The response of striated muscle to extremity lengthening

Theses
of Ph.D. Dissertation

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Introduction

Research on distraction osteogenesis and improvements in lengthening devices have led to increased popularity of limb lengthening. Many previous experimental and clinical studies have reported the astonishing regenerative potential of the lengthened bones, but the regenerative capacity of the surrounding soft tissues appears to be much more limited. Previously it was thought that elongation of muscular tissue is caused by an increase in the length of sarcomeres. It is now supposed that the lengthening of striated muscles is not only passive stretching, but the muscle give an active adaptive response to the lengthening, known as distraction histogenesis. This process contains degenerative and regenerative phases. The fiber necrosis seems to serve as a stimulus for regenerative activity. It may be assumed that the presence of regenerating fibers in the samples, even in the absence of necrotic fibers, is a likely sign of previous necrosis in adjacent muscle that was not sampled. Even before complete removal of the necrotic sarcoplasmic debris by phagocyte cells the process of regeneration may have begun so that myogenesis and phagocytosis can be visualized in the same muscle fiber concurrently.

Present thesis evaluated the histologic changes in the muscle tissue after limb lengthening in skeletally mature and immature rabbits and assessed the most vulnerable level of striated muscle.

Objectives

The aim of the study was to investigate the theoretical background of the complications of limb lengthening on the surrounding soft tissues, especially in the field of muscle. In response to the mechanical force during limb lengthening, a new skeletal tissue is formed. However, there is not too much knowledge about the ability of striated muscles to accommodate to limb lengthening by producing new tissue. In addition, it is not known which region of the muscle-tendon complex is most vulnerable to the lengthening procedure or the ability to regenerate new tissue to mechanical force has a uniform distribution in the muscle-tendon complex.

My thesis consists of two parts: basic and clinical research. In the first part we have evaluated the histological and immunhistochemical changes in the muscle tissue after limb lengthening in skeletally mature and immature rabbits. Then the changes of proliferative activity of muscle progenitor satellite cells were measured by bromodeoxyuridine immunstaining.

The scope of our clinical research was to examine the muscle response after lengthening the humerus in children and young adults.
At the beginning of the research we were seeking for the answers of the following questions:

1. How the striated muscles react to the elongation of the limb?

2. What is the function of the myotendinosus junction during the lengthening?

3. What is the correlation between age and the appearance of complications?

4. Is there any correlation between the daily lengthening rate and complictions?

5. What is the influence of muscle complications on the lengthening of non-weight bearing upper extremity?

6. How does the function of the humerus change during the lengthening?

Methods

Twenty-three male domestic white rabbits divided into six groups, were operated and different lengthening protocols were used. The surgery was followed by 7 days compression in every lengthened group. In group 1 (4 mature rabbits) 0.8 mm distraction once a day was applied till achieving 20% lengthening. In group 2 (5 mature rabbits) the lengthening rate was 1.6 mm(twice 0.8mm per day) till achieving 20% elongation of the leg. In group 3 (5 immature rabbits) 0.8 mm distraction once a day was applied till achieving 20% lengthening. In group 4 (4 mature rabbits) the lengthening rate was 1.6 mm (twice 0.8mm per day), the increase in length: 20%. Group 5 (2 mature rabbits) and group 6 (3 immature rabbits) contained the sham operated animals (the fixator was placed, osteotomy was done, but lengthening was not performed). Young animals were 9 weeks old, mature animals were 28 weeks old (19). All animals were sacrificed immediately after the completion of the lengthening procedure. All animal procedures

conformed to national regulations and approval by the Ethical Committee was obtained.

After 7 days of compression, the apparatus was distracted by 1 mm once a day. The goal was a 20% lengthening of the tibia.

Animal study

Histopathological analysis of rabbit striated muscle

The flexor digitorum longus muscles from all the control and experimental rabbit legs (and from the two sham groups) were dissected and transverse sections were cut from the border between the proximal third and from the border between the middle and distal third of the muscle belly of the flexor digitorum longus. Thereafter routine paraffin embedding method was used and series of 5 μm thick sections were cut from the blocks. The slides were stained with haematoxyline and eosin Weigert Van Gieson trichrome, and Masson trichrome method. The histopathologic changes were analyzed by a semiquantitative method according to the scoring system of Lee et al. (8). This system consists of rating muscle specimens on a scale of 0 – 3, where 0 is normal. The statistical tests of the histopathologic scores, based on the ordinal scale, among the lengthened groups were done by the Kruskal-Vallis test, followed by the Mann-Whitney U test for individual comparisons among the lengthened groups and within each lengthening group between the lengthened side and the control side.

Immunistochemical analysis of rabbit striated muscle

In order to label the dividing cells, the animals were injected with a thymidine analogue, bromodeoxyuridine (BrdU) one hour before they were killed. BrdU competes with thymidine for incorporation into DNA. Monoclonal antibodies were used to detect its presence in the cell nuclei. For counterstaining a weak Mayer's haematoxylin stain was used. The BrdU-labelled muscle nuclei were counted under light microscope, using an eyepiece. The nuclei were counted in 20 fields to determine the main positive staining index, which is the number of positive fibre nuclei divided by the total number of muscle fibre nuclei examined x100. The statistical analysis were done with the help of Kruskal-Vallis and Mann-Whitney U test.

Clinical study – Humerus lengtehning

Between 1984 and 2005, 11(5 male and 5 female) humeri were elongated with Wagner fixator at the Orthopedic Department of Semmelweis University. The average age of the patients at the time of surgery was 17.8 years (12-31). The average amount of shortening was 8.4 (4.5-13.5) cm. In our group the most common reason of humeral shortening was congenital. The lengthening was performed with Wagner midi or normal equipment. The lengthening protocol was 7 days latency period, following with 2x0.5 mm daily distraction. Range-of-motion tests at the elbow and shoulder were done every 2-4 weeks, the axis of the humerus and bony remodellation were checked in every 2-6 weeks.

Results

Results of histopathological analysis

The adult 1.6 mm/day lengthening rate groups (G2) presented significantly higher mean muscle fiber-size variation score level than the adult 0.8 mm/day lengthening rate group (G1) comparing the summarized data of the flexor digitorum longus. The mean score of muscle nuclei internalisation was higher ($p < 0.05$) in the adult G2 group than in the young G4 group according to our datas. The muscle fiber degeneration was significantly increased in the adult group compared to the young ones lengthened either at 0.8mm/day or at 1.6mm/day rate according to the data of flexor digitorum longus. When the muscle fiber regeneration score of the adults lengthened at the rate of 0.8mm/day or 1.6mm/day was compared with young animals, the latter had significantly increased scores($p < 0.001$). Comparing the mean scores of the young 0.8mm/day and the young 1.6mm/day lengthened groups, the G4 group score was double that of the G3 group. The adult animals showed significantly greater amounts of peri-endomysial fibrous tissue than the young ones, and the animals lengthened at the rate of 1.6 mm/day had a greater amount of fibrosis compared to those lengthened at the rate of 0.8 mm/day. The animals lengthened at faster rate had a greater cell density at the MTJ. The young animals lengthened at the rate of 1.6mm/day (G4) had a significantly increased number of capillaries at MTJ while the young 0.8 mm/day lengthened rate group (G3) showed normal occurrence in the number of capillaries in the

above mentioned region.

Features of fibers	Mature		Immature		Mature	Immature
	0.8mm once/day G1	0.8mm twice/day G2	0.8mm once/day G3	0.8mm twice/day G4	Sham G5	Sham G6
Muscle atrophy	1.5	2.35	1.2	1.375	0	0.0833
Internalization of muscle nuclei	0.75	1.35	0.8	0.9375	0.125	0.0833
Muscle degeneration	1.1875	1.95	0.85	1.25	0.125	0.0833
Muscle regeneration	0.375	0.95	0.8	1.6875	0	0
Endomysial and perimysial fibrosis of muscle	1.0625	2.85	0.85	1.4375	0	0
Internalization of muscle fiber nuclei at the MJT	0.4375	1.45	1.2	2	0	0.0833
Cell number at the MJT	0.5625	1.95	0.95	1.625	0	0
Number of the blood vessels at the MJT	0.25	1.1	0	1.375	0.125	0
Haematomas at the MJT	0.1875	1.85	0	0.125	0	0

Results of immunistochemical analysis

In all of the lengthened limbs a significant increase was observed of positive staining index in the muscle belly and at the myotendinosus junction was observed compared to the unlengthened control limb. At both rates of distraction, the response to lengthening in the young far greater than in the mature animals, being over three times more at the myotendinosus junction and over double in the muscle belly. On average, there were nearly six times more dividing myogenic cells in the myotendinosus junction than in the muscle belly in the non-distracted muscles of mature rabbits, and little over three times more in young rabbits. In mature animals we observed a pronounced shift in the distribution of dividing cells during, as although the positive staining index in the

myotendinosus junction remained greater than in the muscle belly, the ratio between the two decreased significantly during the distraction.

Results of humeral lengthening

The average rate of lengthening was 6.2 (4.5-10.5) cm, the achieved lengthening was 27%(16-44%). We found flexion contracture in 18% of our patients. After recognizing the contracture, physiotherapy was begun, with complete return of range of motion. One patient had neuropraxis that resolved in three months after physiotherapy and the planned lengthening achieved in every case. Non-union or other major complication was not found.

Conclusions

1. The previous view was that the growth of muscle fibers occurs exclusively at the end of fibers should be revised, because we detected satellite cell activation through the entire length of the striated muscle. Similar to this appearance of degenerative signs showed the same pattern. At the background of this phenomenon the distraction histogenesis could be stand. This mechanism could help the muscle to maintain its mass and function.

2. We demonstrated an increased activity in the myotendinosus junction. As proliferation of satellite cells result in the formation of myofibrills, then the myotendinosus junction plays a key role in the longitudinal growth of striated muscles.

3. We found strong correlation between the age of the rabbits and rate of the complication. Based on our results, we suggested that the muscle of younger animals damaged less and shown higher regenerative capacity then the older ones.

4. After evaluation of our results it is evident that the higher lengthened rate we used, the more degenerative and regenerative changes could be seen in the rabbits. These mechanisms were mostly in balance, but this is fragile.

5. According to our results, the muscle associated complications were less frequent on the upper extremity then in the lower limb at same lengthening rate.

6. The muscle function during the lengthening was good, and did not inhibit the patients' daily life. We could leave the fixator in the humerus until total bony reconstruction, so there was no need for plate fixation or bone transplantation, and this led a fast functional recovery of the upper extremity after the fixator removed.

Publications related to thesis

Pap Károly, Berki Sándor, Shisha Tamás, Kiss Sándor, Szőke György. Structural chnges in the lengthened rabbit muscle. Int Orthop Published online on 08 Feb 2008. DOI: 10.1007/s00264-008-0514-2. ISSN: 0341-2695.
IF: 0,977

Kiss Sándor, **Pap Károly**, Vízkelety Tibor, Terebessy Tamás, Balla Mária, Szőke György. The humerus is the best place for lengthening Int Orthop. 2007 Feb 24; Epub ahead of print PMID: 17323094 PubMed - as supplied by publisher]
IF: 0,977

Relative ability of young and mature muscles to respond to limb lengthening. Shisha Tamás, Kiss Sándor, Pap Károly, Simpson Hamish, Szoke György.J Bone Joint Surg Br. 2006;88-B:1666-9.
IF: 1,790

Pap Károly, Kiss Sándor, Shisha Tamás, Domos Gyula, Holnapy Gergyely, Szőke György. Hisztopatológiai elváltozások a harántcsíkolt izomban végtaghosszabbítást követően. Magyar Traumatológia, Ortopédia, Kézsebészet és Plasztikai Sebészet. (in press).

Shisha Tamás, Pap Károly, Kiss Sándor, Szoke György. Izomsejt proliferáció az izom-ín átmenetben végtaghosszabbítás során. Magyar Traumatológia, Ortopédia, Kézsebészet és Plasztikai Sebészet. (in press).

Publications not related to thesis

Open adductor tenotomy in the prevention of hip subluxation in cerebral palsy. **Károly Pap**, Sándor Kiss, Tibor Vízkelety, György Szőke Int. Orthop. 2005: (29)18-20.
IF: 0,676

The incidence of avascular necrosis of the healthy, contralateral femoral head at the end of the use of Pavlik harness in unilateral hip dysplasia.

Pap Károly, Kiss Sándor, Shisha Tamás, Marton-Szucs Gábor, Szoke György Int Orthop. 2006 Oct;30(5):348-51. Epub 2006 Apr 26.
IF: 0,977

Osteochondritis (Calve's disease) of a vertebral body-a rare form of vertebraplana. Shisha Tamás, Kiss Sándor, Varga Péter Pál, Bucsi László, **Pap Károly**, Szőke György. Eur Spine J. 2006: (15)377-383.
IF: 1,824

The dangers of intraosseous fibrosing agent injection in the treatment of bone cysts. The origin of major complications shown in a rabbit model. Shisha Tamás, Marton-Szucs Gábor, Dunay Miklós, Pap Károly, Kiss Sándor, Nemeth Tibor, Szendroi Miklós, Szoke György. Int Orthop. 2006 Jul 4; [Epub ahead of print]
IF: 0,977

A nyílt adductor tenotomia eredményessége a spasztikus csípőizületi subluxatio megelőzésében. **Pap Károly**, Kiss Sándor, Vízkelety Tibor, Szőke György Magyar Traumatológia, Ortopédia, Kézsebészet, Plasztikai Sebészet 2004:(3) 216-223

Az ép oldal avascularis necrosisa Pavlik kengyellel kezelt egyoldali csípődysplasiában **Pap Károly,** Kiss Sándor, Matron-Szűcs Gábor, Shisha Tamás, Szőke György Magyar Traumatológia, Ortopédia, Kézsebészet, Plasztikai Sebészet elfogadva

Shisha Tamás, Kiss Sándor, **Pap Károly**, Vízkelety Tibor, Szőke György. A nyílt repozició és rövidítéses varizáló derotációs femur osteotomia hosszú távú eredménye spasztikus betegek csípőluxatiójának kezelésben. Magyar Traumatológia, Ortopédia, Kézsebészet, Plasztikai Sebészet. 2006;49:382-388.