The Mast Cell Stabilizer, Ketotifen Significantly Reduces Joint Capsule α-Smooth Muscle Actin and TGF-β Expression and Collagen Hyperplasia in a Rabbit Model of Posttraumatic Joint Contractures.

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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. Similar to other fibro-proliferative disorders such as Dupuytren’s contracture, scleroderma, hypertrophic wound healing and pulmonary fibrosis, the contracted joint capsule is characterized by excessive collagen deposition and myofibroblast hyperplasia. Myofibroblasts are commonly viewed as the effector cell in this fibrotic process. Our laboratory has documented increased expression of the pro-fibrotic growth factor, TGF-β1 within affect joint capsules in humans and animals. TGF-β1 is a potent fibroblast mitogen and an upregulator of the myofibroblast phenotype. The mast cell is becoming increasingly recognized as a pro-fibrotic cell. This connective tissue resident, is activated by musculoskeletal trauma and is capable of liberating pro-fibrotic mediators such as TGF-β1 into the extracellular environment. Elevated mast cell numbers have been recorded in numerous fibrotic conditions, including Dupuytren’s contracture, myofibroplasia in rats, and various fibrotic disorders 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 by our laboratory to effectively inhibit hypertrophic scar formation in the red Duroc pig.

HYPOTHESIS: Systemic administration of Ketotifen will lessen the synthesis of TGF-β1, α-sma and collagen type I and III within affected joint capsules 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. Intracutaneous injury coupled to internal immobilization of the knee joint was surgically created in skeletal 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 immobilization. Four groups were: a non-operative control group (CON), an operative control 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. The posterior joint capsule was harvested and processed for molecular assessments. Western blots and reverse-transcriptase polymerase chain reaction (RT-PCR) techniques were employed to quantify the protein and mRNA expression of α-sma (myofibroblast specific), collagen type I and III, TGF-β1, and tryptase (mast cell specific) based on techniques previously described by our laboratory. The protein and mRNA expression is presented as a normalized ratio relative to the expression of the basal housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A sample size was calculated prior to commencement of the study and looking for a difference of 2 standard deviations of the means, 7 animals were needed in each study arm to adequately power this study (β=0.9). Statistical calculations were performed using a two-way ANOVA analysis of variance with a post-hoc Tukey for Western blot and RT-PCR 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. Flexion contractures were measured prior to harvesting the joint capsule (data presented in separate abstract). Contractures were most severe in the ORC group and were significantly reduced by 52% and 42%, in the KF0.5 and KF1.0 groups, respectively.

In the ORC group (most severe contractures), a significant increase in the protein and mRNA expression of α-sma, collagen type I, collagen type III and TGF-β was observed compared to the non-operative control group (CON) (p<0.01). This is consistent with previous findings from our laboratory. The expression of these markers was significantly less in the joint capsules of both groups treated with Ketotifen (p<0.01) (Figures 1 and 2). Mast cell tryptase expression was substantially higher in the ORC group compared to the non-operative control (CON) (p<0.05). In both groups treated with Ketotifen, tryptase expression was reduced. This reduction was significant in the KF1.0 group (p<0.01) (Figure 3).

DISCUSSION: Increased expression of α-sma (indicative of the myofibroblast phenotype), TGF-β1 and collagen hyperplasia was observed within contractured joint capsules. The greatest expression of these fibrotic markers was observed in the animals with the most severe contracture (ORC). The mast cell stabilizer, Ketotifen significantly reduced the biomechanical severity of contractures and the expression of all of these markers of fibrosis within the joint capsule. The results of our study suggest that mast cell degranulation may be associated with increased TGF-β1 and α-sma expression and collagen hyperplasia within the joint capsule. TGF-β1 is synthesized by mast cells, is known to induce the myofibroblast phenotype and increase fibroblast collagen synthesis. Inhibition of mast cell degranulation also appears to negatively influence tryptase expression within the joint capsule. Products of mast cell degranulation such as TGF-β1 are mast cell mitogens and chemotactants, which may explain this phenomenon. This work is significant, as we have safely used an FDA approved mast cell stabilizer to reduce both the biomechanical severity and molecular markers of connective tissue fibrosis in an animal model of posttraumatic joint contracture. These results suggest further studies targeting TGF-β or other mast cell mediators and/or stimulators are needed to understand the processes contributing to posttraumatic contractures and provide potential alternative therapeutic modalities.

REFERENCES: