Intra-Articular Tibiofemoral Injection of a Nonsteroidal Anti-Inflammatory Drug has no Detrimental Effects on Joint Mechanics in a Rat Model

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for musculoskeletal injuries due to their analgesic and anti-inflammatory properties. Recent studies have demonstrated the efficacy of injectable NSAIDs in the treatment of intra-articular pathology and postoperative analgesia.[1,2,3] However, little data exist regarding the safety of intra-articular injection on the joint, despite the recent increase in its application.[4] Therefore, the objective of this study is to investigate the effects of intra-articular NSAID injection on articular cartilage, the anterior cruciate ligament, and joint function in the rat knee. We hypothesize that intra-articular ketorolac injection will result in no damage to the articular cartilage and anterior cruciate ligament (ACL), and will not permanently alter joint mechanics.

Methods

Study Design and Animal Use

A total of 64 Sprague-Dawley rats were used to investigate the effects of an intra-articular injection of NSAID. Following anesthetization, injections of saline (0.1 mL) or ketorolac tromethamine (Toradol, Bedford Laboratories, 3 mg/0.1 mL), a commonly used NSAID, were performed bilaterally in the knee (tibiofemoral) joint with both knees receiving the same injection. All rats were returned to cage activity for the remainder of the study. Sixteen rats (8 ketorolac, 8 saline) were sacrificed at each of four time points (2, 7, 28, and 84 days). Following sacrifice, the left tibia was dissected and frozen (−20°C) for cartilage indentation testing. The right hindlimb was frozen (−20°C) for ACL mechanical testing. The 84 day group also underwent knee kinematic evaluation 1 day prior to injection and at 2, 7, 28, and 84 days post-injection prior to sacrifice.

Knee Kinematics

Knee kinematics were quantified by measuring ground reaction forces and paw positioning using a novel ambulation method.[5] All data were collected using LABVIEW and parameters of knee function were determined using a custom MATLAB program.

ACL Mechanical Testing

To assess the mechanical properties of the ACL, the right hindlimb was dissected by removing all surrounding tissue from the tibia and femur except the ACL. Three Verhoeff stain lines were placed on the ACL (Figure 1A) for optical strain tracking. Cross sectional area was determined by taking coronal and sagittal images of the ACL, defining the thickness using Mipav software, and calculating assuming an ellipse. Both the tibia and femur were embedded

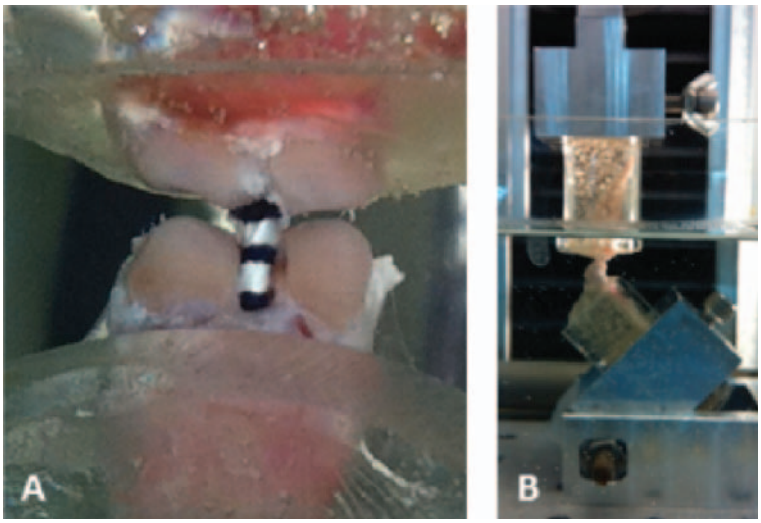

Figure 1. ACL mechanical testing setup. (A) ACL isolated between the tibia (top) and femure (bottom) embedded in PMMA in the holding fixture. Three stain lines mark the two insertions and the center of the ligament for optical tracking. (B) Sagittal view of ACL mechanical testing set-up with custom testing fixtures to hold at 45° knee flexion.

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in holding fixtures using polymethylmethacrylate (PMMA) and inserted into a custom fixture with the joint at 45° flexion (Figure 1B). The specimen was immersed in 37°C PBS bath, preloaded to 0.1N, preconditioned for 10 cycles from 0.1N to 0.5N at 1% /sec and held for 300s. Stress relaxation was performed (ramp to 5% strain at 5%/sec, then held for 600s) followed by a return to initial displacement for 60s, and a ramp to failure at 0.3%/sec. Images were taken during the ramp to failure and 2D Lagrangian strain was calculated by mapping the stain line displacements in MATLAB.

Cartilage Thickness Measurement and Mechanical Testing

Indentation testing of the center region of the medial tibial plateau articular cartilage was performed. The tibia was dissected to remove surrounding tissue and embedded in PMMA. The cartilage surface was scanned in 0.25 mm increments using a 55 MHz ultrasound probe (VisualSonics, Inc) in coronal and sagittal planes. B-Mode images of each scan were segmented and the 3D positions of the cartilage and bony surfaces were reconstructed (Figure 2A).[6] Average thickness was computed in a 0.5 mm diameter region at the center of the thickness map (Figure 2B) and cartilage indentation was performed using a 0.5 mm diameter, non-porous spherical indenter tip in the same region. A stress-relaxation test was performed, with a preload of 0.005N followed by a ramp to 20% strain at −0.05 mm/sec and a 300 second hold. Equilibrium elastic modulus was calculated[7] assuming Poisson's ratio (v=0.3).

Statistics

Significance (set at $p<0.05$) was assessed using 2-way ANOVAs to evaluate the effect of NSAID injection and time post injection.

Results

There were no differences between the ketorolac (NSAID) and saline (SAL) injection groups in any measured parameter at any time point. Specifically, for knee kinematics evaluation, we measured forces (propulsion and vertical ground reaction (Figure 3A-B), medial and lateral, braking, and moment), paw placement (stride width (Figure 3C) and length), and timing (speed (Figure 3D), rate of loading, and stance time) of ambulation over the study time course for both NSAID and SAL groups. There were no differences due to the NSAID compared to the SAL group in any of these parameters. Although not different between treatment groups, walking speed did change over time.

For ACL mechanical evaluation, we measured maximum load, stiffness, percent relaxation, modulus (Figure 4A-D), maximum stress, and cross-sectional area. There were no changes between treatment groups, but changes were observed over time in maximum load, percent relaxation, maximum stress, and cross-sectional area.

Our measurements for cartilage thickness and equilibrium elastic modulus showed no changes between treatment groups with respect to either parameter (Figure 5A, B), but both changed over time.

Discussion

Results indicate that the intra-articular administration of ketorolac in the tibiofemoral joint does not cause detrimental effects to the articular cartilage and the ACL, or cause any detrimental ambulatory changes compared to saline injection. These results support previous findings evaluating the safety of intra-articular injection of NSAIDs.[4] A pre-study power analysis determined that eight animals in each group were sufficient to achieve a power of 80% with p set at 0.05, so we are confident in our findings of "no difference" between treatment groups. Additionally, our delivered dosage of ketorolac was ~30x a normal therapeutic dose in humans. This dose was selected based on the maximum volume allowable in the rat knee without capsule damage using a standard ketorolac concentration. Given the consistent results, it is unlikely that a lower ketorolac dose would cause

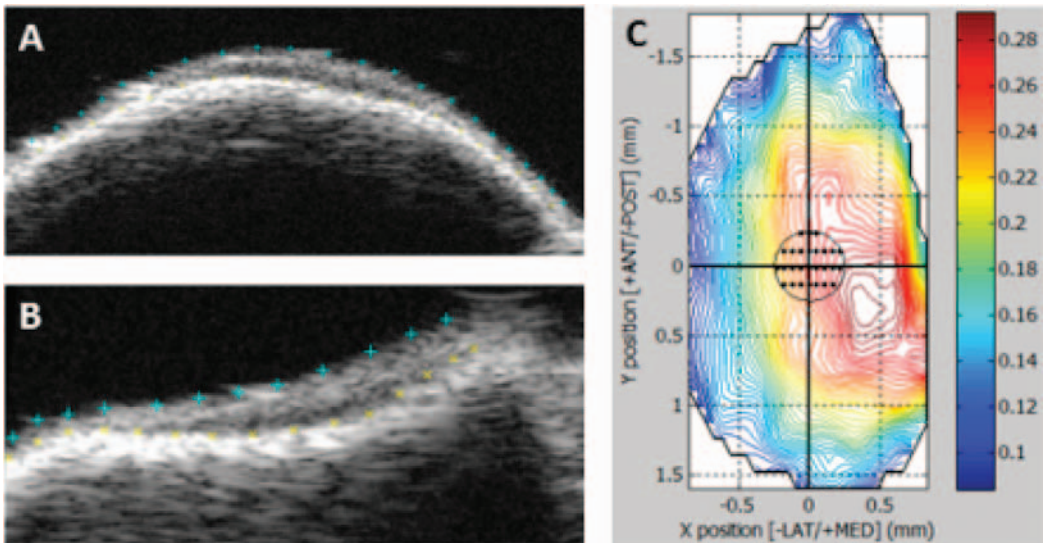

Figure 2. Medial tibial plateau articular cartilage segmentation to determine cartilage thickness. (A) Sagittal and (B) Coronal ultrasound images with markers defining the cartilage with center region of interest defined to average the segmentation points.

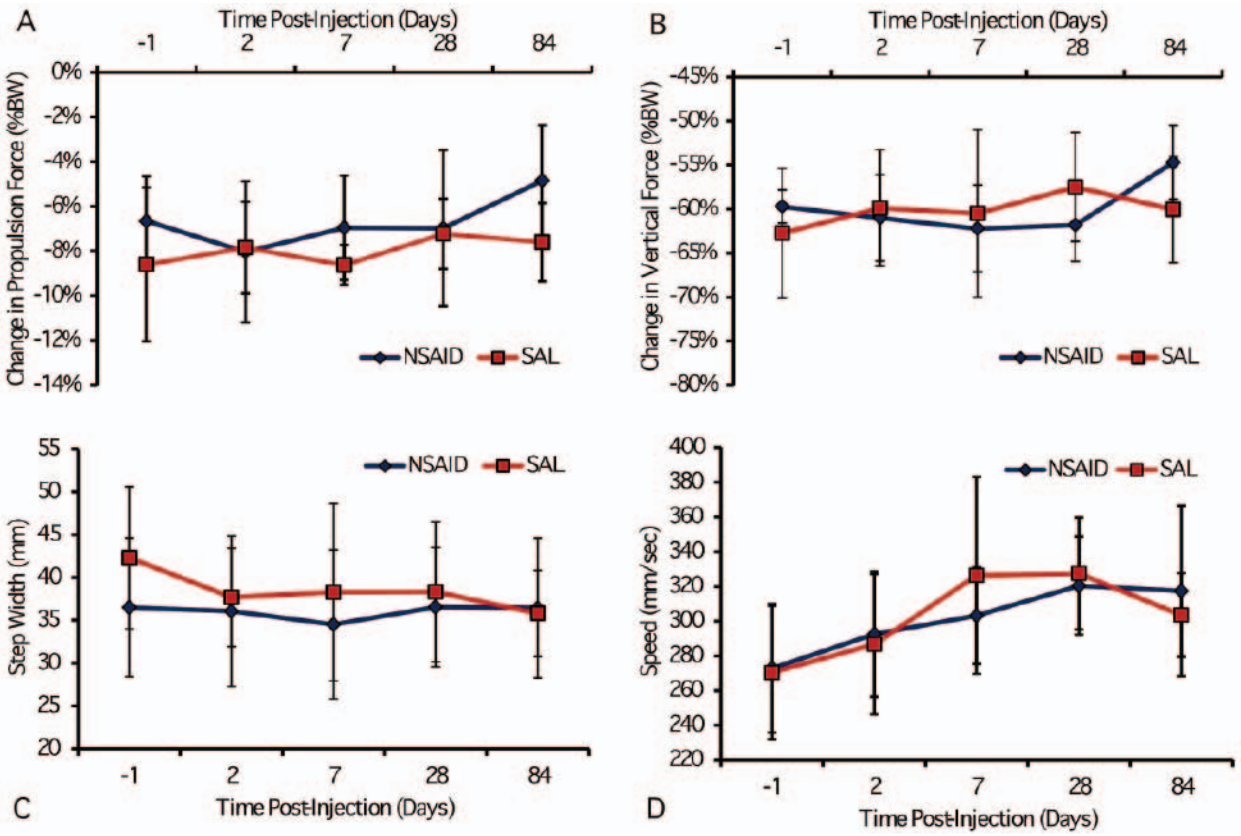

Figure 3. Rat ambulation measures, where "NSAID" is the ketorolac injection group and "SAL" is the saline injection control. (A) Propulsion force and (B) vertical force, normalized by weight and obtained from six-degree of freedom force plates. No differences were found between NSAID and SAL groups in any of these measures at any time point. Note: breaking force, medial/lateral force, momentum, stride length, rate of loading and stance time also showed no differences (data not shown).

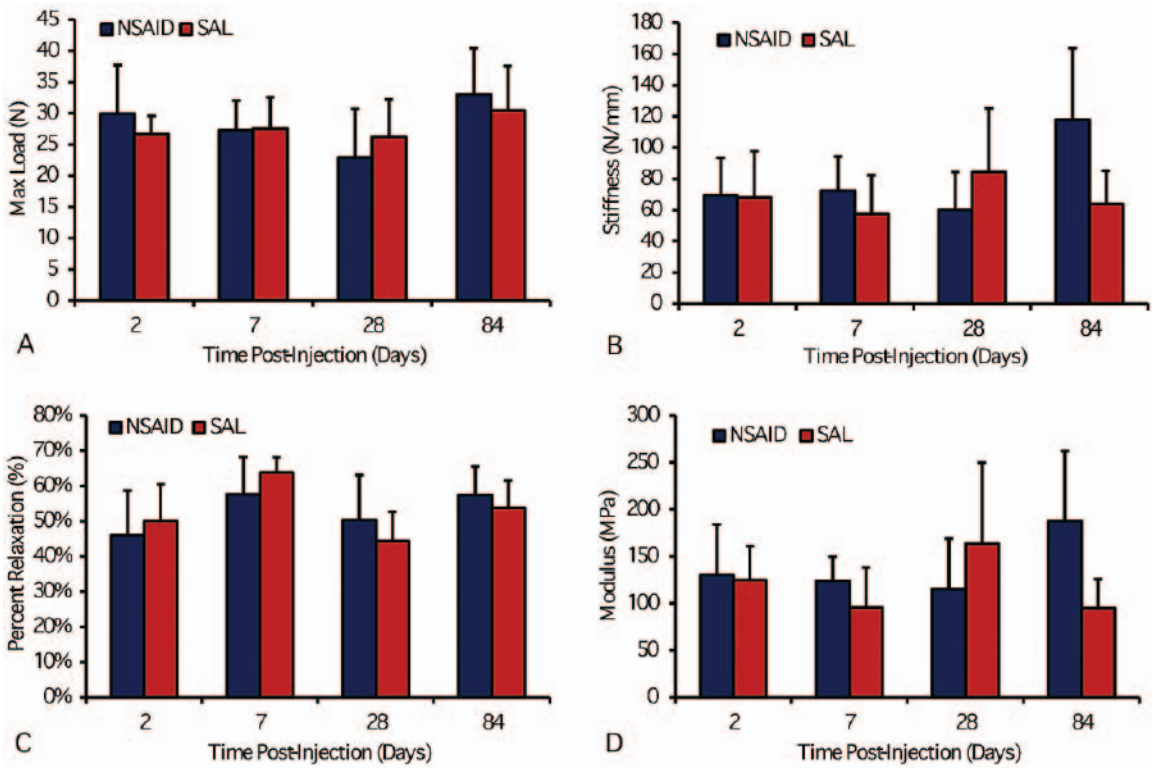

Figure 4. Representative plot of ACL mechanical properties: (A) max load and (B) stiffness determined from the load-displacement curve. (C) Percent relaxation determined from a stress relaxation test, and (D) modulus determined from the stress-strain curve. No differences were found between the NSAID and SAL group in any of these measures at any time point. Note: ACL cross sectional area and max stress also showed no difference (Data not shown).

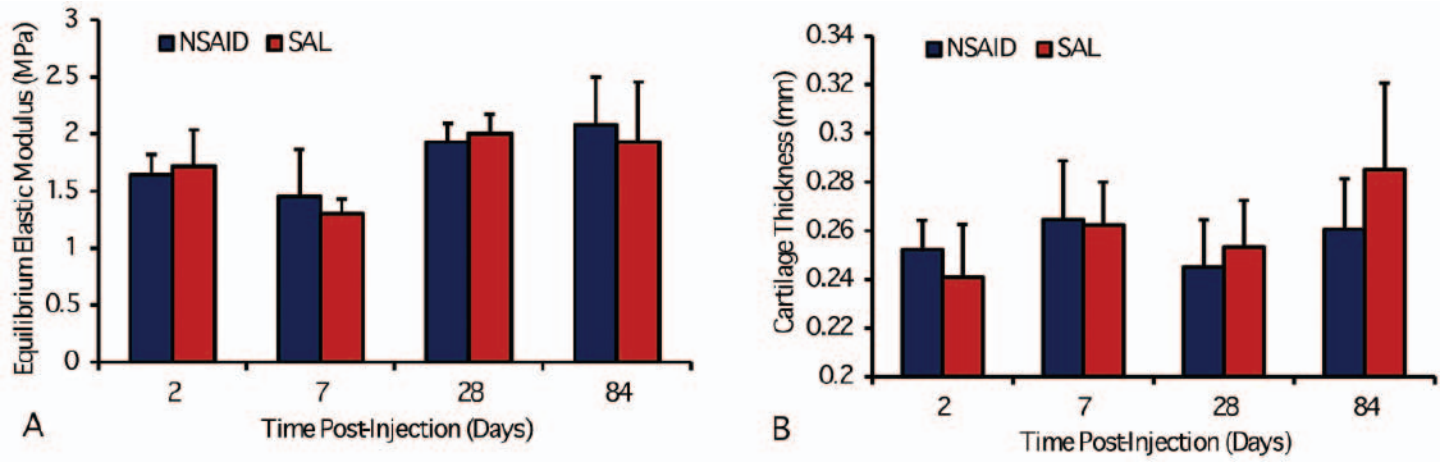

Figure 5. Medial tibial plateau articular cartilage properties: (A) Cartilage equilibrium elastic modulus from a stress-relaxation indentation test, and (B) cartilage thickness of the center region in the medial tibial plateau determined by ultrasound imaging.

tissue damage since this high dose did not. While changes were observed over time in some parameters, these changes were the same in both treatment groups, and therefore most likely due to changes in animal age and/or weight over time as is expected in this type of longitudinal study. In conclusion, since we consistently found no changes in a comprehensive set of structural, mechanical, and ambulatory parameters, we are confident that there are no effects of intra-articular injection of ketorolac. Therefore, it may be safe to use intra-articular ketorolac injection in clinical practice.

Significance

This study supports that no detrimental effects are observed in the articular cartilage, ligaments, and kinematic function of the native knee following intra-articular ketorolac injection in a rat model, demonstrating the safety of this pain management strategy. These findings serve as preliminary data to support future studies examining the therapeutic effects of injectable NSAIDs on intra-articular pathologies.

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