The Mast Cell Stabilizer, Ketotifen Significantly Reduces the Biomechanical Severity of Posttraumatic Joint Contractures and Joint Capsule Myofibroblast Hyperplasia in a Rabbit Model.

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SCIENTIFIC BACKGROUND: In the setting of a congruent articular surface, the joint capsule is the critical causal agent of posttraumatic contractures of the elbow. The affected joint capsule becomes thickened, non-compliant and is characterized by myofibroblast hyperplasia and excessive collagen synthesis. These fibrotic changes parallel other fibrotic conditions such as Dupuytren’s contracture of the hand, scoloderma, hypertrophic wound healing and pulmonary, hepatic and renal fibrosis. The mast cell is a connective tissue resident, is activated by musculoskeletal trauma and is capable of liberating pro-fibrotic mediators into the extracellular environment. Elevated mast cell numbers have been recorded in numerous fibrotic conditions, including posttraumatic joint contractures in animals and humans. Pharmacological agents have been developed to impede mast cell degranulation. Ketotifen fumarate is referred to as mast cell stabilizer as it inhibits normal mast cell degranulation by preventing the intracellular calcium influx associated with this phenomenon. Ketotifen has previously been used to effectively inhibit hypertrophic scar formation in the red Duroc pig and a tight skin mouse model1-3.

HYPOTHESIS: Systemic administration of Ketotifen will lessen the biomechanical severity of contractures and the number of joint capsule myofibroblasts in a rabbit model of posttraumatic joint contracture.

MATERIALS AND METHODS: This study was approved by our institution’s animal care committee prior to commencement. Intra-articular injury coupled to internal immobilization of the knee joint was surgically created in skeletally mature female, New Zealand white rabbits (operative contracture). This technique has been previously developed by our laboratory to create stable joint contractures of the knee despite prolonged periods of remobilization4. Four groups were studied: a non-operative control group (CON), an operative contracture group (ORC) and two-operative groups treated with a mast cell stabilizer, Ketotifen fumarate at doses of 0.5mg/kg (KF0.5) and 1.0mg/kg (KF1.0) twice daily, respectively. After 8 weeks of immobilization, animals were sacrificed and flexion contractures (lack of full extension) were measured biomechanically after the removal of internal fixation and myotendinous structures about the knee. Contractures were tested using an MTS-hydraulic biomechanics device specifically designed for testing motion of the rabbit knee under physiological forces5. Immediately following biomechanical testing, the posterior joint capsule of the knee (the motion limiting structure) was harvested and prepared for immunohistochemistry. A double labeling technique was used. Anti-trypsin, anti-tryptase and anti-alpha smooth muscle actin antibodies were used to identify mast cells and myofibroblasts, respectively4,6. Tryptase is a protease specific to mast cells and is an ideal target used to detect mast cells in tissue. Appropriate positive and negative controls were used. A sample size was calculated prior to commencement of the study and looking for a difference of 2 standard deviations of the biomechanical means, 7 animals were needed in each study arm to adequately power this study (β=0.9). Statistical calculations were performed using a linear regression analysis of repeated measures for biomechanical data and an ANOVA analysis of variance for immunohistochemistry data. Significance was defined at p<0.05 for all statistical tests.

RESULTS: Ketotifen was well tolerated by all animals and no adverse events were recorded such as infection or wound dehiscence. Severe flexion contractures, averaging 58±14° were measured in the operative contracture group (ORC), while the average flexion contractures in the Ketotifen groups were significantly reduced (KF0.5: 42±17° and KF1.0: 45±10°, p<0.02). A small baseline flexion contracture (27±12°) was also present in the non-operative control group (CON) (Figure 1). With the baseline contracture of the CON group subtracted (pure contracture), the ORC group had an average flexion contracture of 31±14°, while contractures were reduced to 15±8,17 and 18±10 in the Ketotifen 0.5mg/kg and 1.0mg/kg groups, respectively. This represents 52% and 42% reduction in contracture severity (p<0.03). Myofibroblasts were observed within the non-operative control (CON) capsules but in small numbers (25±4 cells per field; 11±1% of total cells), where a substantial degree of myofibroblast hyperplasia was observed in the joint capsules of the ORC group (277±98 cells per field; 51±2% of total cells). Myofibroblasts numbers were dramatically reduced in both experimental groups treated with Ketotifen: KF0.5: 66±13 cells per field (23±3% of total cells) and KF1.0: 62±7 cells per field (20±2% of total cells). This was significant (p<0.001) compared to the operative control group (Figure 2).

Mast cells were also observed within the non-operative control capsules (CON) (26±4 cells per field) and similar to myofibroblasts, mast cells were markedly elevated in the ORC group (261±87 cells per field). Mast cell numbers were significantly reduced in the operative groups treated with Ketotifen (KF 0.5: 63±10 and KF1.0: 61±7 cells per field, p<0.001).

DISCUSSION: Using this rabbit model of joint injury and immobilization, severe flexion contractures of the knee were observed. Myofibroblast and mast cell hyperplasia was also observed within the more contracted joint capsules (ORC). The mast cell stabilizer, Ketotifen significantly reduced the biomechanical severity of these contractures. Ketotifen also significantly reduced the number of myofibroblasts and mast cells within the affected joint capsule. The results of our study suggest that mast cell degranulation is an important process in the development of joint capsule fibrosis after joint injury. Mast cell degranulation also appears to promote further mast cell recruitment and proliferation. Mast cells do synthesize their own chemotactic factors such as SCF (stem cell factor) and TGF-β1 (transforming growth factor beta) and NGF (nerve growth factor), which may account for this. This work is significant, as we have safely used an FDA approved mast cell stabilizer to reduce both the biomechanical and histological manifestations of connective tissue fibrosis in an animal model of posttraumatic joint contracture.

Figure 1. Posttraumatic flexion contracture severity

Figure 2. Myofibroblast numbers within the posterior joint capsule.

References