An In Vitro Cartilage Injury Model to Study Drug and IL-1β Effects on the Type II Collagen Response

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Introduction: Osteoarthritis (OA) is a disease involving the gradual deterioration of articular cartilage in joints mostly within the hands, knees, and hips. Osteoarthritis may also occur because of joint injury as seen in athletes (PTOA). PTOA often affects younger and more active patients. Common injuries that can result in the development of PTOA include anterior cruciate ligament (ACL) ruptures, meniscus tears, patellar dislocations, shoulder dislocations, and ankle instability. It is known that after an injury like an ACL rupture, the concentrations of certain inflammatory cytokines, including interleukin 1-beta (IL-1B), tumor necrosis factor alpha (TNF-a), matrix metalloprotease-1,-13 (MMP-1,-13), increase to maximum levels around the joint at about 24 hours post-injury. These cytokines play a critical role in chondrocyte apoptosis and ECM degradation, and these altered conditions are often sustained which can lead to an overall destruction of cartilage. The failure of the joint to repair itself in the face of this damage results in PTOA. Addressing this phenomenon early is considered important to prevent PTOA in younger patients. Unfortunately, methods to prevent the progression of PTOA following injury are scarce. Innovative research identified candidate drugs (aromoline, deserpidine, and ABT-724 (a specific dopamine receptor D4 agonist)) that stimulate type II collagen, a specific marker for articular cartilage[1]. It is hypothesized that each of these drugs will stimulate type II collagen in a proinflammatory environment. This study delineates the temporal response to simulated injury (IL-1B) in an in vitro cartilage model and determined the effect of potential disease modifying therapeutics on that response.

Methods: Neonatal chondrocytes (IIAM), engineered to secrete a type II collagen luciferase reporter, were treated with aromoline (5µM), deserpidine (5µM), ABT-724 (5µM), and IL-1B (125 pg/ml) alone or in combination. Chondrocytes were seeded into 96-well non-adherent u-bottom plates at a density of 50,000 cells per well and centrifuged (5 min, 500RCF) to form cell aggregates. Cell aggregates were cultured in defined chondrogenic media[1] and stimulated with IL-1B, aromoline, deserpidine, and ABT-724 to comprehensively examine the effects of these drug compounds **(Fig 1, Fig 2)**. Over 22 days, media was sampled and changed every 2 days, luminescence was measured, and pellet images taken. On day 22, cell aggregates were split for histology or biochemical assays.

Results: On days 1-9, neonatal chondrocytes demonstrated a significant increase in luminescent expression when cultured with aromoline compared to being cultured in chondrogenic media alone ($P<0.05$). Following the addition of IL-1B to the remaining groups on day 10, each test group, when compared to aggregates stimulated with only IL-1B, expressed a significant difference in luminescent expression on day 12; the aggregates stimulated with only IL-1B displayed the greatest luminescence. On day 14, aggregates stimulated with only IL-1B significantly expressed greater luminescence than aggregates treated with basal media alone ($p=0.0078$) **(Fig 3)**. There was no significant difference in type II collagen promoter-driven luminescence on day 16 and day 21 between aggregates treated with only IL-1B and any other test group.

Discussion: This study examined type II collagen production under the stimulation of drug compounds in proinflammatory conditions with the idea that they will simulate pre-injury and post-injury treatment. The early stage of our study demonstrated that cell aggregates stimulated with aromoline generated greater levels of type II collagen compared to aggregates in basal media, supporting previous research[1]. After the addition of IL-1B, only aggregates treated with IL-1B alone had a significant increase in type II collagen vs. aggregates treated with basal media alone. This preliminary data suggests aromoline, deserpidine, and ABT-724 do not significantly stimulate more type II collagen production in a proinflammatory environment. Given the literature reviewed, IL-1B stimulation of type II collagen was an unexpected result; however, the concentration of IL-1B used in this study more closely mimics physiological conditions, which is about 80-fold less than that typically reported in similar studies. More analyses such as aggregate size, DNA/GAG assay, and MMP assay are being conducted to determine more clearly whether there are any positive effects of these potential drug compounds within an in vitro proinflammatory environment.

Significance/Clinical Relevance: Aromoline, deserpidine, and ABT-724 have been documented to stimulate type II collagen production in the absence of an inflammatory signal. This research aimed to examine whether the application of these compounds may help to promote chondrogenesis within an acute or chronic model of post-traumatic osteoarthritic environment. Results suggest that physiological concentrations of IL-1B effectively promotes type II collagen production within a cartilaginous injury.

References:

1. Cruz MA, Karimzadeh M, Martyniak K, Medam R, Kean TJ. Disease Modifying Osteoarthritis Drug Discovery Using A Temporal Phenotypic Reporter In Primary Human Chondrocytes. BioRxiv. 2023:2023.03.10.532073. doi: 10.1101/2023.03.10.532073.

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Treatment Groups
A: Basal Media (BM) (No IL-1B)
B: BM w/ aromoline (No IL-1B)
C: BM w/ deserpidine (No IL-1B)
D: BM w/ ABT - 724 (No IL-1B)
E: BM w/ IL-1B added @ Day 10
F: BM w/ aromoline; IL-1B added @ Day 10
G: BM w/ deserpidine; IL-1B added @ Day 10
H: BM w/ ABT - 724; IL-1B added @ Day 10
I: BM w/ both aromoline + IL-1B added @ Day 10 and removed @ Day 12
J: BM w/ both deserpidine + IL-1B added @ Day 10 and removed @ Day 12
K: BM w/ both ABT - 724 + IL-1B added @ Day 10 and removed @ Day 12
L: BM w/ both aromoline + IL-1B added @ Day 10; only IL-1B removed @ Day 12
M: BM w/ both deserpidine + IL-1B added @ Day 10; only IL-1B removed @ Day 12
N: BM w/ both ABT - 724 + IL-1B @ Day 10; only IL-1B removed @ Day 12

Figure 1: Treatment groups in the study

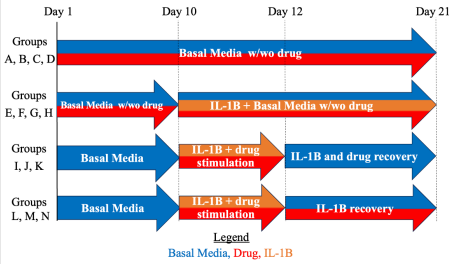

Figure 2: Treatment schedule for each treatment group.

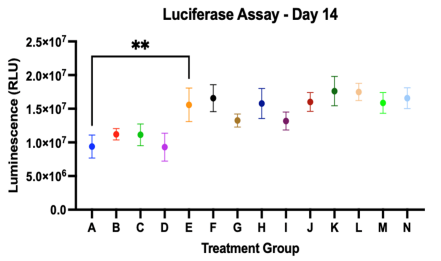

Figure 3: Day 14 luminescence results. n = 6 ± S.D. ** $p = 0.0078$