JOINT IMMobilIZATION COMPROMISES THE EXPRESSION OF SENSORY NEUROPEPTIDE RECEPtoRS AND IMPAIRS HEALING AFTER ACHILLES TENDON RUPTURE IN A RAT MODEL

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INTRODUCTION

Clinically, it is well known that mobilization is essential for the healing after connective tissue injury. Though, the exact mechanisms for this mechano-biological transduction are still largely unknown. Accumulating data, however, suggest that a variety of neuronal mediators, so called neuropeptides, and their receptors play an important role in tissue repair [1]. Recently we demonstrated that ruptured tendons in rats exhibit extensive new nerve fibre ingrowth during the early regenerative phase of healing (week 1-4) [2]. This is followed by a specific temporal expression of sensory neuropeptides, i.e. substance P (SP) and calcitonin gene related peptide (CGRP), suggesting an essential role in tendon repair. These neuronal mediators have previously been reported to promote angiogenesis and tissue regeneration by stimulating proliferation of endothelial cells and fibroblasts [1].

The present study was designed in order to investigate whether the possible effects of mobilization after Achilles tendon rupture could be explained by changes in the peripheral sensitivity to sensory neuropeptide stimulation, i.e. in the tissue expression of SP- (NK), and CGRP- (CRLR and RAMP-1) receptors.

METHODS

Sixty-five male Sprague Dawley rats were used. Fifty-two rats had their right Achilles tendon subjected to a blunt rupture and were subsequently allocated to cage mobilization or immobilization using a plaster. The remaining rats served as controls. Dissections and tissue collection were performed at 8 and 17 days post rupture for mRNA analysis, while tissue for histology was harvested at 14 and 25 days.

Statistics were based on the Mann-Whitney U and Tukey’s test. The experiments were approved by the local animal ethics committee.

**Histology:** The tissues were fixed in 4% paraformaldehyde solution containing 0.2% picric acid, sectioned (15μm) and stained according to the Hematoxylin-Eosin method. The maturation of the specimens were judged by the amount of edematous tissue, presence of inflammatory cells, quantity of blood vessels and the amount, orientation and maturation of collagen fibers.

**mRNA gene expression:** The tissues were frozen in liquid nitrogen, powdered and treated with Trizol™, chloroform and 70% EtOH, followed by the column fractionation step of the RNeasy® Total RNA Kit. Finally the mRNA was eluted. All cDNA was reverse transcribed at the same time. RT-PCR was performed for each individual sample. The PCR amplification was kept within the range of exponential progression. The PCR reactions were all run in parallel. Glyceraldehyde 3-phosphate-dehydrogenas (GAPDH) was used as an internal control. Negative and positive controls were included in the experiments. All primers were validated and the amplicons sequenced to ensure primer specificity. The PCR products were separated on a 2% agarose gel, scanned and quantified. All assays and assessments were in the linear range of detection.

RESULTS

The mRNA levels for many extra cellular matrix proteins (Table) were significantly increased in both the mobilized and immobilized groups at 8d post rupture. However, at 17d the mRNA levels continued to increase in the mobilized group, while the expression in the immobilized group decreased rapidly.

Histological examination confirmed the molecular analysis by demonstrating a more mature repair tissue in the mobilized groups.

The expression of mRNA for SP receptors (NK, Fig. A) was increased at 8d and 17d post rupture in the immobilized group. Between 8 and 17d, however there was an immense increase in the mobilized group, while the expression in the immobilized group decreased to levels similar to those in the control group. The mRNA expression for CGRP receptors (CRLR and RAMP-1, Fig. B and C) did not show any significant changes at 8d in either of the two groups. Though, between 8 and 17d the mobilized group exhibited a significant increase of the expression of both CRLR and RAMP-1, while the expression in the immobilized group remained unchanged.

**REFERENCES**


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