CROSSLINKED HYALURONAN GEL (HYLASTAN): CLEARANCE FOLLOWING A SINGLE INTRA-ARTICULAR INJECTION IN RABBITS AND BIOCOMPATIBILITY ACCORDING TO ISO 10993

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Purpose: Clearance and biocompatibility studies were conducted on radiolabeled and unlabeled crosslinked hylastan gels, respectively. This study objective was to determine the temporal distribution and concentration of 14C-labeled gel in rabbit hindlimbs following a single intra-articular injection. 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 µCi/animal) into both hindlimbs. Two animals were euthanized at 1 hour and 7, 28, 84 and 85 days post-dose. Following sacrifice, each carcass was deep frozen. The left hindlimbs were removed and solubilized in a toluo- and radioactivity determined by liquid scintillation spectroscopy. 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 the testing was conducted pursuant to GLP 21 CFR 58. 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 (521±98 µg eq/g) and the fat pad (79±18 µg 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 tissue samples analyzed by QWBA generally ranged from 21.6 to 27.3 µg eq/g and the exception of the 56 day time point where the mean limit of quantification was 1213±180 µg eq/g. The left hindlimb which included the bone marrow (femur and tibia), condyle of the femur (proximal) and patella had concentration values that were below the limit of quantification at all timepoints. Although radioactive gels could still be detected up to 6 months post-injection, no radiolabel was detected above the limit 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, 95.9% 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 6.2 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.

AN EX VIVO MODEL FOR DEGENERATIVE CARTILAGE DISEASE

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Purpose: In osteoarthritis (OA), the breakdown of extracellular matrix components exceeds the synthesis of new matrix, which results in the degeneration of cartilage. In the search for new therapeutics for OA, we aim at screening of libraries to identify small molecules that either stimulate the synthesis of new cartilage matrix, or inhibit the degradation of the matrix. For this purpose we have developed an ex vivo model for osteoarthritis using mouse fetal metatarsals. To mimic an osteoarthritic environment, we treat the metatarsals with TNFα or IL1β or a combination of the two cytokines.

Methods: Mouse fetal metatarsals were isolated at day 17 of gestation, and cultured for 2 days prior to addition of TNFα and IL1β. The metatarsals were treated with TNFα, IL1β or a combination for 7 days and metatarsals were examined morphologically before harvesting for histological analysis.

Results: Morphological analysis of the metatarsals after treatment with TNFα and IL1β for 7 days showed no significant changes in the size of the metatarsals. In marked contrast, treatment with a combination of both TNFα and IL1β causes severe degeneration of the cartilage. Histological examination of the metatarsals showed a decrease in Alcian Blue and Safranin O staining after treatment with both TNFα and IL1β, indicating a loss of glycosaminoglycans in the matrix. Glycosaminoglycan content of the extracellular matrix was quantified. Treatment with TNFs reduced glycosaminoglycan content with 60%, IL1β with 90% and the combination of both with almost 100%.

Conclusions: Based on the results of these experiments, it can be concluded that treatment with either TNFα, IL1β or both can cause cartilage degeneration in mouse fetal metatarsals, resulting in an osteoarthritic-like situation. We will use this model to screen libraries of small molecules to identify new therapeutics for treatment of degenerative cartilage disease.

BIOMARKERS

INFLAMMASOME ACTIVATION IN OSTEOARTHRITIS BY URIC ACID

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Purpose: The production of mature active IL-18 and IL-1β 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 (STOP) study with 138 returning for 3 year follow-up. The study included white and black volunteers from Clinics from various geographic locations and various socioeconomic backgrounds. Participants met radiographic criteria and ACR criteria for symptomatic OA of at least one knee with a Kellgren-Lawrence (KL) grade of 1-4.

Radiographic 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 1 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 for women (68.9±7.1 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.