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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/DRYK1A inhibitor, demonstrated disease-modifying potential for knee OA via Wnt pathway modulation in preclinical studies. Further studies were performed to evaluate 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. These were treated with LOR (10 nM, 30 nM) or DMSO and stimulated with either IL-1β (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 were measured by qPCR and levels of MMP-1, MMP-3, MMP-13, ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs), and ADAMTS-5 proteins 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-1β 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-1β-stimulated or 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 catabolic 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.

A

	IL-1β stimulation			TNF-α+OM stimulation		
Protein levels (Supernatants)	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
MMP-1	4.49 (0.2)	**1.24 (0.03)**	**1.1 (0.02)**	8.23 (0.44)	**1.01 (0.01)**	**1.0 (0.01)**
MMP-3	5.97 (0.37)	**1.02 (0.03)**	**1.35 (0.07)**	5.17 (0.32)	**1.1 (0.02)**	**1.08 (0.04)**
MMP-13	14.8 (0.49)	**4.57 (0.7)**	**1.93 (0.2)**	70.78 (3.31)	**2.56 (0.17)**	**1.47 (0.08)**
ADAMTS-4	1.28 (0.02)	1.04 (0.01)	1.09 (0.02)	1.45 (0.03)	1.03 (0.01)	0.91 (0.02)
ADAMTS-5	1.91 (0.1)	**1.03 (0.03)**	**1.15 (0.03)**	2.28 (0.11)	**1.24 (0.02)**	**0.87 (0.03)**

B

	IL-1β stimulation			TNF-α+OM stimulation		
Gene expression (Explants)	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
MMP1	1.04 (0.01)	0.81 (0.04)	0.71 (0.05)	1.04 (0.01)	**0.67 (0.03)**	**0.50 (0.03)**
MMP3	1.02 (0.01)	0.82 (0.02)	**0.56 (0.02)**	1.03 (0.01)	**0.73 (0.03)**	**0.71 (0.03)**
MMP13	1.05 (0.01)	**0.60 (0.02)**	**0.60 (0.05)**	1.05 (0.01)	**0.60 (0.03)**	**0.63 (0.03)**

C

	IL-1β stimulation			TNF-α+OM stimulation		
Supernatants	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
GAG	1.35 (0.04)	1.05 (0.03)	**0.87 (0.02)**	2.4 (0.07)	2.02 (0.05)	**1.54 (0.04)**
NO	490.46 (18.69)	**0.89 (0.05)**	**0.44 (0.03)**	144.2 (9.6)	**19.79 (2.71)**	**20.09 (2.65)**

Table: A) Enzyme protein levels, B) Enzyme gene expression, C) GAG and NO levels

Numbers represent normalized versus unstimulated. ***P*<0.05**, one-way ANOVA