Objectives: This study aims to assess the chondroprotective and anti-inflammatory effects of the selective human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride and the selective human MC3 receptor agonist PG-990 on S-Nitroso-N-acetyl-DL-penicillamine (SNAP) activated chondrocytes.

Methods: The human chondrocytic cell-line C-20/A4 was seeded at 25.0×10^6 viable cells/ml (5 μ l droplet was transferred into individual wells of a 96-well plate). Micromass cultures [6] were stimulated with SNAP (1.0 mM) and after 2h treated with Dexamethasone (1.0 μ M), selective human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride (10.0 μ g/ml) or selective human melanocortin MC3 receptor agonist PG-990 (10.0 μ g/ml) for 6h. Cell viability was determined by MTT assay, Caspase -3 and -7 activity determined by Caspase-Glo 3/7 apoptosis assay. Glycosaminoglycan (GAG) content determined by alcian blue staining and anti-inflammatory heme-oxygenase-1 (HO-1) protein expression was determined by western blot. Data are expressed as Mean $\pm$ S.E.M of $n=4$ samples repeated in triplicate. $\#p \leq 0.05$ vs control or $*p \leq 0.05$ vs stimulus.

Results: Cell viability analysis showed SNAP stimulation caused a maximal cell death of 23% ($\#p \leq 0.05$). Dexamethasone, BMS-470539 dihydrochloride and PG-990 inhibited cell death by 2%, 98% and 129% respectively ($*p \leq 0.05$). SNAP stimulation caused a significant increase in Caspase -3 and -7 activity, which was inhibited by Dexamethasone, BMS-470539 dihydrochloride and PG-990 by 8%, 5% and 19% respectively ($*p \leq 0.05$). GAG content was significantly reduced by SNAP by 29% ($\#p \leq 0.05$), which was inhibited by Dexamethasone, BMS-470539 dihydrochloride and PG-990 by 1%, 3% and 14% respectively ($*p \leq 0.05$). SNAP also caused a significant decrease in HO-1 protein expression, which was increased by Dexamethasone, BMS-470539 dihydrochloride and PG-990 by a 1.0-fold, 1.1-fold and 2.1-fold increase respectively ($*p \leq 0.05$).

Conclusion: The selective human melanocortin MC3 receptor agonist PG-990 exhibited enhanced chondroprotection and modulation of inflammatory and tissue destructive mediators following SNAP activation compared to Dexamethasone and the selective human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride. This suggests that melanocortin peptides display enhanced chondroprotective and anti-inflammatory effects at the MC3 receptor sub-type in this cell line.

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AB0069

LORECIVIVINT (SM04690), AN INTRA-ARTICULAR, SMALL-MOLECULE CLK/DYRK1A INHIBITOR THAT MODULATES THE WNT PATHWAY, AS A POTENTIAL TREATMENT FOR MENISCAL INJURIES

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Background: Meniscal injuries, associated with pain, stiffness, and localized swelling, are the most common pathology of the knee with a prevalence of 61 per 100,000.¹ Meniscal damage is a frequent finding on MRI images of knee osteoarthritis (OA)²; while a meniscal tear can lead to knee OA, knee OA can also lead to a spontaneous meniscal tear.³ Efforts to repair meniscal damage have been largely unsuccessful and do not prevent the progression of degenerative changes that lead to knee OA.⁴ The Wnt signaling pathway has been shown to be regulated during meniscal development,^{5,6} suggesting that manipulation of this pathway may influence the regenerative capacity of the meniscus. Lorecivivint (LOR; SM04690) is an intra-articular (IA), small-molecule CLK/DYRK1A inhibitor that modulates the Wnt pathway.

Objectives: LOR was evaluated in preclinical studies to determine its protective and anabolic effects in ex vivo explants and in a rat model of chemically induced inflammatory meniscus degeneration.

Methods: Effects of LOR (30 nM) on expression of matrix metalloproteinases (MMPs) in cultured rat menisci treated with IL-1B were measured by qPCR. In vivo, LOR activity was evaluated in a rat model of monosodium iodoacetate (MIA) injection-induced inflammatory meniscus degeneration. A single IA injection of MIA was immediately followed by a single IA injection of LOR (0.3 μ g) or vehicle. Knees were harvested on Days 1, 4, and 11 and menisci were isolated. Anti-inflammatory effects were evaluated by measuring *TNFA* and *IL6* expression by qPCR. Meniscus protection was evaluated by qPCR for MMPs and aggrecanase and anabolic effects by qPCR for collagens.

Results: In ex vivo meniscal explants, LOR inhibited expression of *MMP1*, *MMP3*, and *MMP13* compared to DMSO ($P < 0.01$). In vivo, LOR significantly decreased expression of these MMPs and aggrecanase ($P < 0.05$) compared to vehicle in the rat model of inflammatory meniscus degeneration at Day 4 after MIA injection. In addition, LOR reduced expression of inflammatory cytokines *TNFA* and *IL6* at Day 4 compared to vehicle. Finally, LOR increased expression of collagen types I, II, and III at Day 11 after MIA injection.

Conclusion: LOR exhibited protective effects in the meniscus ex vivo and in vivo by reducing the expression of catabolic enzymes compared to control. Anti-inflammatory effects of LOR were demonstrated by inhibition of inflammatory cytokine expression. Compared to vehicle, LOR increased expression of collagens in vivo, indicating potential meniscal anabolic effects. These data support further investigation of LOR as a potential disease-modifying therapy for meniscal injuries.

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AB0070

LORECIVIVINT (SM04690), A POTENTIAL DISEASE-MODIFYING TREATMENT FOR KNEE OSTEOARTHRITIS, DEMONSTRATED CARTILAGE-PROTECTIVE EFFECTS ON HUMAN OSTEOARTHRITIC EXPLANTS

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Background: Wnt pathway upregulation contributes to knee osteoarthritis (OA) through osteocyte differentiation, cartilage thinning, and inflammation. Lorecivivint (LOR; SM04690), a novel, small-molecule CLK/DYRK1A inhibitor that modulates the Wnt pathway, demonstrated disease-modifying potential for knee OA in preclinical studies.¹ However, the specific mechanisms by which LOR protects cartilage in knee OA are unclear.

Objectives: To evaluate the cartilage-protective effects of LOR on human OA explants from total knee replacement (TKR) donors.

Methods: Knee joint tissue from 22 TKR donors was obtained. IRB approval was obtained from Scripps Health. Cartilage was scored using the Outerbridge classification system based on gross appearance (grade 1=least-damaged tissue, grade 4=most-damaged tissue). Cartilage explants (4 mm in diameter) with Outerbridge grades 2–3 were harvested and cultured for 48 hours to reach metabolic stability. They were then treated with LOR (10 nM, 30 nM) or DMSO and stimulated with either IL-1 β (10 ng/ml) or TNF- α (20 ng/ml)+oncostatin M (OM) (10 ng/ml) or left unstimulated. After 72 hours, supernatants and explants were collected. Gene expression of matrix metalloproteinases (MMPs) 1, 3, and 13 was measured by qPCR and protein levels of MMP-1, MMP-3, MMP-13, and thrombospondin-motif-containing disintegrins/metalloproteinases ADAMTS-4 and ADAMTS-5 were measured in supernatants by ELISA. Glycosaminoglycan (GAG) and nitric oxide (NO) levels were measured in supernatants using the dimethylmethylene blue assay and Griess assay, respectively. One-way ANOVA was used for multiple group comparisons.

Results: Treatment with IL-1 β or TNF- α +OM led to statistically significant increases in gene expression of *MMP1*, *MMP3*, and *MMP13* and increased secretion of GAG, MMP-1, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, and NO in supernatants compared to unstimulated control. Treatment with LOR decreased both IL-1 β -stimulated and TNF- α +OM-stimulated gene expression of *MMP1*, *MMP3*, and *MMP13* and secretion of GAG, MMP-1, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, and NO in supernatants compared to treatment with DMSO.

Conclusion: LOR demonstrated potent inhibition of cartilage catabolism enzyme production in human OA explants compared to controls. These cartilage-protective effects support the development of LOR as a potential disease-modifying treatment for knee OA. Human trials are ongoing.

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