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"Use of a Novel IL11 Blocking Agent to Modulate Hypertrophic Dermal Scarring"

INTRODUCTION: Keloids are aesthetically distressing and often itchy or painful hypertrophic scars that form by aberrant growth and differentiation of myofibroblasts that excessively deposit mostly collagen (COL)1 primarily mediated by transforming growth factor beta (TGFβ)1 and connective tissue growth factor (CTGF). Though recurrence is common, the standard of keloid care is monthly intralesional corticosteroid injections (ICSI) until the lesions improve. Still, recurrent use of ICSI may result in lipoatrophy, hypopigmentation, and dermal atrophy. Controlled interleukin (IL)11 signaling plays an important role in the differentiation of healthy human skin fibroblasts (HSFs) into myofibroblasts during healing but shows increased expression in fibrotic diseases such as keloids and idiopathic pulmonary fibrosis. Therefore, this project investigates whether novel NMX compounds can disrupt the cascades that differentiate HSFs into keloid-forming myofibroblasts (KFMs) and related dysfunctional COL1 deposition. NMX structural analogs are designed to selectively block the TGFβ1-dependent, autocrine IL11 JAK/STAT3 signaling cascade to successfully reduce heart fibrosis and preserve cardiac function. We hypothesize that NMX will similarly interfere with the IL11-mediated activity of KFMF, effectively suppressing myofibroblast phenotype and normalizing COL1 production.

METHODS: HSFs and KFMs (ATCC) were grown in duplicate wells at 37ºC and 5% CO_2 in low-serum growth media to ~70% confluency and treated with NMX compounds (1μM, 5μM) or DMSO vehicle control for 24 hours. Cell lysates and conditioned media were collected to quantify COL1, CTGF, and IL11 levels by analyte-specific enzyme-linked immunosorbent assay (ELISA; Abcam) in triplicate wells normalized to total protein content from a bicinchoninic acid assay. Similarly, the effect of NMX1 and NMX5 (10μM) on HHSF and KC was evaluated after 24 hours concurrent with TGFβ1 stimulation at 4ng/mL. Results were obtained as optical densities (OD) using a microplate reader (BioRad). Results were analyzed by one-way ANOVA with Prism (GraphPad) and $\alpha=0.05$.

RESULTS: When treated with NMX2 (5μM), we observed lower COL1 and CTGF values in KFMs and HSFs but calculated significant decreases only in KFMs for COL1 (53.29%; P=0.036) and IL11 (38.14%; p=0.0559). CTGF was significantly lower only in NMX2-treated HSFs (46.06%; p=0.0016).

DISCUSSION: The data indicates that NMX2 mainly affects a CTGF-dependent process in HSFs and an IL11-dependent process in KSFs. However, this study only used one HSF cell line and one KFM patient cell line, so a larger sample size is needed. Furthermore, since there is no animal model of keloid formation, future studies should focus on developing keloid organoids in vitro to gain a better understanding of the impact of NMX on growth, differentiation, and epithelial-stromal transition dynamics.

CLINICAL SIGNIFICANCE: Improving pharmacotherapeutics for early modulation of key inflammatory stimuli of fibrotic processes and transformation of HHSF into contractile myofibroblasts that lead to non-compliant keloids could improve current treatment paradigms and more effectively reduce the painful discomfort imparted by hypertrophic scarring.

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