FIRST SOUND EVIDENCE OF MUSCLE REGENERATION IN RECOVERY OF FUNCTION OF HUMAN PERMANENT DENERVATED MUSCLES BY A LONG-LASTING FUNCTIONAL ELECTRICAL STIMULATION (FES) TRAINING: BIOPSY FINDINGS

PRVI DOKAZ O REGENERACIJI IN POVRNITVI FUNKCIJE KRONIČNO DENERVIRANE MIŠICE PRI ČLOVEKU S POMOČJO DOLGOTRAJNE FUNKCIONALNE ELEKTRIČNE STIMULACIJE (FES): IZVID BIOPSIJE

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Arrived 2004-02-16, accepted 2004-05-10; ZDRAV VESTN 2004; 73: Suppl. II: 29–31

Abstract – *Contrary to general believe, in one case of 18-month cauda equina lesion four-month electrical stimulation of thigh muscles (impulse energy 1.92 Joule) increased stimulation frequency from 2 to 20 Hz, i. e., up to tetanic contractions. After 2 years of treatment, CT-cross sectional area of quadriceps improved 58.3% (right) and 44.4% (left) with increased muscle density. Mean myofiber size was 37.2 ± 24.8 µm (right) and 40.5 ± 24.9 µm (left). Improvement of stimulated knee torque, from zero to 12.0 Nm and 10.5 Nm, respectively, enabled to stand up trials. Surviving myofibers undergo re-growth (they show the chess board appearance of normal muscle), and dying myofibers continuously regenerate (up to 3% are embryonic myosin positive 3-year post-FES). Regeneration events are essential components of the FES rehabilitation protocol due to superior excitability of regenerated myofibers in comparison to long-term denervated, degenerated myofibers, which were almost not excitable before FES training.*

Izvleček – *V nasprotju z uveljavljenim prepričanjem o primernosti/učinkovitosti metode, smo s štirimesečno električno stimulacijo stegenskih mišic (energija impulza 1,92 J) pri pacientu s sindromom kavde ekvine 18 mesecev po okvari povečali frekvenco draženja z 2 na 20 Hz oziroma do tetaničnega zlitja. Po 2 letih zdravljenja se je prečni presek štiriglavih stegenskih mišic, ki smo ga določili z računalniško tomografijo, povečal desno za 58,3% in levo za 44,4% ob hkrati povečani gostoti mišičja. Povprečna velikost myofibril je bila desno 37,2 ± 24,8 µm in levo 40,5 ± 24,9 µm. Navor v kolenu ob stimulaciji se je izboljšal od nič do 12,0 Nm desno oziroma 10,5 Nm levo, kar je pacientu omogočilo poskuse stoje. Miofibrile, ki so preživele dolgotrajno denervacijo, so regenerirale (ponovno se je vzpostavil vzorec šahovnice na prečnem prerezu kot pri normalni mišici), proces regeneracije pa smo opazili tudi pri miofibrilah, za katere smo glede na morfološke in biokemične spremembe menili, da odmirajo (do 3% pozitivnih na embrionski miozin 3 leta po FES). Potrdili smo, da so ugodni regeneracijski dogodki po kronični denervaciji najverjetneje posledica rehabilitacijskega programa temelječega na FES, ki vpliva na povečano vzdražnost regenerirajočih miofibril.*

Introduction

Mr. Z., a 47 year old man suffered a traumatic cauda equina lesion at the vertebral level of the D-12 with complete and sustained denervation of quadriceps femoris muscles. One year after trauma, thigh muscles were severely atrophic. Clinical neurological examination and assessment by needle EMG, Lumbosacral Evoked Potentials and Somatosensory Brain Evoked Potentials revealed permanent complete loss of motor and sensory functions of the L-1 to L-4 spinal nerves. Measurements of chronaxie longer than 20ms (compare to 0.1–0.7 ms of normal muscle) confirm lower motoneuron denervation. The CT scan showed marked atrophy and fat content of the thigh muscles. The cross sectional area of the quadriceps muscles were 58.9% (right) and 59.1% (left) of healthy persons (1). Knee extension force (isometrically measured in sitting position with 90° knee flexion) was zero.

Electrical stimulation training

A year and a half after injury a Functional Electrical Stimulation (FES) program of both completely denervated quadriceps muscles has been introduced to Mr. Z.
The electrical stimulation program started with 15-min a day, 5 days a week 2 Hz twitches elicited by biphasic rectangular pulses of 120 ms, and 200 mA. These impulses are1000 times longer than for innervated muscles (1, 2). To reach the all

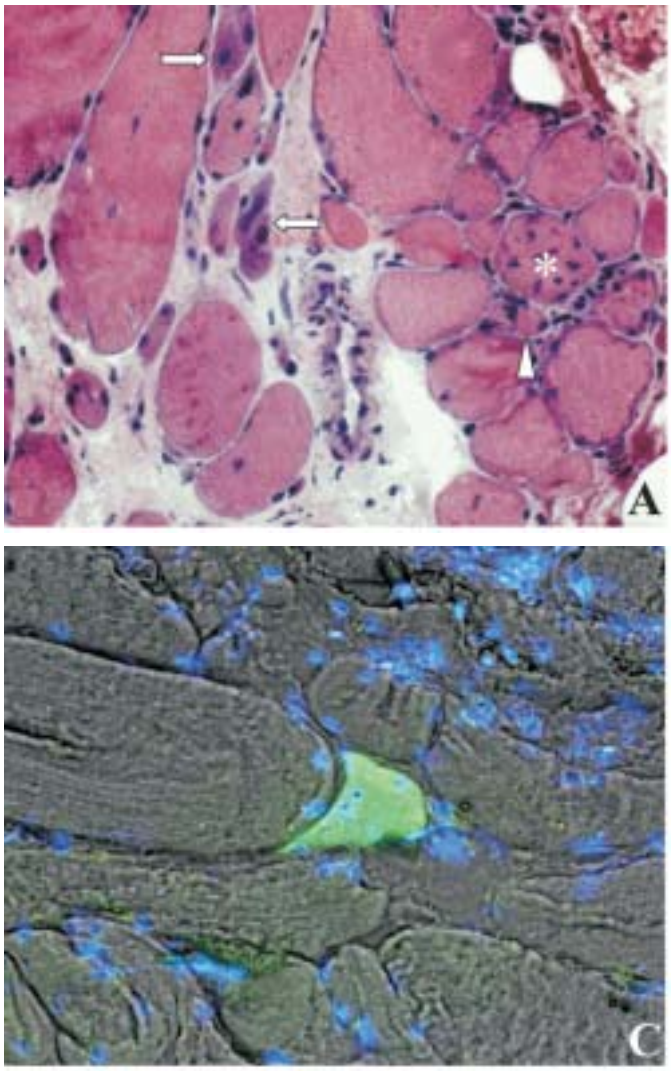

Figure 1. *Muscle size and regeneration after 2-y FES. A – H-E stains, C – Immunohistochemistry with monoclonals against MHCemb.*

quadriceps muscle fibers by surface electrical stimulation high impulse energy is needed (impulse energy 1.92 Joule). At this stage of long-term denervation atrophy, electrical stimulation can not elicit tetanic contractions.

Muscle excitability increased after 4 months of 2 Hz stimulation. Thereafter, a second FES protocol was added to elicit tetanic contractions (20 Hz, by bursts of 40ms pulsewidth and10ms pause) 2 sec ON – 2 sec OFF (2).

Clinical results

After 26 months of electrical stimulation, the cosmetic appearance of the Mr. Z.'s thighs was similar to those of healthy sedentary subjects. CT-cross sectional area confirmed that this is the result of muscle mass restitution: cross sectional area of the quadriceps muscle went from cm^2 36.0 to 57.9 (right) and 36.1 to 52.4 (left), i. e., they became 94.7 (right) and 85.7 (left) of healthy subjects. Muscle density in Hounsfield Units rised from 11.04 to 26.36 (right) and 10.73 to 24,14 (left).

In spite of the structural achievements, knee extension torque reached 12.0Nm for the right and 10.5Nm for the left quadriceps, i. e. less than 10% of normal subjects. Using this FES-induced torque force Mr. Z. is able to extend the knee in

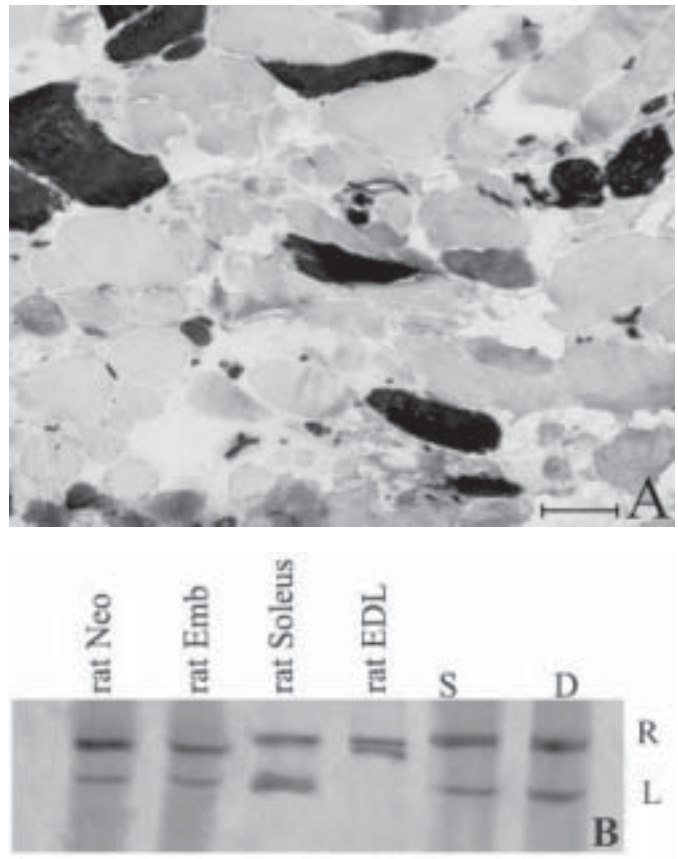

Figure 2. *Myosin ATPase and SDS PAGE of MHC confirm that most of the myofibers recovered from severe atrophy.*

sitting position and to perform electrically induced stand up trials supported by the upper extremities.

Biopsies

After 26 months of FES, needle muscle biopsies were taken from the right and left vastus lateralis muscle. Cryosections were stained with hematoxilin-eosin and anti-MHCemb monoclonals (Novo Castra) (3). Biopsies show round large myofibers [mean diameter 37.2 ± 24.8 μm (right) and 40.5 ± 24.9 μm (left)], while adipocytes and collagen sheets are rare. Some small myofibers (diameter less than 10 mm) are present. They are either severely atrophic myofibers (arrowhead) or basophilic central-nucleated regenerated myofibers (arrows in Figure 1A). Small myofibers with central nuclei are MHCemb positive, i. e., myofibers not older than one-week. Larger MHCemb positive myofiber (30 μm diameter) with subsarcolemmal myonuclei are also present (Figure 1C). These features suggest they are one-month old, since they remind 3 week-old myofibers in myotoxin-induced muscle regeneration of permanent denervated rat muscles (4, 5). Overall, the MHCemb positive myofibers are 8.7 % in right and 2.3 % in left quadriceps.

In conclusion, we have shown that recovery of myofibers trophism and of muscle force could be achieved by a multistage FES stimulation protocol. Figure 2 shows by ATPase histochemistry (panel A) and SDS PAGE of the Myosin Heavy Chains (panel B) that the regain of the muscle mass is the product of both the re-growth of surviving fibers (the majority of the muscle fibers show the chess board appearance of normal adult fibers) and of the continuous regeneration of dying myofibers.

The stimulation protocol has to be improved to reach all the fiber-population and to increase fatigue resistance. Within the next 3 years, the EU-supported RISE project aims to identify optimized stimulation protocols, which could produce early beneficial effects in spite of a reduce burden of daily stimulation for patients.

Acknowledgments

Supported by EU Commission Shared Cost Project RISE (Contract n. QLG5-CT-2001-02191), and by C. N. R. Institute of Neuroscience, University of Padova, Italy.

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