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Methods: Biocompatibility testing of the unlabeled gel was conducted according to standard ISO 10993 methods to determine safety of the medical device.

Methods: Hylastan gels were prepared by crosslinking high molecular weight HA at an initial polymer concentration of 5.25% using 0.035 Meq of unlabeled or 14C-labeled divinyl sulfone. The gels were washed with acid and saline then swollen in phosphate buffered saline (pH 7.5) to a concentration of 9.1 mg/mL. In the clearance study, a total of 12 male New Zealand White rabbits, approximately three months of age with a body weight of 1.8 to 2.3 kg, were given a single intra-articular injection of 14C-labeled gel (0.1 μg/animal) into both hindlimbs. Two animals were euthanized at 1 hour and 7, 28, 56, 84 and 85 days post-dose. Following sacrifice, each carcass was deep frozen. The left hindlimbs were removed and solubilized in toto and radioactivity determined by liquid scintillation spectrometry. The right hindlimbs were removed and embedded in carboxymethylcellulose and frozen. Each block was sectioned serially at a 30 μm thickness and freeze-dried for quantification. Radioactivity distribution in tissues was determined by quantitative whole body autoradioluminography (QWBA). Biocompatibility testing with non-radiolabeled gel was performed in accordance with ISO 10993 guidelines for bone and tissue contacting medical devices. All non-clinical animal studies were reviewed and approved by IACUC.

Results: Following intra-articular administration of 14C-labeled gel, any clinical signs observed throughout the study were considered to be incidental. The highest mean radioactivity concentration values were observed in the saccus (5218 μg/g eq/g) and the fat pad (798 μg/g eq/g) at 1 hour post-dose. Moderate levels of radioactivity were also seen in the lymph node (popliteal) at 7 days post-dose and meniscus (medial) at 1 hour post-dose. The mean limit of quantification for tissues analyzed by QWBA generally ranged from 21.6 to 27.3 μg/g eq/g with the exception of the 56 day timepoint where the mean limit of quantification was 1213 μg/g eq/g. The left hindlimb which included the bone marrow (femur and tibia), condyle of the femur (distal) and condyle of the tibia (proximal) and patella had concentration values that were below the limit of quantification at all timepoints. Although radioactivity was still be detected up to the 56 day timepoint, radioactivity concentrations were below the limits of quantification for all tissues of interest by 28 days post-injection. The calculated half-life of the gel was 6.23 days. Overall, the mean content of radioactivity in the left hindlimbs was 99.6% at 1 hour post-injection, 45.9% at 7 days post-injection and below the limit of quantification for subsequent timepoints. Therefore, most of the radioactivity administered was eliminated by 28 days post-injection. The limit of quantification for the hindlimbs was approximately 8% of the administered dose. Biocompatibility testing showed the unlabeled hylastan gel to be non-cytotoxic, non-sensitizing, non-mutagenic, slightly reactive (short and long term implantation), a negligible irritant (intradermal), non-toxic (acute systemic) and non-pyrogenic.

Conclusions: Following a single intra-articular injection of 14C-hylastan gel, the test material cleared from the joint space with a half-life of 6.23 days. There were no adverse events related to the administration of the gel. The battery of biocompatibility testing on the crosslinked hylastan gel showed that the material was safe.

Biomarkers

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INFLAMMASOME ACTIVATION IN OSTEOARTHRITIS BY URIC ACID

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Purpose: The production of mature active IL-1β and IL-18 requires inflammasome activation. One mechanism for the production of inflammasomes is the activation of the NOD-like receptor protein NALP3 by uric acid (UA). We sought to determine whether in persons with osteoarthritis (OA) elevated synovial fluid (SF) UA might be associated with elevated levels of IL-18 and IL-1β, indicative of inflammasome activation and OA severity.

Methods: Patients: A total of 159 participants (118 female, 41 male) were enrolled in the NIH sponsored Strategies to Predict Osteoarthritis Progression (POP) study with 138 returning for 3 year follow-up. The study was approved by the institutional review board and informed consent was obtained from all subjects. Participants met radiographic criteria and ACR criteria for symptomatic OA of at least one knee with a Kellgren-Lawrence (KL) grade of 1-4.

Radioigraphic Imaging: Posteroanterior fixed-flexion knee radiographs were obtained and read for KL grade and individual radiographic features of OA, including joint space narrowing (JSN) and osteophyte (OST).

Scintigraphic Imaging: Scintigraphic images of the knee were obtained at 2.5 hours (late phase) after injection of technetium-99m methylene diphosphonate. The intensity of bone uptake was scored for each compartment (medial and lateral) of the tibiofemoral knee joint.

SF analysis: UA was analyzed using HPLC. Cytokines were quantified using the MBL Human IL-18 ELISA and the Quantikine Human IL-1β High Sensitivity ELISA kits, both from R&D Systems (Minneapolis, MN).

Pain Scores: Knee symptoms were ascertained by the NHANES I criterion of pain, aching or stiffness on most days of any one month in the last year.

Statistical Analysis: Descriptive statistics and univariate analyses were performed using Graphpad Prism software (San Diego, CA). Relationships between SF analytes and between SF analytes and OA were analyzed using the GenMOD procedure, to control for within subject correlation of knee data, with addition of a repeated statement (GLM, SAS Enterprise Guide, Cary, NC).

Results: The synovial fluid analyses were limited to 69 study participants (49 women and 20 men) with sufficient synovial fluid volumes for uric acid analyses. The mean (±SD) age of this subset was 64.5 ± 10.1 years (68.9 ± 7.17 years for men, and 62.7 ± 10.6 years for women). The mean (±SD) body mass index was 32.4 ± 7.1 kg/m2 and similar between the sexes.

Knee OA ranged from 1-4 in severity (23.1%, 14.6%, 49.2%, 13.1% for each disease state).
Conclusions: and progression. The association of synovial fluid IL-18 with OA progression lends strong support to the potential involvement of the innate immune system in OA pathology and IL-1β known to be produced by UA activated inflammasomes and the line synovial fluid uric acid, IL-18 and IL-1β all correlated independently with x-ray features of OA represented by OST scores and IL-1β also correlated with JSN (Table 1). Synovial fluid UA and IL-18 also correlated strongly with x-ray features of OA represented by OST scores and IL-1β additionally correlated with JSN (Table 1). Baseline synovial fluid UA and IL-18 also correlated strongly with late phase bone scan scores (Table 1). IL-1β additionally correlated with knee pain (Table 1). Three-year follow-up bone scans, radiographs and pain scores were available for 37, 31, and 38 knees respectively. Models were run using this 3 year follow-up subset to determine if the baseline concentrations of synovial fluid measures were associated with changes in bone scans, radiographs, and pain over time (Table 1). Baseline IL-1β was associated with 3-year change in OST scores. Baseline synovial fluid uric acid showed a borderline association with 3-year change in pain (p=0.05) while IL-1β showed no associations.

Conclusions: This study shows that SF UA is a marker of joint inflammation and OA severity. The correlation of SF UA with the two cytokines (IL-18 and IL-1β) known to be produced by UA activated inflammasomes and the association of synovial fluid IL-18 with OA progression lends strong support to the potential involvement of the innate immune system in OA pathology and progression.

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COMBINATION OF BIOMARKERS AND BONE IMAGING MARKERS FROM LOW-FIELD MRI

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Purpose: The pathogenesis of osteoarthritis (OA) is complex including structural and morphometric changes of several tissues. Cartilage loss and overall bone remodeling are central in the progression of OA, and have been studied in many ways including radiographs, magnetic resonance imaging (MRI), and from systemic fluids. Previous research has demonstrated the superiority of a combination marker compared to single biomarkers in diagnosis of OA. In this study, we investigated a combination of a biochemical marker with cartilage and bone structure markers from MRI for diagnosis of OA.

Methods: The 21-month longitudinal study included 159 subjects prospectively selected to include a large range of ages and degrees of OA. After exclusion of scans due to acquisition errors, 311 knee scans were included. The population characteristics were: age 56±16, BMI 26±4, 47% female, and 19% with radiographic OA (Kellgren and Lawrence, KL ≥ 1). The KL score was determined from load-bearing radiographs in semi-flexed position using the SynaFlex (Synarc). MRI scans with near-isotropic voxels were acquired from a Turbo 3D T1 sequence from a 0.18T Esaote scanner (40° FA, TR 50ms, TE 16 ms, scan time 10 minutes, resolution 0.7mm x 0.7mm x 0.8mm).

Urinary levels of collagen type II C-telopeptide fragments (uCTX-II) were measured by the CartiLaps ELISA assay and corrected for urinary creatinine levels. We used log(uCTX-II) to have approximately normally distributed marker values. The cartilage MRI markers consisted of the cartilage thickness, surface smoothness, volume, homogeneity (the entropy of the cartilage intensity distribution), and surface curvature. They were all quantified by a fully automatic, computer-based framework in the medial tibial compartment. The bone structure MRI marker was quantified by a machine learning system from a selection of 20 features extracted in a region-of-interest in the subchondral medial tibia from 3 to 14 mm below the cartilage covering a load-bearing region of the trabecular bone. An aggregate biomarker was made by combining all biomarkers. They were evaluated with respect to their ability to discriminate healthy and OA knees by the area under the receiver-operator characteristics curve (AUC) individually and in combination. The statistical significances of AUC scores and differences in-between were tested using DeLong’s test.

Results: The highest AUC among the single biomarkers was 0.79 for the cartilage smoothness marker. The AUC for the combination marker was 0.85, p=0.00059 which was significantly better compared to cartilage thickness, cartilage homogeneity, cartilage volume, and uCTX-II.

Table 1. Analyte associations with severity and progression of knee OA (adjusted for age, gender, and BMI)

<table>
<thead>
<tr>
<th>Parameter Estimate</th>
<th>(95% CIs)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF UA (95% CIs)</td>
<td>0.12</td>
<td>(0.05/0.19)</td>
</tr>
<tr>
<td>SF ln IL-18 (95% CIs)</td>
<td>0.57</td>
<td>(0.57/0.82)</td>
</tr>
<tr>
<td>SF ln IL-1β (95% CIs)</td>
<td>0.09</td>
<td>(0.06/0.12)</td>
</tr>
</tbody>
</table>

P values adjusted by GLM (generalized linear modelling to control for within subject correlation of knee data). Additional adjustment for baseline OA status.

Bone Scan, JSN = joint space narrowing; OST = osteophyte; BMI = body mass index; Δ change over 3 years in knee OA status.