Cartilage Regeneration in a Rat Model of Knee OA by SM04690, a Potential Disease Modifying Wnt Pathway Inhibitor

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Background

- Knee osteoarthritis (OA) is characterized by destruction of articular cartilage, subchondral bone alterations, and synovitis.
- At a cellular level, Wnt signaling affects OA pathogenesis by modulating inflammation, cartilage breakdown, and bone/cartilage formation. Increased Wnt signaling induces stem cell differentiation into osteoblasts and inhibition shifts lineage fate towards chondrogenesis.
- Samumed is developing a small molecule, intra-articular (IA) Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug (DMOAD). Preclinical evidence of its effects on chondrogenesis, cartilage protection, inflammation, and joint health are reported here.

Methods

- In vitro chondrogenesis was evaluated in human mesenchymal stem cells (hMSCs) treated for 21 days by immunocytochemistry and qRT-PCR. Matrix production in chondrocytes was measured as sulfated glycosaminoglycan (sGAG) with a dimethylthelyene blue (DMMB) assay. Cytokine induced GAG breakdown in chondrocytes was measured with a DMMB assay.
- Pharmacokinetics were evaluated following a single IA injection of SM04690 into Sprague-Dawley rats and measurement in cartilage, bone, and plasma by liquid chromatography-mass spectrometry (LC-MS).
- In vivo activity of a single IA injection of SM04690 was evaluated in 2 rat OA models:
  1. Anterior cruciate ligament transection with partial medial meniscectomy (ACLT-pMMx): One week after surgery, vehicle or SM04690 (0.3 μg) was injected into the knee. Protease and chondrogenic gene expression in cartilage was measured by qRT-PCR and sGAG by DMMB at Week 5. Cartilage health was evaluated using blinded Osteoarthritis Research Society International (OARSI) histology scoring and thickness in Safranin O stained sections at Week 13. Doublecortin was measured by immunohistochemistry. Biomarkers (cartilage oligomatrix protein [COMP]; N-propeptide of collagen IIA [PIIANP]) were measured in plasma by ELISA.
  2. Monosodium iodoacetate (MIA) injection-induced model: 3 days after IA MIA (3 mg) injection, vehicle or SM04690 (0.3 μg) was injected. Joint inflammation was evaluated histologically and by qRT-PCR measurement of pro-inflammatory markers (TNF-α, IL-1β, IL-6) on Day 11. Pain was measured as paw withdrawal threshold using Von Frey apparatus on Days 0-22.

For parametric data, t-tests were used to compare 2 groups and one-way ANOVA for >2 groups. Mann-Whitney U test was used to analyze non-parametric data.

Animal studies were approved by the Samumed, LLC Animal Committee and performed in accordance with the U.S. Department of Agriculture’s (USDA) Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and Samumed, LLC protocols.

Results

SM04690 protected cartilage in the ACLT-pMMx model of rat OA

- Vehicle
- SM04690 (0.3 μg)

Figure 3. ACLT-pMMx induced OA in rats treated with IA vehicle or SM04690 (0.3 μg) at one week. (a) Protease gene expression in rat cartilage at Week 5. (b,c) Circulating COMP and PIIAPP measured by ELISA. (n=12 rats, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001).

SM04690 regenerated cartilage in the ACLT-pMMx model of rat OA

- Vehicle
- SM04690 (0.3 μg)

Figure 4. ACLT-pMMx induced OA in rats treated with IA vehicle or SM04690 (0.3 μg) at one week. (a) Representative images of rat knee stained with Safranin O-Fast Green. (b) Cartilage thickness from Safranin O stained sections. (c) OARSI Joint scores (d) Gene expression of chondrocyte markers in rat cartilage. (e) Total sGAG levels relative to tissue weight. (f) Representative images of rat knee stained for Doublecortin (Dcx)-expressing chondrocytes in the superficial zone of the articular cartilage. (g) Quantification of Dcx-positive chondrocytes in (f). (n=12, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001).

SM04690 attenuated acute inflammation and reduced pain in an MIA model of rat knee OA

- Vehicle
- MIA+Vehicle
- MIA+SM04690

Figure 5. IA MIA injection-induced OA in rats treated with IA vehicle or SM04690 (0.3 μg) at Day 3. (a) Representative images of H&E stained knee sections on Day 11. (b) Gene expression of inflammatory markers in the rat knee on Day 11. (n=10 rats, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001). (c) Pain in the MIA-injected limb measured as Von Frey paw withdrawal threshold.

Conclusions

- SM04690, a Wnt pathway inhibitor, induced chondrogenesis, protected chondrocytes from catabolic breakdown, increased cartilage thickness, and improved joint health in a rat ACLT + pMMx model of knee OA.
- In the MIA model, SM04690 showed potent anti-inflammatory effects that improved pain responses and structure.

Significance

- OA currently has limited therapeutic options that focus on alleviating pain until knee replacement without impacting disease processes.
- A single IA injection of a small molecule Wnt pathway inhibitor, SM04690, demonstrated reduced inflammation, increased cartilage protection, and cartilage regeneration in rodent knee OA models.
- These data suggest SM04690 may potentially be an effective disease modifying treatment for OA. Clinical studies are ongoing.