Anti-inflammatory Properties Of Nell-1 On Human Articular Chondrocytes In Vitro.

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Introduction: Osteoarthritis remains one of the most common diseases of advanced age and can result in disability and diminished quality of life. Accordingly, there has been substantial interest in the development of biologic agents which may diminish the inflammation and cartilage degradation that defines osteoarthritis. Nell-1 has previously been shown by our lab to have cartilage regeneration properties as well as anti-inflammatory properties, by means of increased anabolic activity and decreased catabolic activity in both in vitro and in vivo rabbit chondrocyte models. In the current experiment, we hypothesize that Nell-1 treated, primary human articular chondrocytes (ARC's), will have a significantly lower catabolic response when subjected to a well established arthritis inducing protocol utilizing the pro-inflammatory cytokine IL-1β.
Methods: Primary human articular chondrocytes (ARCs) were obtained from tibial plateau or femoral condyle tissue samples obtained at the time of joint replacement surgery under a standing IRB approved protocol at UCLA. The cartilage was sectioned into 1mm3 specimens, subjected to a 50 unit of activity collagenase digestion at 37o, and collected at 3hr, 6hr, and 12 hour intervals. For treatment purposes, ARCs were grown to confluecy in 24-well plates and serum starved for a period of 24 hours prior to any treatment. After the serum starvation period, cells were pre-treated with Nell-1 (doses: 0ng/mL, 800ng/mL and 2000ng/mL) for 2 hours preceding the addition of IL-1β (doses: 0ng/mL, 1ng/mL and 10ng/mL) for a total of 12 different treatment groups and controls. After a 24 hour incubation period, the cells were either harvested using Trizol for qPCR analysis or fixed in 4% PFA for immunohistochemistry and subsequently labeled with IL-6 at a dilution of 1:200. Additionally, tissue sections were prepared from the surgical specimens for histological analysis with H&E, Safranin O as well as Nell-1 antibody. These tissue sections were then graded using the Mankin Histological Grading Scale as well as the OARSI scale to determine their current levels of arthritis.
Results: Immunohistochemistry using IL-6 as a surrogate marker for inflammation, demonstrated significantly higher levels of signal intensity in the IL-1β positive control groups. It also demonstrated that increasing doses of Nell-1 diminished the inflammatory effects experienced by the chondrocytes in response to IL-1β stimulation as evidenced by lower signal intensity on fluorescent microscopy.
H&E staining of the tissue sections for Nell-1, showed an increased expression pattern primarily located at the articular surface of the sections which had received a higher Mankin HGGS or OARSI score.
Additionally, qPCR data demonstrated that the addition of IL-1β to ARCs resulted in up regulation of proinflammatory cytokines TNFα. IL-6 and MMP13. The addition of Nell-1 to ARCs in the presence of IL-1β resulted in a relative downregulation of TNFα. IL-6 and MMP13 as compared to IL-1β controls (Fig 1). A similar downregulation of proinflammatory cytokines was noted with the addition of Nell-1 to ARCs in the absence of IL-1β, however, this effect was less profound.
Discussion: Our data demonstrates that the previous findings of Nell-1's anti-inflammatory effects on rabbit in vitro and in vivo models translate accordingly to a human in vitro model; this is demonstrated by a clear, dose dependent, downregulation of inflammatory factors in the Nell-1 treated human ARCs. Based on the qPCR data, it appears that the optimal Nell-1 dose is 800ng/mL, given that the downregulation seen with 2000 ng/mL is not as pronounced as with 800ng/mL. We believe that the endogenous upregulation of Nell-1 at the surface of arthritic cartilage, may indicate a normal response at an attempt to slow down or halt the chronic degradation associated with osteoarthritis.
Significance: The clinical implications of these findings suggest that the addition of exogenous Nell-1, as a biological agent, has the potential to diminish the progression of the degenerative disease.
Acknowledgments: UCLA Orthopaedic Surgery Department

References:

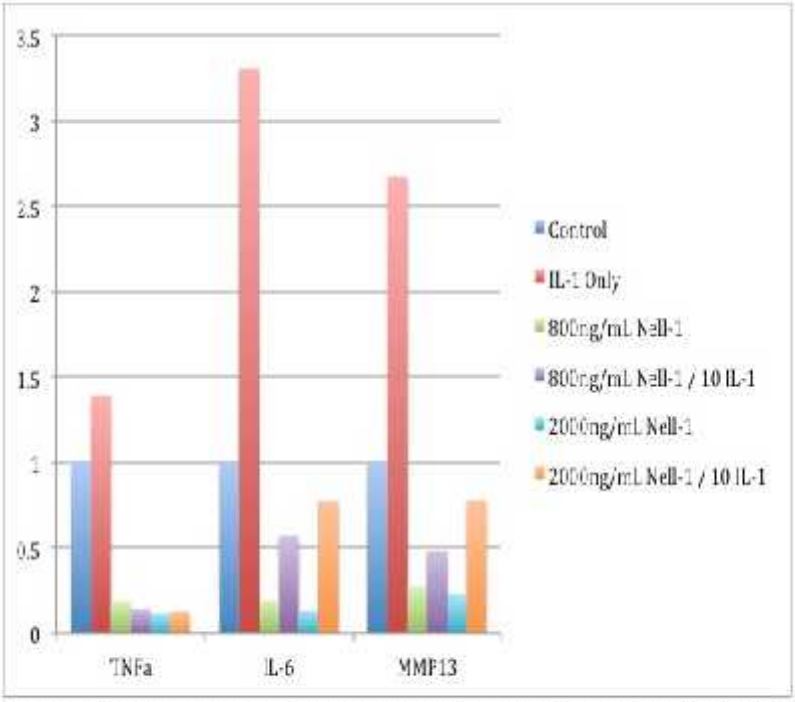

Fig 1. qPCR results demonstrating the upregulation of inflammatory factors, TNFα, IL-6 and MMP13 in the IL-1 only sample and downregulation in the Nell-1 sample, when compared to the control.

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