The response of muscles to limb lengthening

Ph.D. thesis

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INTRODUCTION

Limb lengthening procedures are performed more and more frequently with the progress of surgical techniques. They are usually applied when the difference of length between the two contralateral limbs leads to a loss of function. The cause behind the difference of length may well be a trauma, tumour or osteomyelitis damaging the growth plate of the epiphysis of the bone. In other cases it is a congenital disorder or developmental abnormality, which requires surgical correction.

The most frequent and most sever complications during distraction osteogenesis are related to the non-compliance of the muscle tissues. Such complication may be the contracture of the muscles, loss of range of motion, subluxation, dislocation or pain. Therefore, the factor limiting the maximum rate of distraction is primarily the muscle tissue.

This makes it understandable, that in last several years there have been many study teams doing research in the field of muscle reactions during limb lengthening. With a better understanding of the physiopathology, we expect to find ways to lower the frequency and severity of the complications caused by the intolerance of the muscle tissue.

As a consequence of chronic distraction, the mytotic activation and proliferation of the ever-present satellite cells is immediately initiated. This may be done through well-described signal transduction channels and by means of mediator molecules, described in the literature; and may also be done through mechanisms not yet discovered. These satellite cells are indispensable for the genesis of new myofibrils. During this process the length of muscle fibres increases by the formation of new sarcomers.

These processes are observed in every muscle. However, when the muscle tissue cannot adapt to the lengthening rate, there are other pathological reaction that can be observed. One of the most frequent among these pathological reactions is the parallel activation of fibroblasts and

consequent fibrosis; interstitial bleeding near the musculo-tendinous junction; oedema; and the partial or complete rupture of the muscle. Most of the clinical complications (e.g. loss of range of motion) are a result of the above described tissue reactions. Understanding the *early* processes is of paramount importance, since this may give us a chance to react *before* the occurrence of clinical symptoms. By adapting the distraction rates during the lengthening process, we may be able to prevent some of the complications.

Measuring the proliferative activity of myogenic precursor satellite cells by means of immunohistochemistry is a well-described method. Several studies have proven that the number of satellite cells increases during chronic distraction. It was not clear, however, what pattern the proliferative activity of satellite cells shows in the musculo-tendinous junction, which plays a key role in formation of new muscle fibres. It was also not clear what differences may exist between young and mature muscles. This difference may help us understand why young muscles adapt better to lengthening.

In the long-term, we expect the increase of muscle mass during distraction. However, during or immediately after the lengthening procedure we often observe a loss of bulk. There are several reasons to explain this finding. First of all, the operated and lengthened limb is always in a relative immobilisation compared to the healthy contralateral side. This immobilisation leads to a certain degree of atrophy. Secondly, the production of significant quantities of muscle fibres and satellite cells is time consuming; and therefore does not have an important impact on weigh change, at this stage.

On the other hand, there are other processes, which may lead to an increase of bulk. Such can be the above mentioned pathological tissue reactions: oedema, fibrosis or bleeding. Therefore, the mass change of muscles can be an early indicator of these pathological reactions. Still, there have only been few publications about mass changes observed in the distracted muscles; and these articles reported cases of one specific muscle

distracted at one specific rate. As a consequence, the results showed unexplained inconsistency. For the statistical evaluation and interpretation of the results, several groups of young and mature rabbits had to be examined, distracted at different lengthening rates.

GOALS

1. The proliferation of myogenic precursor satellite cells

1.1 Satellite cell activation at the myotendinous junction

Our goal was to observe, the proliferative activity of satellite cells at the myotendinous junction. Based on previous reports, we expected an increase of the activity. We also wanted to demonstrate possible differences between the young and mature animals.

1.2 Satellite cell activation in the muscle belly

Similarly to the myotendinous junction, we wanted to measure to proliferative activity of satellite cells in the muscle belly, both in young and mature animals. Previous studies suspected that the formation of new myofibrils and proliferation of satellite cells might occur exclusively in the myotendinous junction. We therefore presumed, that we might not observe any increase of activity at all.

1.3 Change of distribution of satellite cells.

The number of proliferating satellite cells may increase both in the muscle belly and at the myotendinous junction, but the increase in the two regions may be disproportional. Therefore it is possible that not only there is an increase of the absolute number, but also in the distribution of satellite cells. The drift in the distribution can be an indicator of pathological processes

2 Change of muscle mass

2.1 The change of muscle mass in the different groups

We presumed that different muscles might react differently to distraction. Longer muscle tendon complexes may tolerate better the lengthening, since the same amount of lengthening is relatively more for the shorter muscles. We wanted to identify the mass changes of muscles with good and bad compliance.

2.2 Change of muscle mass in young and mature animals

Based on clinical experience and previous reports we presumed that young rabbits would tolerate better the distraction. Our goal was to find out whether the age of the animal has an effect on the observed mass changes.

2.3 The rational of the change of bulk observed in muscles

Our goal was not only to register the data on mass change but also to be able to provide a rational for the changes observed.

2.4 The change of muscle mass as a factor predicting complications

As a conclusion of the above investigations our goal was to show if change of bulk, or a parameter strongly correlating with it (e.g. circumference or volume of the limb) could be used to predict complications frequently seen during lengthening procedures.

3. Histopathology

Our goal was to perform the histological examination of the samples taken from young and mature animals, distracted at different rates, in order to get a subtle picture of the reactions seen in the different regions of the muscle (i.e. myotendinous junction, muscle belly proximal and distal third).

We wanted to evaluate the results in order to have an understanding of different tissue reactions and to be able to compare the different regions with each other. Also, it was important for us that knowing the histopathlology of the muscles could help us interpret the results of cell proliferation and mass change.

METHODS

The young (9-11 weeks) and mature (27-29 weeks) rabbits were randomly allocated to the groups. The external fixator was positioned and the osteotomy performed in the middle portion of the diaphysis. The bony ends were distanced by 0.8 mm/day, 1.6 mm/day or 3.2 mm/day to reach a total length increase of either 20% or 30%. After reaching the required lengthening, the animals were humanly killed and the muscle tendon complexes of the operated and control side were dissected and prepared. We performed the measurement of length and the weighing immediately.

Indirect immunohistochemistry: A thymidine analogue was used to label the dividing cells. Bromodeuxiuridine (BrdU) was given intravenously one hour before the sacrifice of the animal. BrdU competes with thymidine to be incorporated into DNA. After incorporation the BrdU is stable. The BrdU can be made visible by a specific anti-BrdU antibody (Bu20a).

We counted the positive staining muscle cell nuclei under the microscope. The Positive Staining Index (PSI) was defined as the number of positive staining muscle cell nuclei divided by the total number of muscle cell nuclei multiplied by one hundred. Therefore, the PSI shows what percentage of all muscle cell nuclei are proliferating. The PSI is a good indicator of the proliferative activity of muscle cells.

The histopathological changes were evaluated by Lee's semi-quantitative technique. Thus, the slices were scored from 0 to 3. On this scale 0 always meant physiological histology of the observed tissue and 3 stood for severe structural alterations. We examined nine parameters, adding another four to the five parameters suggested by Lee. The nine parameters: 1. size variation of muscle fibres, 2. internalisation of the nuclei of muscle fibres, 3. degeneration of muscle fibres, 4. regeneration of muscle fibres, 5. endomysial and perimysial fibrosis of muscle, 6. internalisation of the muscle fibre nuclei at the myotendinous junction, 7. cell number at the myotendinous junction line, 8. the number of the blood vessels at the

myotendinous junction, 9. haematomas at the myotendinous junction. The histopathological signs were counted in 20 fields to determine the average occurrence.

RESULTS

Proliferations of myogenic precursor satellite cells

- The proliferative activity of satellite cells was increased in the distracted muscles compared to the control. The positive staining index (PSI) was elevated during the lengthening both in the muscle belly and at the myotendinous junction.
- The proliferative activity of satellite cells is greater in the young than in the mature muscle.
- The proliferative activity of satellite cells is greater in the myotendinous junction than in muscle belly.
- At high distraction rates, the increase of PSI is greater in the muscle belly than in the myotendinous junction. This shows a drift in the distribution of satellite cells, from the myotendinous junction to muscle belly.

Change of muscle mass

- In the muscles of operated but not lengthened limbs („sham") we observed a significant decrease of bulk.
- In the short muscles of anterior compartment (m. peroneus quartus, m. tibialis anterior) we observed a significant increase of mass; or in other cases the partial or complete rupture of the muscles.
- In the long muscles of the posterior compartment (m. flexor digitorum longus; m. peroneus longus) we observed a significant decrease of bulk.

Histopathology

- According to expectations, in young muscles and in muscles distracted at low rates we observed less structural changes than in adult muscles or in muscles distracted at high rates.
- In the short muscles of the anterior compartment (m. peroneus quartus) we observed significantly more pathological signs than in the long muscles of the posterior compartment (m. flexor digitorum longus).
- Though some differences have been observed between the proximal and the distal third of the muscle belly, these differences did not reach the level of statistical significance.

CONCLUSIONS

The myogenic precursor satellite cells react to lengthening with an increase of their proliferative activity. During natural growth or well tolerable lengthening this activity was observed almost uniquely at the myotendinous junction. However, at high distraction rates there is significant increase of activity in the muscle belly, too. At very high distraction rates, damaging the muscle, with which the cell proliferation at the myotendinous junction cannot comply, we observe a more significant increase of activity in the muscle belly, than in the myotendinous junction. This change in the distribution of satellite cells is certainly unfavourable, since, in physiological circumstances, the genesis of new muscle fibres occurs in the musculotendinous junction. During the activation of satellite cells in muscle belly, other cell lines get activated as well, probably through the same mediators. The proliferation and activation of fibroblasts can lead to peri- and endomyseal fibrosis.

The better compliance of young muscle to distraction may therefore be based on the better maintenance of the physiological satellite cell balance between the muscle belly and the musculotendinous junction. In young animals, though the absolute number of satellite cells increases in both regions, the proportions are not altered significantly.

The muscles that tolerate well the distraction reacted with a loss of bulk. The reason for this is the relative immobilisation of the lengthened limb, and the consequent atrophy. This fact points to the importance of early active physiotherapy. The muscles that do *not* tolerate well the distraction racted by an increase of bulk. The reasons behind this increase are pathological structural changes such as fibrosis, oedema and bleeding. The proliferation of myogenic cells and the formation of new muscle fibres does

not have a significant impact on mass at this stage. This suggests, that follow-up of mass change can help identify pathological tissue reactions before the appearance of clinical symptoms. In clinical practice, other parameters correlating with muscle mass could be used, which can be measured by non-invasive methods. By observing the circumference of limb or volume change of the limb or muscle we have a chance to act before the occurrence of clinical symptoms, by adapting the distraction rate during the lengthening process. This could help us prevent some of the severe muscle related complications during distraction osteogenesis.

The candidate's publications in the field of the thesis:

All confirmed impact factors: 6.545

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