

ACE Inhibitors do not Significantly Stimulate Type II Collagen Expression in Primary Human Chondrocytes

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Disclosures: Scott Gronowicz (N), Tarek Obeid (N), Janapriya Vijayakumar (N), Sean Stapleton (N), Otto Phanstiel (N), Thomas Kean (4; ABT, AZN, FATE, FUJII, GILD, GWW, MRK, VSTM. 9; Innovation Network Committee Member, Cartilage Topic Co-Chair)

INTRODUCTION: Osteoarthritis is a widespread joint disease with debilitating effects that worsen over time. The current standard in restorative treatment involves resurfacing the affected joint using synthetic or biological implants or micro-fracture techniques that typically elicit the production of fibrocartilage. While these treatments address the defective joint surface and may reduce symptoms, artificial materials and fibrocartilage lack the regenerative ability of the hyaline cartilage that forms the articular joint surface. Deserpidine, a natural product that is an angiotensin-enzyme (ACE) inhibitor, was previously shown to have a stimulatory effect on chondrogenesis in human articular chondrocytes (1 of 2 donors [1]). If ACE inhibitors stimulate type II collagen in general, their effects could be further evaluated to develop potential treatments to stimulate new cartilage growth in patients with joint degeneration due to osteoarthritis or other conditions. To investigate the effects of other known ACE inhibitors on chondrogenesis, a 22-day assay of neonatal articular chondrocytes transfected with type II collagen promoter-driven Gaussia luciferase in media containing various ACE inhibitors at their IC90 concentrations was performed. The effects of the ACE inhibitors on the chondrogenesis of these cells were assessed by quantification of luciferase luminescence associated with type II collagen production and cartilage pellet area analysis over time.

METHODS: Primary neonatal chondrocytes (Male), genetically engineered to express Gaussia luciferase under the control of the COL2A1 promoter cells were used to assess the response to various ACE inhibitors. Engineered cells were seeded in a non-adherent 96-well u-bottom plate 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 [2] containing an ACE inhibitor (benazeprilat, captopril, enalaprilat, lisinopril, quinaprilat, ramiprilat, trandolapril, and deserpidine) at its IC90 concentration in an active form or deserpidine (5 μ M) [1]. A negative control consisting of basal media alone and a positive control with TGF β 1 (10ng/ml) added to the basal media were employed for comparison. The basal media control represents minimal chondrogenic stimulation while the TGF β 1 positive control represents maximal stimulation. An ACE inhibition assay (Sigma-Aldrich ACE1 Colorimetric Activity Assay Kit) was used to experimentally determine the inhibition curves to determine the IC90 concentrations of each of the ACE inhibitors used. There were 10 test conditions total with 6 replicates per condition. Over 22 days, media was changed every 2-3 days with luciferase assessment occurring at the 2-day intervals. After each media sampling, COL2A1 promoter-driven luminescence was measured (PerkinElmer EnVision 2104 Multilabel reader). A third of the cell aggregates were formalin-fixed then paraffin embedded. Histology for glycosaminoglycan (Safranin-O) and immunohistochemistry for type II collagen is in progress. The other two-thirds of the cell aggregates will be used for the biochemical analysis of glycosaminoglycan, DNA, and hydroxyproline contents of the cartilage pellets.

RESULTS: The COL2A1 promoter-driven luminescence and pellet area data was analyzed using a two-way ANOVA and Dunnett's multiple comparisons tests vs. the negative control across treatment groups and data collection days. There was no statistically significant effect of the drug treatment on the source of variation between the pellet data or luminescence means for the experimental groups. Using Dunnett's multiple comparisons tests, the only significant difference between treatment groups and the negative control was in the positive TGF β 1 control versus the negative control for both the pellet area and luminescence data. There were no significant differences between the means of the ACE inhibitor treatment groups and the negative control for the pellet area.

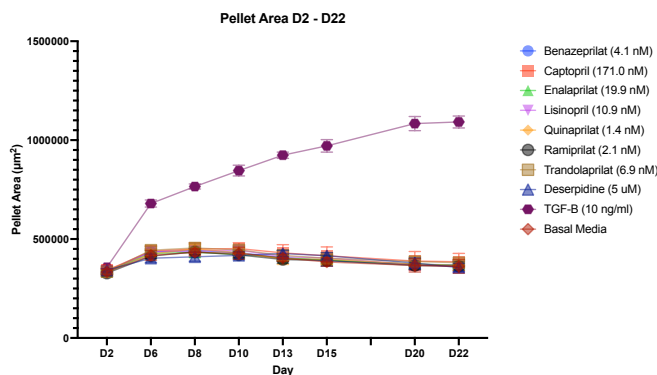


Figure 1: Day 2 through 22 pellet area results. n=6, +/- SD

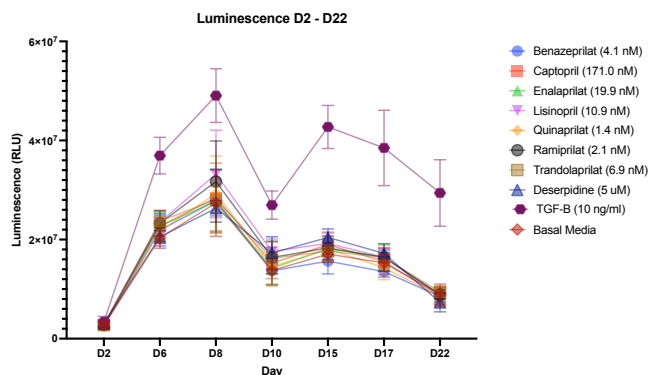


Figure 2: Day 2 through 22 luminescence results. n=6, +/- SD

DISCUSSION: This study evaluated chondrocytes in the presence of ACE inhibitors to investigate the resulting effects on chondrogenesis. The genetic engineering of the chondrocytes to express Gaussia luciferase under the control of the COL2A1 promoter cells allowed for temporal evaluation of the chondrogenesis throughout the assay. The results showed no significant effects on type II collagen production or metabolism in these cells after 22 days in response to ACE inhibitors at their IC90 concentrations. Given that the tested cells were all from the same donor, a 4-day-old male with alobar holoprosencephaly, there may be donor-dependent effects that were not evaluated. Barring donor-dependent effects, ACE inhibitors do not show promise as a potential area of investigation for stimulation of type II collagen production in human articular chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: ACE inhibitors were shown to have no appreciable effect on chondrogenesis in neonatal articular chondrocytes. This data should steer investigators away from ACE inhibitors as a mechanism to treat OA allowing us to focus on more promising targets. We could also expect that ACE inhibitors can be safely used in OA patients. This knowledge will inform investigation into drugs to stimulate chondrogenesis of articular cartilage and treat osteoarthritis in the future.

REFERENCES:

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