MECHANICAL FORCE PROMOTES FIBROBLAST SYNTHESIS IN THE POSTERIOR TIBIAL TENDON TREATED WITH TRIAMCINOLONE

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INTRODUCTION:
The posterior tibial tendon (PTT) inverts the foot and plantarflexes the ankle while serving as the main stabilizer of the midtarsal joint during forward propulsion. Repeated local corticosteroid injections as well as systemic corticosteroids have been implicated as an etiologic factor for the degeneration and spontaneous rupture of the PTT. Early activity following injury promotes faster healing and recovery from muscular-skeletal injuries. This phenomenon has been demonstrated in studies that show cyclic strain to be essential to tendon health. However, using the mechanical force to promote gene expression in PTT fibroblasts treated with corticosteroid has not yet been characterized. The aim of this study was to assess the gene expression of type I collagen and decorin under combined exposure to mechanical loading and various concentrations of triamcinolone acetonide (TA).

METHODS:
Cell culture
PTT cultured fibroblasts were obtained from three-month-old porcine ankle sections. The culture medium consisted of Dulbecco’s Modified Eagle medium (DMEM)/F-12 with 10% fetal bovine serum, 100 U/ml penicillin and 100µg/ml streptomycin. The culture plates were kept at 37 °C in a humidified incubator with 5% CO2 and 95% air. Mechanical compression force application
Mechanical loading was applied on the PTT cell cultures using Flexercell® Tension Plus™ system. The tenocytes underwent an equibiaxial cyclic tension stress at a frequency of 0.5 Hz with 5% strain for 24 h. The combined effect of steroid and mechanical load was achieved by applying the 5% strain to fibroblasts with differing TA concentrations.

Triamcinolone acetonide administration
To compare different conditions, three concentrations of TA (Sigma Chemical)—10⁻⁷ M, 10⁻⁶ M, and 10⁻⁵ M—were used along with a control without TA [1].

RNA isolation procedure and reverse transcript polymerase chain reaction (RT-PCR)
Total cellular RNA was extracted from the tenocytes. The template for RT-PCR amplification was a 1µg sample of total RNA. Primers specific to decorin and β-actin were used as an internal control. Base sequences for these genes are provided in Table 1. Electrophoresis in 4% agarose gels was then performed. Following electrophoresis, densitometric analyses were completed with AlphaEase™.

Statistical analysis
Groups 1-4 received 0% strain. Group 1 acted as the control group treated with 0.1% ethanol only. Groups 2-4 were treated with TA at concentrations of 10⁻⁷ M, 10⁻⁶ M, and 10⁻⁵ M, respectively. Groups 5-8 underwent 5% strain. Group 5, the control for the second set, was treated with 0.1% ethanol only. Groups 6-8 were treated with TA at concentrations of 10⁻⁷ M, 10⁻⁶ M, and 10⁻⁵ M, respectively. The analysis of variance (one-way ANOVA) with Tukey’s post hoc honestly significant difference test was used to compare the groups. All differences were considered to be significant with values of p<0.05.

RESULTS:
Without mechanical load, TA decreased the mRNA of type I collagen in a dose-dependent manner from 1.36 at the control to 1.30 at 10⁻⁷ M (p<0.05), 1.19 at 10⁻⁶ M (p<0.05), and 1.12 at 10⁻⁵ M (p<0.05) (Fig. 1). When the 5% strain group was compared to the control group, the mRNA expression was increased to 1.54 (p<0.05) in the control group. The TA groups at 5% strain had increased mRNA expression to 1.49 at 10⁻⁷ M (p<0.05), 1.36 at 10⁻⁶ M (p<0.05), and 1.18 at 10⁻⁵ M (p<0.05). In the strain control groups (0% strain), TA decreased the mRNA expression by 0.37 at 10⁻⁷ M (p<0.01) with the extent of the decrease corrected to the TA level. With 5% mechanical force, differences were also seen between the higher TA concentration groups: 10⁻⁷ M (p<0.05), 10⁻⁶ M (p<0.01), and 10⁻⁵ M (p<0.01).

DISCUSSION:
The results of this study indicate that mechanical load on porcine PTT tenocytes improves type I collagen and decorin expression even in the presence of a corticosteroid. The mechanical load showed a significant increase in type I collagen mRNA expression at 10⁻⁷ M and 10⁻⁶ M concentrations of TA. These results suggested that increased gene expression of extracellular matrix component molecules regulated by combined effects of mechanical load and TA might play an important role in tenocyte metabolism [2]. Type I collagen and decorin have major biological roles in tendon or ligament formation. Decorin also mediates the growth of both fiber diameter and length, ensuring optimal formation of type I collagen fibers. These results implicate that a possible tissue-specificity of the interaction type I collagen / decorin correlated to the structure of the proteoglycan to organize and maintain the tissue integrity [3]. In summary, this study demonstrates that mechanical force increases gene expression of type I collagen and decorin even with varying concentrations of corticosteroid. These findings suggest that mechanical force plays an important role in the corticosteroid regulation of gene expression for extracellular matrix component molecules, thereby highlighting the complexity of interaction between corticosteroids and mechanical force.

REFERENCES:

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<table>
<thead>
<tr>
<th>Target gene</th>
<th>Forward primer/ Reverse primer</th>
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<tr>
<td>Collagen I</td>
<td>GGCTCCCTGGCTCCCTTATTAGC / CATGGTACCCTGAAGCCG TTC</td>
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<td>Decorin</td>
<td>GATGCACGTAGCTCAGAAAGG / TCAACCAGAATAAGAGAAGGC</td>
<td>274</td>
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<td>β-actin</td>
<td>GGTGTCCAGAAGCCGCTCT/ TCCACGTGCCACCTCAGAT</td>
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Table 1: Sequences of porcine primers for RT-PCR

Type I collagen (TA only)

Type I collagen (Strain +TA) β-actin

Fig. 1: Typical image of the RT-PCR products of type I collagen. Group 1 was the control without TA and Groups 2-4 received TA in concentrations of 10⁻⁷ M, 10⁻⁶ M, and 10⁻⁵ M, respectively.

Decorin (TA only)

Decorin (Strain + TA) β-actin

Fig. 2: Pictured is a result of the mRNA expression of decorin from RT-PCR, with β-actin as the internal control. Group 1 was the control without triamcinolone acetate (TA) and Groups 2-4 received TA in concentrations of 10⁻⁷ M, 10⁻⁶ M, and 10⁻⁵ M, respectively.

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