Therapeutic Potential of TRPC6 Inhibition in Painful Intervertebral Disc Degeneration

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INTRODUCTION: Low back pain is the leading cause of global disability, with discogenic etiology accounting for up to 42% of cases[1]. Intervertebral disc (IVD) degeneration is characterized by elevated pro-inflammatory cytokines, catabolic enzyme activity, and nerve and blood vessel ingrowth, all of which exacerbate pain[2]. The degenerated disc microenvironment is further compromised by extracellular matrix breakdown, reduced load-bearing capacity, and accumulation of metabolites such as diacylglycerol (DAG) and lysophosphatidylcholine (LysoPC), which accelerate disease progression. Despite the high prevalence of discogenic chronic back pain (DCBP), no pharmacological therapy directly targets these underlying mechanisms.
Transient receptor potential (TRP) channels are cation-permeable transmembrane proteins that have emerged as promising therapeutic targets in various diseases. The canonical subtype TRPC6, known to regulate inflammation and pain in multiple tissues, has not yet been investigated in the context of DCBP. TRPC6 is upregulated in painful, degenerated discs and can be activated by hypo-osmotic stimuli, mechanical loading, and DAG[3, 4]. We hypothesize that in degenerated IVDs, elevated diacylglycerol (DAG) activates TRPC6, driving inflammation and catabolism, hallmarks of DCBP. Conversely, pharmacological inhibition of TRPC6 could mitigate these pathological processes, positioning TRPC6 as a novel non-opioid therapeutic target for the management of discogenic chronic back pain.

METHODS: Human IVD tissue was obtained from patients undergoing surgery for disc herniation or degenerative disc disease (DDD) under institutional ethical approval. TRPC6 mRNA expression was quantified in degenerated (n=22) and non-degenerated (n=12) IVD samples using RT-qPCR. DAG levels were assessed in isolated human IVD cells (n=2) using a fluorometric assay (CellBiolabs). TRPC6 channel activity was measured by calcium influx assays (FlexStation-3, Fura-2 QBT™) in human IVD cells treated with TRPC6 activators (OAG, Hyp9; n=3) or inhibitor (larixyl acetate [LA]; n=1). For gene expression studies, IVD cells were treated with Hyp9 (1 µM, 18 h; n=7) or pretreated with Hyp9 (1 µM, 2 h) followed by LA (10 µM, 18 h; n=4). Untreated and vehicle controls were included. mRNA expression was analyzed by RT-qPCR, protein secretion quantified by ELISA (normalized to DNA), and pathway activation (Hyp9 1µM for 15 or 30 minutes) examined by Western blot (phospho/total ratios). Statistical analyses (normality test, t-test, ANOVA) were performed using GraphPad Prism 10.0.2.

RESULTS SECTION: TRPC6 mRNA and protein levels were significantly elevated in painful, degenerated human IVDs (data not shown)[5]. Isolated human IVD cells produced DAG (Fig. 1A), and TRPC6 activation by OAG or Hyp9 induced a time-dependent increase in intracellular calcium, whereas inhibition with larixyl acetate (LA) attenuated this response (Fig. 1B). Hyp9 stimulation significantly upregulated inflammatory and catabolic genes (Fig. 1C), as well as neurotrophic and angiogenic factors (data not shown). At the protein level, Hyp9 increased IL-8 secretion across 18–48 h (Fig. 1D), with additional cytokines including IL-6 and VEGF also being elevated (data not shown). Hyp9 further activated the ERK pathway (Fig. 1E). Importantly, LA treatment following Hyp9 pre-activation significantly reduced inflammatory and catabolic gene expression (Fig. 1F) and neurotrophic and angiogenic factors (data not shown).

DISCUSSION: Our data highlight TRPC6 as a key mediator of inflammatory and catabolic signaling in the degenerated intervertebral disc. Consistent with its upregulation in painful human IVDs, pharmacological activation of TRPC6 elevated intracellular calcium, induced pro-inflammatory cytokine release, and activated MAPK/ERK signaling, linking TRPC6 activity directly to hallmarks of DCBP. Importantly, inhibition of TRPC6 with larixyl acetate reversed these pathological effects, underscoring its therapeutic potential. These findings align with the emerging role of TRP channels as modulators of pain and inflammation in other tissues and extend this paradigm to the IVD.

SIGNIFICANCE/CLINICAL RELEVANCE: The annual treatment cost of low back pain is estimated at $314 billion in the US alone. Targeting TRPC6 may allow intervention upstream of multiple pathological cascades, offering a disease-modifying, non-opioid therapeutic strategy for DCBP. Ongoing studies will validate these findings in vivo and explore the clinical translatability of TRPC6 inhibition.

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IMAGES AND TABLES:

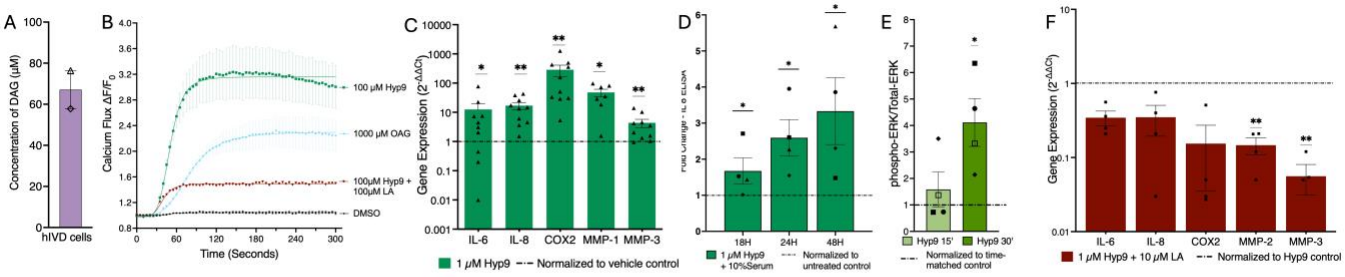

Figure 1: Human IVD cells produce DAG, and pharmacological modulation of TRPC6 regulates calcium flux, inflammatory-catabolic signaling, and ERK pathway activation.
(A) Human IVD (hIVD) cells produce ~70 μM DAG from ~10^7 cells grown in 2D *in vitro* culture (n=2). (B) TRPC6 activation by Hyp9 (100 μM, n=3) or OAG (1000 μM, n=3) induced Ca^{2+} influx, while larixyl acetate (LA; 100 μM, n=1) reduced this response. (C) TRPC6 activation with Hyp9 (1 μM for 18 hours, serum-free) increases the mRNA expression of selected inflammatory-catabolic genes (n=10). (D) TRPC6 activation with Hyp9 (1μM for 18 hours, 24 hours and 48 hours,10%serum) show time-dependent increase in the release of IL-8 (n=4) (E) TRPC6 activation with Hyp9 (1μM for 15 or 30 minutes, serum-free) show increase in ratio of phospho-ERK to total-ERK (n=4) (F) TRPC6 inhibition with LA (2 hour pre-treatment with 1 μM Hyp9 followed by co-treatment with 10 μM for 18 hours, serum-free) decreases the mRNA expression of selected inflammatory-catabolic genes (n=4). Data are presented as mean ± SEM from biological replicates (n as indicated); $^*p < 0.05$, $^{**}p < 0.01$.