Dose-Dependent Analysis of FDA-Approved Compounds for Lubricin Stimulation in a 3D Human Chondrocyte Model of Post-Traumatic Osteoarthritis

Najla A. Saleh[1], Swathi Menon[1], Rachel Kemp[1], Aditi Thorat[1], Chloe Watson, Janapriya Vijayakumar, Thomas J. Kean[1]
[1]Biionix Cluster, Internal Medicine, College of Medicine, University of Central Florida, Orlando, FL
Email of Presenting Author: najlla.adelsaleh@ucf.edu

INTRODUCTION:

Osteoarthritis (OA) is a chronic, degenerative joint disease driven by cartilage breakdown, inflammation, and loss of joint function. Post-traumatic OA (PTOA) accounts for ~12% of cases. Current therapies provide only symptomatic relief, underscoring the need for disease-modifying interventions. Lubricin, encoded by PRG4, is a mucinous glycoprotein that reduces cartilage friction and protects the joint surface. Its depletion in OA contributes to cartilage wear and progressive joint damage. Enhancing lubricin levels has therefore emerged as a potential therapeutic strategy. In a previous screen of 52 FDA-approved compounds using a PRG4-luciferase reporter system, nine drugs were identified as PRG4 stimulators and seven as inhibitors. To further evaluate their therapeutic potential, we conducted dose-response validation of three top hits and additionally assessed the other eleven candidates using a 3D human chondrocyte aggregate model that mimics PTOA conditions.

METHODS:

To model cartilage tissue in vitro, primary human chondrocytes expressing a PRG4-Gaussia luciferase reporter (HuPRG4-GLuc) were cultured as 3D spheroids under physioxia (5% O_2). Two plate formats, 96-well (50,000 cells/spheroid) and 384-well (5000 cells/spheroid) were assessed. By day 12, when lubricin production reaches a plateau, baseline luminescence was recorded. To simulate PTOA, aggregates were exposed to IL-1β (1 ng/mL) for 24 hours. Drug treatment with serial dilutions (0.00078–0.2 µM) of C5, C6, D4, D7, E2, E4, E5, or F2 stimulators and B5, B7, D8, D10, F5, F10 or G6 inhibitors began on day 13 and was repeated every 48 hours through day 19. Luminescence was measured on days 13, 15, 17, 19, and 21 and normalized to pre-injury values. On day 21, aggregates incubated with E2, E5 and F2 were collected for biochemical analysis of DNA, glycosaminoglycans (GAG), and hydroxyproline (HDP), and processed for histology. The cell culture basal media and dimethyl sulfoxide (DMSO – drug vehicle) were used as negative control and TGF- β1 (10ng/ML) was used as positive control.

RESULTS:

Spheroid loss was reduced from ~75% in early 384-well experiments to 0.3% by altering aspiration height and using breathable plate sealing membranes. For E2, E5, and F2, IL-1β injury was successfully confirmed by a measurable drop (~50%) in luciferase signal 24h post-injury, enabling proper evaluation of compound efficacy. All three compounds transiently increased lubricin expression, with a peak on day 15. E2 at 0.1 µM induced the strongest PRG4 activation, surpassing the TGF-β1 positive control, but this effect declined by day 21 and was not accompanied by ECM enhancement. E5 produced moderate increases in PRG4 activity (**Figure 1A**) but showed consistent improvement in GAG and HDP content (**Figure 1B, C**), particularly at 0.2 µM, suggesting matrix preservation. F2 yielded minimal changes in both lubricin expression and matrix components. DNA content remained stable across all treatments, and histological findings were consistent with the biochemical data. In contrast, for the others tested compounds, luciferase activity did not decrease following IL-1β exposure, indicating that the inflammatory injury failed to initiate in these plates. Their analysis is ongoing and consequently, their therapeutic effects could not be evaluated at this time.

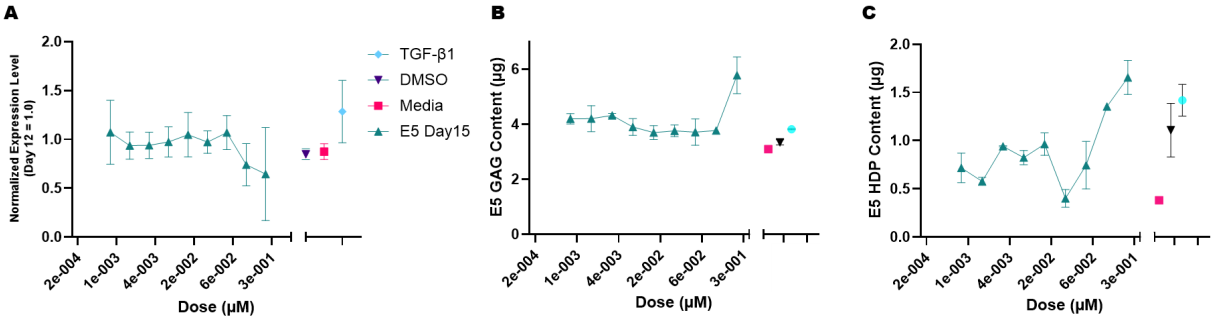

Figure 1. Representative Figure of Dose-dependent effects of FDA-approved compounds (E5) on lubricin expression and matrix components in IL-1β-injured 3D chondrocyte aggregates. **(A)** PRG4-luciferase activity normalized to baseline (Day 12 = 1.0) shows transient upregulation following treatment with E5 on Day 15. Biochemical quantification of glycosaminoglycan (GAG, **B**) and hydroxyproline (HDP, **C**) in E5-treated aggregates reveals dose-dependent matrix enhancement, most notably at 0.2 µM.

DISCUSSION:

Together, these results reveal that lubricin induction is complicated by other ECM factors. E2 stimulated PRG4 strongly but lacked ECM support. In contrast, E5 balanced moderate PRG4 activation with enhanced matrix output, potentially making it a promising disease-modifying osteoarthritis drug (DMOAD) candidate. F2 offered limited efficacy. Overall, E5 demonstrated the most favorable balance of bioactivity and matrix preservation. Future studies should explore combining PRG4-enhancing agents with ECM-supportive therapies to optimize OA treatment. The eleven additional compounds could not be properly evaluated due to the failure of IL-1β-induced suppression of PRG4 activity, a critical feature for mimicking PTOA. Their reassessment is currently underway to ensure consistent inflammatory challenge and reliable data.

SIGNIFICANCE/CLINICAL RELEVANCE:

This study highlights the power of 3D phenotypic screening to identify candidate DMOADs based on both lubricin stimulation and matrix preservation. Among the compounds tested, E5 showed the strongest therapeutic potential and warrants further investigation for OA drug in combination therapies.