500. **Fig. 3.** E group, right MLT (MLTd). Low-power electron-microscopical picture of an "overtrained" muscle, showing both the LESS-unaffected (to the left) and LESS-affected (to the right) muscle fibers, the latter with a clearly visible "zig-zag"-shaped Z lines and some dilated cisternae of the sarcoplasmic reticulum. × 15 500. **Fig. 4.** E group, MLTs. Dilatation of intermyofibrillar space (large arrows), proliferation and dilatation of sarcoplasmic reticulum (SR), irregular course of the Z line (small arrows), depletion of the myofilaments (within the circle). × 15 500. **Fig. 5.** E group, MLTs. Empty space in the left upper corner. Disintegration of the Z line (asterisks) and its irregular course (arrowheads), depletion of the myofilaments (within the circle). T-tubules and terminal cisternae are also visible (arrows). × 19 500. **Fig. 6.** E group, MLTs. Space filled with lamellar structures in the left upper corner, disorganization of the Z line. Visible clusters of glycogen. × 27 000.

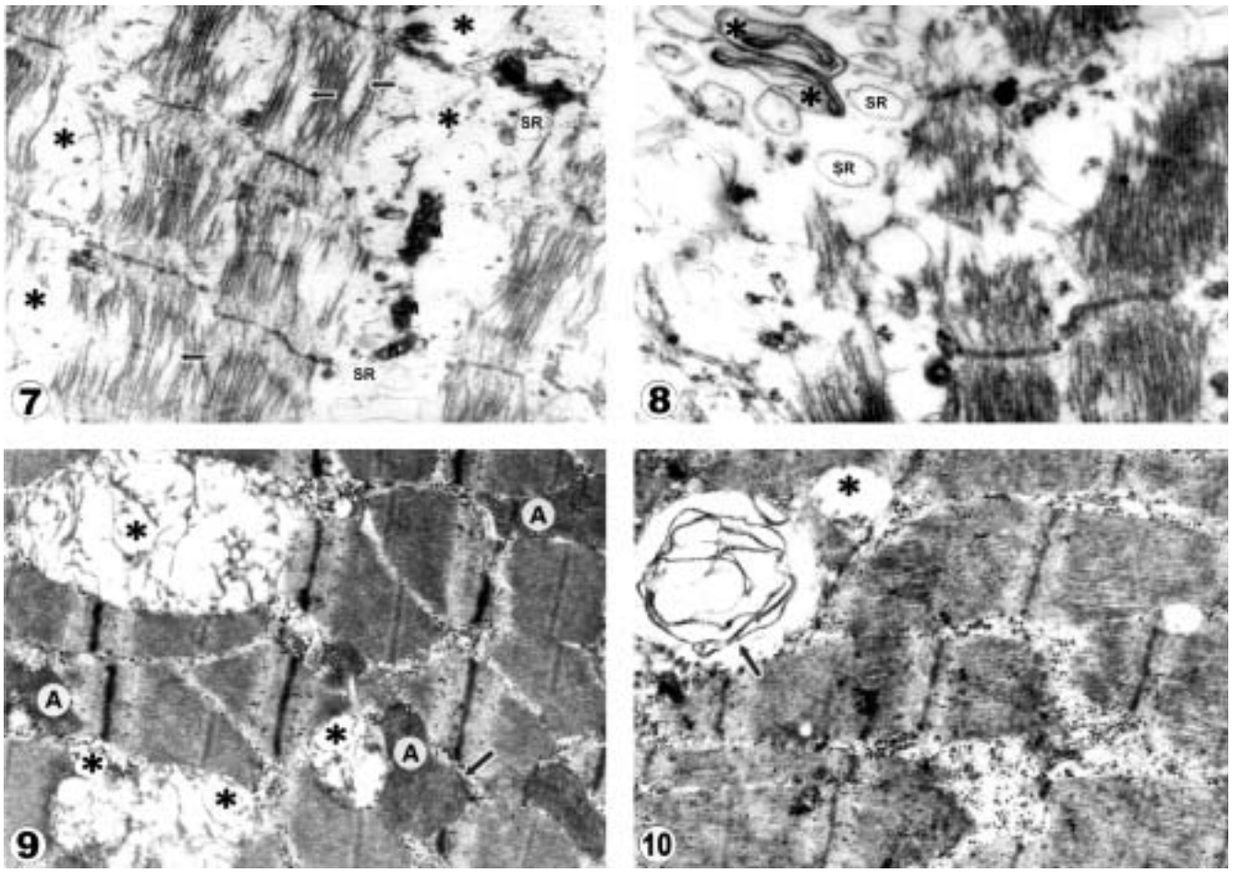

Fig. 7. E group, MLTd. Depletion (asterisks) and degradation of myofilaments in the myofibrils (arrows), dilatation of sarcoplasmic reticulum (SR) and swollen mitochondria (M). × 27 000. **Fig. 8.** E group, MLTd. Depletion of some sarcomeres, proliferation and dilatation of the sarcoplasmic reticulum (SR), myelin-like structures (asterisks). × 27 000. **Fig. 9.** E group, MLTd. Spaces filled with disintegrated lamellar structures (asterisks). Glycogen granules are visible in the sarcoplasm. Moreover, a thickened Z line can also be observed. × 15 500. **Fig. 10.** E group, MLTd. A large intracellular space filled with a myelin-like, lamellar structures (arrow) and a smaller one, devoid of any lamellae (asterisk). Visible glycogen granules. × 15 500.

spinal circuit contributing to the IS etiology [5-7, 10, 13, 16, 18, 20, 24]. This appears to be in line with secondary morphological lesions described in muscles by Grimby and co-workers [4], who also suggested their interrelationship with destructive action of long-term LESS on this afferent pathway.

This view may further be substantiated by observation that the atrophy of the muscle fiber was paralleled by the widening of the sarcoplasmic reticulum, a phenomenon that is thought to be characteristic for simultaneous lesion of muscle fiber and nerve terminal [8, 9, 17-19, 23, 24]. Since the results of the present study closely mirrored the above-mentioned situation, it is tempting to assume that at least part of the adverse influence(s) of long-term LESS may be explained by such a mechanism(s). From the etiological point of view, the chronic injury of the lower motor neuron must also be taken into consideration as a possible cause of the observed changes [9]. It should also be pointed out, that the factor responsible for the observed degenerative changes was most probably the functional overloading of the supraspinal muscle due to the LESS "overstimulation". This may be judged not only from the increase in the number of mitochondria present in the muscle fibres, changes in their shape and structure (organization of the mitochondrial cristae) but also from the destruction of the Z line, what is regarded as a sign of severe damage of the integrity of the skeletal muscle fiber (for details see [11]).

Thus, it could be assumed that the lesions observed are, at least partly, responsible for the failure of "classical" LESS therapy mentioned in the literature, especially due to the "overloading" of the neuro-muscular control mechanisms by too long electrostimulation. The period of stimulation was far too long for any of compensatory mechanisms of the muscle, thus it evoked a "myasthenia-like" status rather, than inducing the strengthening of the muscle entity and, as a result, it was not only

being unable to reverse the IS, but even worsened the condition of patients.

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