IMMOBILIZATION OF THE IMMATURE RABBIT MCL LEADS TO THE DEVELOPMENT OF A CATABOLIC ENVIRONMENT IN THE TISSUE

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Introduction:

Skeletal ligaments represent one of the important classes of tissues that are involved in maintenance of joint structural integrity and homeostasis by serving as connectors of bone across joints. The growth and maturation of an immature ligament requires a regulated process of anabolism and catabolism, such that anabolic processes exceed catabolic activity to allow for ligament growth. Immobilization has been used as clinical modality following joint injury. In the immature rabbit MCL model, a long term biochemical effect of immobilization involves a decrease in collagen synthesis and an increase in a serine proteinase activity (1). In this model, immobilization also lead to arrest of the biomechanical maturation of the tissue (2). The purpose of the present study was to assess the effects of immobilization on mRNA levels for a subset of matrix molecules, as well as mRNA levels for enzymes/enzyme inhibitors, involved in regulation of matrix synthesis, degradation and remodeling.

Methods:

Animal model: 38 New Zealand White Rabbits (Reiman’s Furriers) at 3 months of age were entered into the study. The right hindlimb of 14 animals were surgically pinned in flexion and maintained immobilized for 1.5 months (1.5MI) (n=7) and 3 months (3.0MI) (n=7). Age matched control groups (AMC) (n=7) (4.5 month and 6 month old, respectively), as well as a 3 month old control group (n=10) were used for comparison in the molecular analysis. Wet weights (g) of each MCL were taken after surgical excision.

Molecular analysis: Total RNA from the MCLs of both age-matched control and immobilized study groups was extracted using the TRIspin method as described previously (3). 1µg of total RNA from each tissue sample was reverse transcribed using the Stratagene RT kit in order to convert mRNA to complementary DNA (cDNA). Semi-quantitative RT-PCR was performed using specific primer sets for collagen types I and III, biglycan, decorin, MMP-1, MMP-13, the tissue inhibitor of metalloproteinases (TIMPs) TIMP-1, TIMP-2, TIMP-3, and GAPDH (internal control). 1.5 ul aliquots of cDNA were then amplified by the polymerase chain reaction in a final volume of 50uL containing 20mM Tris-HCL, 50mM KCL, 1.5mM MgCl2, 0.5mM of each specific PCR primer and 2.5 units Taq DNA polymerase (Pharmacia; Piscataway, NJ). Amplification was performed using a PT-100 Programmable thermocycler (MJ Research Inc.) with variable PCR cycling parameters. All reactions were determined to be in the linear range of amplification. Densitometric analysis was used in the semi-quantitative assessment. Statistical analysis was performed using one-way analysis of variance (ANOVA).

Results:

Both gross morphological analysis and wet weight measurements of immobilized MCLs demonstrated that these ligaments were significantly smaller and weigh less than AMC at both timepoints (p<0.05). The semi-quantitative RT-PCR data for matrix molecules and metalloproteinase inhibitors in the MCL are summarized in Table 1. For the matrix molecules, decreased mRNA levels were evidenced for biglycan, collagen III, and decorin at both timepoints of immobilization. Depressed mRNA levels were also found for the TIMPs (with the exception of TIMP-3 after 1.5MI) In contrast to the enzyme inhibitors, increased mRNA levels were found for the matrix remodeling/degrading enzymes MMP-13 and MMP-1 (Figure 1). MMP-1 levels were significantly higher (p<0.05) after 1.5MI compared to AMCs but approached control levels after 3MI. MMP-13 mRNA levels were significantly elevated in both 1.5MI and 3MI groups compared to AMCs, which were significantly decreased in the MCLs of 4.5 month old control animals and undetectable in the MCLs of 6 month old control rabbits.

<table>
<thead>
<tr>
<th>Matrix Molecules</th>
<th>1.5 MI</th>
<th>3.0 MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biglycan</td>
<td>D*</td>
<td>D*</td>
</tr>
<tr>
<td>Decorin</td>
<td>D</td>
<td>D*</td>
</tr>
<tr>
<td>Collagen I</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>Collagen III</td>
<td>D*</td>
<td>D*</td>
</tr>
</tbody>
</table>

Table 1. Genes depressed (D) or unchanged (U) with immobilization (* = p<0.05)

Discussion:

The growth of ligament tissue to maturity can be expected to be a function of regulated catabolic and anabolic activity whereby matrix synthesis would exceed degradation until a homeostatic, normal state is attained at skeletal maturity. The requirements for mechanical stimuli in attaining this state is evidenced by the altered morphological (4), biochemical (1) and biomechanical (2) properties of immobilized ligament tissue. Walsh et al suggested that immobilized ligaments achieve a metabolic state whereby anabolism is decreased in skeletally immature animals, resulting in the inhibition of ligament maturation (1). The decrease in mass observed in the immobilized ligaments in this study is consistent with these data, since decreased expression of mRNAAs specific for the matrix building molecules decorin,biglycan and collagen III was observed, while increases in mRNA levels for two matrix degrading/remodeling enzymes, MMP-1 and MMP-13, was also evidenced. The lack of a significant decrease in mRNA levels for collagen type I in immobilized ligaments implies that other mechanisms may be operative to regulate the production of this matrix molecule. Thus, in the immobilized MCL, there is a shift to lower mRNA levels for most matrix molecules assessed, a decline in mRNA levels for inhibitors of matrix metalloproteinases (TIMP-1, TIMP-2, TIMP-3) and an increase in mRNA levels for two enzymes capable of degrading collagen (MMP-1 and MMP-13).

Thus, the immobilization of the MCL likely leads to development of a catabolic environment in the tissue which may account for the failure of the tissue to mature biomechanically, and ultimately atrophy.

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References: