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The Mast Cell Stabilizer Ketotifen Fumarate Lessens Contracture Severity and Myofibroblast Hyperplasia: A Study of a Rabbit Model of Posttraumatic Joint Contractures

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Abstract

Background—The propensity of the elbow to become stiff after trauma is widely appreciated and in this setting, the joint capsule is commonly recognized as the major motion-limiting anatomical structure. Affected joint capsules become fibrotic, characterized by myofibroblast hyperplasia and excessive collagen deposition. Mast cell hyperplasia is common within fibrotic tissue and mast cells are known to synthesize many profibrotic mediators. We have hypothesized that mast cell inhibition after skeletal injury will lessen the degree of contracture severity and will reduce myofibroblast hyperplasia within the joint capsule.

Methods—Posttraumatic contractures of the knee were created using a combination of intra-articular injury coupled to internal immobilization in skeletally mature, New Zealand white rabbits. Four groups of animals 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, flexion contractures were measured biomechanically and the posterior joint capsule was harvested for quantification of myofibroblast and mast cell numbers.

Results—Flexion contractures developed in the ORC group ($58 \pm 14^\circ$) and the severity of contracture was significantly reduced in both groups treated with *Ketotifen* (KF0.5: $42 \pm 17^\circ$ and KF1.0: $45 \pm 10^\circ$, $p < 0.02$). Joint capsule myofibroblast and mast cell numbers were significantly increased within the operative contracture group ($p < 0.001$). In both surgical groups treated with *Ketotifen*, myofibroblast and mast cell numbers were significantly reduced ($p < 0.001$).

Conclusions—The use of a mast cell stabilizer, *Ketotifen* was effective in reducing the biomechanical and cellular manifestations of joint capsule fibrosis in a rabbit model of posttraumatic joint contracture. This is suggestive that an inflammatory pathway, mediated by mast cell activation is involved in the induction of joint capsule fibrosis after traumatic injury.

Clinical Relevance—These results suggest mast cell activation is an important event in the genesis of posttraumatic joint contractures. Further work is needed to determine if mast cell inhibition has a role in the prevention of posttraumatic joint contractures in humans.

Introduction

It is well known that disabling motion loss can complicate elbow injuries. Despite this awareness, loss of functional range of motion about the elbow leads to joint ankylosis in 5% of elbow injuries and further surgical procedures are required for the treatment of motion loss in 10–15% of patients suffering a traumatic elbow injury.,.

In the setting of a congruent joint surface the joint capsule is regarded as the critical causal agent of contracture formation. Surgical resection or division of the joint capsule is often required for contractures resistant to aggressive physical therapy and splinting yet despite excellent intra-operative motion gains, the trend is for only a 50% return of the preoperative deficit over the long-term.,.

In patients with chronic posttraumatic contractures of the elbow, the joint capsule becomes thickened and markedly less compliant compared to unaffected elbows.,. Histological preparations have documented a significant degree of myofibroblast hyperplasia within contracted capsular tissue. We have demonstrated that elbow motion in the flexion-extension arc is inversely proportional to the number of myofibroblasts identified within the joint capsule. The myofibroblast is a specialized contractile lineage of the fibroblast, which is characterized by the expression of a contractile protein, alpha-smooth muscle actin (α -SMA). mRNA and protein expression of collagen type I and III, both of which are major synthetic products of myofibroblasts and fibroblasts are also increased in affected joint capsules.

Our laboratory has recently developed a rabbit model to study the process of posttraumatic contracture formation. In this model, the combination of intra-articular joint injury and eight weeks of immobilization promotes the development of stable flexion contractures of the knee, despite prolonged periods of remobilization. Joint capsule preparations harvested from contracted knees in this animal model are also characterized by myofibroblasts hyperplasia and excessive collagen deposition. These biomechanical and histological changes parallel the human condition and support the use of this model to further study and manipulate the process of posttraumatic contracture formation.

Myofibroblast and collagen hyperplasia are characteristic of connective tissue fibrosis and are observed in many human fibrotic conditions such as adhesive capsulitis of the shoulder, Dupuytren's contracture of the hand, hypertrophic wound healing and scleroderma—. It has been postulated that excessive myofibroblast activity and collagen secretion lead to the development of permanent contracture of the extracellular matrix (ECM) in these fibrotic conditions.,. The mechanisms behind this deleterious myofibroblast hyperplasia are unknown, but it is speculated that excessive signals promoting myofibroblast differentiation and inhibition of apoptosis are at work.

Numerous animal and human models of fibrosis have documented increased mast cell densities within fibrotic tissue, prompting the hypothesis that mast cell activation may be of importance in the pathogenesis of fibrosis—. Mast cells synthesize and secrete proteases, growth factors and cytokines, many of which have mitogenic effects on fibroblasts and

myofibroblasts. Our laboratory has recently documented elevated mast cell numbers in the joint capsules of chronic posttraumatic contractures in humans and an animal model.

Medications known to inhibit mast cell activation are commercially available and have been extensively studied. *Ketotifen fumarate* is an FDA approved medication for the treatment of asthma, physical urticarias and allergic conjunctivitis of the eye and has both anti-allergic and anti-histamine properties,. *Ketotifen* non-competitively blocks the binding of histamine to its H1 receptor and inhibits calcium-dependent vesicle degranulation of activated mast cells. Recently, *Ketotifen* has been effectively used to prevent hypertrophic scar formation in the red Duroc pig and esophageal stenosis and fibrosis in a rabbit model of caustic esophageal injury.

The purpose of the present study was to determine if mast cell activation plays an important role in the genesis of joint capsule fibrosis and subsequent motion loss after joint injury in a rabbit model of posttraumatic joint contracture. The hypothesis is that joint contracture severity and myofibroblast numbers will be significantly decreased in rabbits receiving *Ketotifen*.

Methods

Group allocation

Animals were randomly assigned to one of four groups: the first (control arm) consisted of rabbits untouched by any surgical or pharmacological interventions (non-operative control group, "CON"). In the second arm (operative contracture group) surgical intra-articular joint injury and 8 weeks of immobilization was used to create stable posttraumatic joint contractures ("ORC"). No pharmacologic interventions were given to this study arm. The third and fourth study arms (experimental #1 and experimental #2) were treated with identical surgical interventions as the ORC group, but received a subcutaneous twice-daily dose of a mast cell stabilizer, *Ketotifen fumarate* (KF; Sigma, Chicago, IL), of either 0.5mg/kg or 1.0mg/kg ("KF0.5" and "KF1.0", respectively). All rabbits were sacrificed exactly 8 weeks from the initial day of surgery and all surgeries took place over the duration of four weeks.

Joint interventions

Animal ethics approval from our local university animal care committee was obtained prior to study initiation. Surgical joint interventions were performed under general anesthesia on the right or left knee, which was randomly selected in advance, as described previously. A lateral skin incision was made over the thigh and the interval between the lateral quadriceps and the hamstrings musculature was developed. The lateral femur was exposed and the quadriceps were bluntly elevated off the anterior aspect of the femur. The mobile skin of the rabbit permitted the use of the same lateral skin incision to expose the medial and lateral aspect of the distal femur, from which medial and lateral arthrotomies were made. The femoral condyles were visualized through the arthrotomies and bilateral, 5mm × 5mm cortical windows were removed from the non-articular cartilage portion of the medial and lateral femoral condyles, taking care not to injure the collateral ligaments. The creation of

intra-capsular yet extra-articular cortical windows created a traumatic hemarthrosis of the joint mimicking a traumatic intra-articular bony injury, while ensuring intra-articular incongruity was not a confounding variable. A second incision was then made over the subcutaneous border of tibia, roughly 3–4cm distal to the tibial tubercle. The tibia was drilled in an anterior to posterior direction, angled 30° cephalad using a 1.6mm drill bit. A 1.6mm diameter Kirschner wire (Zimmer, Mississauga, ON) was passed through the tibia, behind the knee and was then bent around the lateral aspect of the femur. The knee was then flexed to 150° and the tibial portion of the Kirschner wire was bent and cut to solidify the immobilization. After each surgery, the rabbits were allowed free cage activity (0.1 m³). All rabbits were immobilized in this fashion for a total of eight weeks (Figure 2). A broad-spectrum antibiotic, Baytril (Bayer Health Care, Toronto, ON) was given to all surgical animals at a dose of 5mg/kg daily for a total of 3 days. A morphine derivative, *Buprenorphine* (McGill University, Montreal, QC) was also given post-operatively (0.03mg/kg) for a total of 3 doses to provide adequate post-operative analgesia.

Joint range of motion measurements

Eight weeks from the initial surgery date rabbits were sacrificed with an overdose of Euthanyl (MTC, Cambridge, ON) and the joint range of motion measurement was performed the same day of sacrifice. A custom rabbit knee gripping device was attached through a rack and pinion arrangement (Figure 3A) to an MTS TestStar II hydraulic materials testing machine (MTS, Eden Prairie, MN). The lower extremity was removed from the carcass by disarticulating the hip joint; the muscle, tendons and skin were then removed from the femur and tibia. All tissue from the superior border of the patella to 1 cm distal to the patellar tendon insertion on the tibia was left intact to preserve the joint capsule. The tibia and femur was potted in a cylindrical cast of polymethyl-methacrylate (PMMA) to allow consistent mounting within the rabbit knee gripping device. Maximum knee joint extension was measured as the posterior soft-tissue knee joint structures are the source of contracture in this animal model. To ensure consistent mounting of the knees, the femur clamp on the pinion was placed at 90° to the tower housing the 2nd clamp (tibia). Consistent positioning of the knee joint relative to the pinion axis of rotation was ensured by placing the femoral epicondyles, located just superior to the medial collateral ligament (MCL) insertion, over the pinion axis. Quasi-static testing (0.4 mm/s) was used to minimize inertial effects and performing the flexion-extension measurements in a plane perpendicular to gravity negated any gravitational forces. Testing began with the knee joint at 90° of flexion. An extension torque was applied to a maximum of 0.2 Nm or to a maximum of 55 mm displacement of the MTS actuator. The maximum torque was calculated based on in vivo loading of the intact rabbit hind limbs. The soft-tissues were kept moist with a continuous flow of humidified air warmed to 37°C. Extension is recorded as a number between 0° and 90°, with 0° representing full extension. Negative numbers represent hyperextension. Thus, a larger extension angle actually represents a more severe contracture, or loss of motion (Figure 3B). The lack of full extension is referred to as a flexion contracture. Each limb was cycled 5 times, with the values of each cycle averaged.

Immunohistochemistry

A double-labeling immunohistochemical methodology as previously described was used to identify and quantify myofibroblasts and mast cells in joint capsule preparations. Alpha-smooth muscle actin (α -SMA) is used as a marker for myofibroblasts as it is not present in fibroblasts or primitive myofibroblasts. Tryptase is mast cell specific, is the most abundant protein synthesized by mast cells and virtually all mast cell phenotypes express tryptase based on data from humans and rodents. Sections (8 μ m thickness) of the frozen capsule samples were cut using a cryostat, mounted on precoated glass slides and pretreated with hyaluronidase (Seikogaku Corporation, Code no. 100740, Tokyo, Japan) for 20 min at 37°C. Endogenous peroxidase activity was quenched using 0.3% hydrogen peroxide in methanol for 20 min at room temperature. Ten percent normal bovine serum in phosphate buffered saline (PBS) was applied for 20 min at 37°C as a blocking agent. The mouse monoclonal α -SMA antibody (clone asm-1, Roche Molecular Biochemicals, Laval, QC) was applied and incubated for 60 min at 37°C and subsequently washed with PBS. The secondary antibody, a sheep anti-mouse immunoglobulin (IgG) horseradish peroxidase (HRPO) conjugate (Röche), was applied to the sections for 60 min at room temperature. This was followed by application of the diaminobenzidine (DAB)/peroxide substrate (Röche) for 13 min. The reaction was stopped by washing with PBS, followed by the application of the polyclonal goat anti-tryptase antibody (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. A donkey anti-goat antibody conjugated with Alexa Fluor 488 (Molecular Probes, Eugene, OR) was applied the following day for 60 min at room temperature. Finally, 4'-6-diamidine-2-phenyl indole, or DAPI, (Vector Laboratories, Burlington, ON) was applied to label all cell nuclei and cover slips were placed on the sections. The sections were stored at 4°C in darkness. Appropriate controls were assessed in all experimental series. Positive controls for α -SMA were blood vessels in capsular tissue and skin sections for tryptase. Negative controls included substituting primary antibodies with 3% bovine serum albumin. The sections were viewed under a light microscope (Zeiss, Axioskop 2 plus, Toronto, ON). Images were captured (200 $\times$) from five randomly selected areas from each of four sections for each specimen with a digital camera (Zeiss, AxioCam). Image-Pro Plus (Media Cybernetics, Silver Spring, MD) was used to analyze cell numbers. Immunostaining and cell counting was performed by a laboratory technician blinded to the animal allocation and treatments. Cell nuclei associated with α -SMA or tryptase were counted as myofibroblasts or mast cells, respectively. Total cell counts also were obtained. The data was collected as an average of myofibroblast and mast cell numbers per high power field and as a percentage of myofibroblasts and mast cells to total cell numbers.

Statistics

Data is presented as mean $\pm$ standard deviation. For the biomechanical experiments, data was analyzed using a random effects regression model to account for the repeated contracture measures sampled from each limb. Comparisons of contracture severity between individual groups were analyzed by testing linear combinations (of model estimates) of the coefficients. Immunohistochemistry data was interpreted using a two-way ANOVA analysis of variance with a post-hoc Bonferroni to analyze differences between individual groups. Significance was defined as $p < 0.05$.

Source of funding

An external award (Robert Salter Award) was received from the Canadian Orthopaedic Foundation (Innisfil, Ontario) for this project. Funds were used for animal related costs and the acquisition of primary and secondary antibodies used for immunostaining.

Results

A total of 44 rabbits were included in the study. Five animals were excluded for the following reasons: one rabbit experienced an intra-operative tibia fracture, two rabbits were euthanized for post-operative failure to thrive, another rabbit was euthanized because of early hardware failure noted 5 days post-operatively and a fifth rabbit was excluded secondary to a tibia fracture encountered during biomechanical testing. Table 1 categorizes demographics and average flexion contractures according to group allocation. Randomization was successful as there was no statistical difference between groups regarding allocation of right and left legs and pre-operative weights. All rabbits treated with *Ketotifen* tolerated the medication and there were no observed cases of wound dehiscence or deep infection.

Flexion contractures of the four animal groupings are illustrated in Figure 4. In the operative contracture group (ORC, n=13), the average flexion contracture measured $58^{\circ} \pm 14$, while average flexion contractures in the KF0.5 mg/kg (n=9) and KF1.0 mg/kg (n=9) group measured $42^{\circ} \pm 17$ and $45^{\circ} \pm 10$, respectively ($p < 0.02$). A baseline flexion contracture was also present in the non-operative control group (CON, n=8), which averaged $27^{\circ} \pm 12$.

Flexion contractures measured in the operative contracture group (ORC) and *Ketotifen* groups relative to the non-operative control (control flexion contracture subtracted) were calculated to give a representation of the true lack of extension secondary to the surgical interventions in isolation. Relative to the non-operative control (CON), the operative contracture group (ORC) had an average flexion contracture of $31^{\circ} \pm 14$, while contracture severity were reduced to $15^{\circ} \pm 17$ and $18^{\circ} \pm 10$ in the *Ketotifen* 0.5mg/kg and 1.0mg/kg groups, respectively. This represents 52% and 42% reduction in contracture, respectively ($p < 0.03$). Factors such as animal weight and right or left leg allocation did not significantly influence the biomechanical contracture values.

Table 2 summarizes the immunohistochemistry data for all treatment groups. The averaged total cell count within the joint capsule was significantly increased in the operative contracture group (ORC) compared to the non-operative control (CON), 543 ± 124 and 244 ± 35 cells per field respectively ($p < 0.001$). Total cell counts in the *Ketotifen* treated groups were significantly decreased compared to the ORC group (KF0.5: 278 ± 47 and KF1.0: 297 ± 53 cells per field, $p < 0.001$). Total cell counts were increased in the *Ketotifen* treated groups relative to the non-operative control (CON), but this increase was not statistically significant.

All immobilized groups had increased myofibroblast counts in the posterior capsule compared to the non-operative control (CON), but the *Ketotifen* groups (KF0.5: 62 ± 15 cells per field and KF1.0: 51 ± 15 cells per field) had a significant reduction in

myofibroblasts compared to the operative contracture (ORC) group (267 ± 61 cells per field), $p < 0.001$. Myofibroblasts represented $12 \pm 1\%$ of the total cell population in the non-operative control (CON) group and increased to $49 \pm 2\%$ in the operative control (ORC) group ($p < 0.001$). In the *Ketotifen* 0.5mg/kg and 1.0mg/kg treated groups, myofibroblasts represented $21 \pm 3\%$ and $17 \pm 2\%$ of the total cell population, respectively ($p < 0.001$), indicating a 57% and 65% reduction in myofibroblast numbers within the joint capsule, respectively (Table 2).

A very similar trend was noted for mast cell counts within the joint capsule (Table 2). Compared to the non-operative control group (CON), which averaged 29 ± 7 mast cells per field, mast cell numbers were significantly increased ($p < 0.001$) to 259 ± 56 cells per field in the operative contracture group (ORC). Relative to the operative contracture group, mast cell numbers were significantly reduced in the *Ketotifen* 0.5mg/kg and 1.0mg/kg groups to 60 ± 13 and 50 ± 14 cells per field ($p < 0.001$). Mast cell percentage of total cells within the capsule sections was increased to 47% in the operative contracture group (ORC) compared to 12% in the non-operative control group ($p < 0.001$). In the *Ketotifen* 0.5mg/kg and 1.0mg/kg treated groups, mast cell percentage of total cells was reduced to 21% and 17%, representing a 55 and 64% reduction, respectively ($p < 0.001$). Figures 5 show examples of the immunostaining of myofibroblasts and mast cells and sections of negative controls.

Discussion

From these experiments, we have demonstrated that animals treated with the mast cell stabilizer, *Ketotifen* had significant reductions in both the severity of joint contractures and the degree of joint capsule myofibroblast and mast cell hyperplasia. It should be noted that contractures in the *Ketotifen* treated groups were not completely reversed values of the non-operative control group. Numerous mast cell mediators have been implicated in the development of myofibroblast differentiation and fibrosis and include histamine, tryptase, chymase, basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and transforming growth factor-beta1 (TGF- β 1),-. The synthesis and storage of these mediators is not homogeneous. Some mediators are stored in preformed granules (histamine, tryptase and chymase) while others are newly synthesized upon mast cell activation (bFGF, PDGF, TGF- β 1),-. *Ketotifen* inhibits mast cell mediator release by a combination of blocking the rapid intra-cellular calcium influx from the extra-cellular environment and by blocking the transient drop in cyclic-AMP (cAMP) concentrations needed for the terminal events of vesicle degranulation,,-. Calcium dependent granule release is the primary method by which mast cell mediators are released, but it is also quite likely that some of the profibrotic mast cell mediators may also be secreted by calcium-independent mechanisms. The complexity of mast cell signaling is only partially understood and is an area of ongoing research.

Another point to consider is that the *Ketotifen* doses used in this study likely did not completely inhibit mast cell mediator release. The *Ketotifen* doses used were calculated based on previous *in vivo* animal studies testing the anti-anaphylactic properties of *Ketotifen* over a wide range of doses,,-. Even at the highest *Ketotifen* doses (10mg/kg) used in these aforementioned *in vivo* studies, anaphylaxis-induced mortality rates were only reduced by a maximal 70%, implying that even with large doses of *Ketotifen*, the inhibition of activated

mast cell degranulation is incomplete. Based on the information from these studies, the *Ketotifen* doses chosen for this present study were designed to be well tolerated by the animals while maximally impeding mast cell mediator release without causing a paradoxical destabilization of the mast cell like more traditional anti-histamines when administered at high concentrations,. We initially postulated that by using two different doses of *Ketotifen* a dose response might have been observed, although this was not the case as the biomechanical and histological results were similar in both *Ketotifen* groups. In retrospect, both 0.5mg/kg and 1.0mg/kg are similarly large doses of *Ketotifen* and ideally, doses should have differed by an order of 10 or 100. Despite this, it was encouraging to note that both *Ketotifen* 0.5mg/kg and 1.0mg/kg groups responded in an identical fashion to the drug treatment.

A distinctive feature of this rabbit model of posttraumatic joint contractures is that motion loss is irreversible, despite prolonged periods of remobilization (up to 4× the length of immobilization). In contrast, other animal models of atraumatic immobilization have shown that contractures are reversible after periods of remobilization equal to the duration of immobilization—. This brings to question, what pathobiology unique to traumatic injury renders the joint susceptible to irreversible motion loss. Based on findings in humans and animals, we have hypothesized that the joint capsule fibrosis observed in chronic posttraumatic joint contractures may account for this irreversible motion loss. The results of this study have demonstrated a significant reduction in joint capsule fibrosis in animals treated with a mast cell stabilizer and this information will need to be supplemented by future studies addressing motion loss in *Ketotifen* treated animals which are allowed to remobilize.

The myofibroblast is often viewed as an “effector cell” in the development of connective tissue fibrosis and our results would support this claim as the joint capsules harvested from the stiff operative contracture (ORC) group were densely populated with myofibroblasts. Treatment with *Ketotifen* significantly reduced both joint capsule myofibroblast and mast cell numbers. It is possible that the products of mast cell degranulation are responsible not only for the differentiation and recruitment of myofibroblasts, but may also function in the recruitment and/or proliferation of additional mast cells. Traditionally, mast cell recruitment and differentiation has been attributed to the interaction between the mast cell membrane-bound receptor, c-kit and its soluble ligand stem cell factor (SCF), although recently growth factors synthesized by the mast cell, such as bFGF, PDGF, nerve growth factor (NGF) and TFG-β1 have been documented to promote mast cell chemotaxis and proliferation,. Interestingly, these are the same growth factors proven to induce myofibroblast differentiation and proliferation,,,.

The results from this study suggest that joint capsule fibrosis and subsequent motion loss is mast cell mediated, but many questions pertaining to the pathobiology of this process remain unclear. In particular, we are uncertain of what cellular or molecular events are driving mast cell activation after trauma. One hypothesis is that neurogenic factors may be involved. Anecdotally, we have observed that patients who present with joint contractures after injury commonly have a history of increased or prolonged experiences of pain prior to the formation of joint stiffness. Neuropeptides such as substance P are important mediators in

the transmission of pain within the central nervous system. These peptides are also released peripherally into joints and connective tissue such as skin, muscles and tendon and are recognized as important pro-inflammatory agents and modulators of the immune system. Interestingly, substance P is also a potent mast cell activator and is secreted both peripherally and centrally in response to pain and injury,. Our laboratory has documented elevated counts of substance P containing nerve fibers within contracted joint capsules of humans and rabbits, which would support this hypothesis. Increased expression of substance P has also been documented in other models of fibrosis, such as Dupuytren's contracture of the hand, scleroderma and pulmonary fibrosis,.,

The results of this study are quite encouraging, as we have used a medication known to inhibit mediator release from activated mast cells to successfully reduce joint contracture severity and myofibroblast hyperplasia in an animal model that closely parallels the human condition of posttraumatic elbow contractures. This information provides further insight into the pathobiology driving the genesis of posttraumatic joint contractures and may also aid in the development of prevention strategies targeted towards at risk patient populations. The determination of "at risk" patients will be of great importance as certainly not all patients who suffer an elbow injury will develop a contracture. We have previously documented in this animal model that the expression of various markers of fibrosis, such as myofibroblast hyperplasia, increased TGF- β 1 expression and upregulated collagen synthesis occur as early as 2 weeks after injury, highlighting the importance of identifying at risk populations and implementing prevention strategies soon after injury.

Despite the documented safety of *Ketotifen* administration to human subjects, theoretical side effects of *Ketotifen* administration to acute fracture patients could include delayed wound healing and blunting of the inflammatory and proliferative phases of fracture healing. Mast cells appear to have a role in normal skin healing and there is also histological evidence that mast cell numbers are increased within the organized fracture hematoma and subsequent fracture callus, often in close association with osteoblasts,. Should mast cell inhibition eventually be considered as a preventative modality for contracture development, especially in the acute phases of injury, these concerns will need to be further evaluated.

This study does have numerous limitations. Firstly, most of our knowledge of mast cells comes from studies in humans and rodents, with very little from studies examining rabbit mast cells. The distribution of rabbit mast cells does not seem to completely parallel rodents and man, especially within the gastrointestinal tract and circulatory system. Although, like humans and rodents, rabbit mast cells are abundant within connective tissues such as the skin, ears, nose and paws.

The specificity of *Ketotifen* for mast cells is another potential uncertainty. Although *Ketotifen* is described as a mast cell stabilizer, it is known to affect other cells of the immune system such as the inhibition of basophil degranulation and decreased neutrophil migration. Because of this, we cannot be certain that the results of our study are exclusively the product of inhibited mast cell degranulation.

The results of this study suggest that mast cell activation is an important event in pathogenesis of joint capsule fibrosis and subsequent motion loss after intra-articular injury. The specific mast cell mediators involved in the induction of joint capsule fibrosis and the mechanisms promoting enhanced mast cell activation after joint injury will need to be determined as we search for novel prevention and treatment strategies targeted towards posttraumatic joint contractures.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

Table 1

Animal demographics and flexion contractures

	CON	ORC	KF0.5	KF1.0
n	8	13	9	9
Pre-op animal weight (kg)	5.2 ± 0.4	5.1 ± 0.4	5.1 ± 0.3	5.2 ± 0.3
Post-op animal weight (kg)	5.3 ± 0.6	4.6 ± 0.3	4.6 ± 0.2	5.1 ± 0.3
Weight change (kg)	+0.1 ± 0.03	−0.6 ± 0.03	−0.5 ± 0.03	−0.08 ± 0.01
Right/Left	3/5	6/7	4/5	5/4
Flexion contracture (average)	27 ± 12°	58 ± 14°	42 ± 17°	45 ± 10°
T1 – T5: (1 st cycle – 5 th cycle)	2.2 ± 0.69 (p>0.05 b/w groups)	3.3 ± 2.2 (p>0.05 b/w groups)	3.4 ± 2.4 (p>0.05 b/w groups)	4.8 ± 2.7 (p>0.05 b/w groups)

Table 2

Immunohistochemistry Data Summary

Animal Group	Total Cells (per hpf)	Myofibroblasts (per hpf)	Myofibroblasts (%)	Mast Cells (per hpf)	Mast Cells (%)
CON	244 ± 35	30 ± 7	12 ± 1%	29 ± 7	12 ± 1%
ORC	543 ± 124 [#]	267 ± 61 [#]	49 ± 2% [#]	259 ± 56 [#]	47 ± 3% [#]
KF0.5mg/kg	278 ± 47 [*]	62 ± 15 [*]	22 ± 3% [*]	60 ± 13 [*]	21 ± 2% [*]
KF1.0mg/kg	297 ± 53 [*]	51 ± 15 [*]	17 ± 2% [*]	50 ± 14 [*]	17 ± 2% [*]

hpf: high powered field

%: Percent of total cell numbers

^{*} Significantly different compared to ORC (p<0.001)[#] Significantly different compared to CON (p<0.001)