Combined Anti-TNFα and Anti-depressant Treatments have Additive Effects Alleviating Pain in Male and Female Rats in a Chronic In-vivo Discogenic Pain Model

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INTRODUCTION: Back pain is a leading cause of global disability; discogenic pain accounts for ~40% of back pain cases and is driven by intervertebral disc (IVD) degeneration (IVDD) [1]. Annulus fibrosus (AF) injury induced IVDD in a rat model caused persistent IVD inflammation as well as sensitization and neuroinflammation of the spinal cord (SC) dorsal horn highlighting the complexity of discogenic pain [2]. Nevertheless, most current treatments focus on pain management or broad anti-inflammatory approaches with unsatisfactory outcomes. We postulate that treating discogenic pain requires simultaneous targeting of IVDD and SC neuropathy. Etanercept, a TNFα inhibitor, has shown promise reducing inflammation associated with IVD degeneration [3,4]. Duloxetine, a serotonin–norepinephrine reuptake inhibitor, has efficacy reducing neuropathic and chronic low back pain, and attenuating SC glial activation in both animal and clinical studies [5–7]. This study applies Duloxetine and Etanercept as combined and individual treatments since these drugs have no known interactions. Furthermore, sex-differences are reported in pain sensitivity, inflammatory responses, and therapeutic outcomes [8]. Therefore, the aims of this study are to assess the effects of Etanercept, Duloxetine, and their combination on chronic discogenic pain in male and female rats using an in-vivo discogenic pain model using measurements of behavior, IVD, and SC.

METHODS: With IACUC approval, 49 male and 20 female skeletally mature (5-6 month old) Sprague–Dawley rats were randomized into Male; Naïve (n=12), Vehicle (n=13), Duloxetine (n=5), Etanercept (n=4), or Combined (n=13) groups and Female; Naïve (n=6), Vehicle (n=7) or Combined (n=7) groups. All groups except Naïve received AF puncture injury (26G needle with PBS at anterior and left and right lateral punctures with transverse sweeps to increase IVD disruption) to induce severe IVDD at L3-4, L4-5, and L5-6 levels. Naïve animals served as controls. Four weeks post-injury, systemic treatments were administered for 2 weeks: Duloxetine (20 mg/kg, i.p. daily), Etanercept (5 mg/kg, s.c. every 3 days), their Combination, or Vehicle (equivalent saline injections as Combination). Euthanasia occurred at 8 weeks, 2 weeks after treatment. Outputs included von Frey hindpaw mechanical allodynia, IVD height loss, IVDD grading (Safranin-O/Fast-green), spinal macrophages (CD68); and SC staining for neuropeptide (SubP), astrocytes (GFAP), microglia (Iba1), and TNFα. ANOVA with Tukey's post-hoc tests determined effects of treatment for all variables. Random Forest regression identified predictors of withdrawal thresholds using all outputs.

RESULTS: IVDD decreased hindpaw withdrawal threshold in male and female rats at 2 weeks post-injury and persisted through 4 weeks. Combined therapy restored the withdrawal thresholds to Naïve levels in both male and females (**Fig. 1**). Individual treatment (evaluated only in the male cohort) showed Duloxetine was more effective than Etanercept in restoring the withdrawal threshold to Naïve levels. In the SC, SubP-, GFAP-, Iba1-, and TNFα-ir in the SC dorsal horn were significantly increased in the Vehicle group compared to Naïve (**Fig. 2**). Duloxetine reduced GFAP, Iba1, and TNFα; Etanercept selectively reduced TNFα; and Combined treatment broadly suppressed all four markers (**Fig. 2**). Random Forest identified male SC GFAP, Iba1, and SubP as the top predictors of hindpaw mechanical hypersensitivity, surpassing IVDD grade and spine inflammation measures (**Fig. 3**). No treatments altered male IVD height, IVD degeneration, or spine CD68-ir (not shown). Female cohort SC and spine histology and IVD height measures are in progress.

DISCUSSION: Combination treatment reduced pain-related behaviors in both sexes although the kinetics of effects was slower in females (even though dosing by weight was identical), and all SC biochemical markers were reduced (in males; female measures are ongoing) further demonstrating combined treatment reduced central sensitization. Individual treatment interventions (in males) showed additive effects, in agreement with animal models where simultaneous targeting of neurotransmitter and cytokine pathways enhanced behavioral recovery in neuropathic injury [6,10]. Duloxetine's modulation of GFAP and Iba1 is consistent with similar findings in radiculopathy and neuropathic pain models [5]. Etanercept's selective TNFα effect parallels clinical and preclinical evidence that systemic anti-TNFα therapy has limited efficacy in established pain unless administered early or locally [9]. Importantly, neither treatment altered IVD height, IVD degeneration, or spine CD68-ir, paralleling clinical observations where symptom improvement can occur without structural repair [6]. Furthermore, Random Forest identified SC GFAP, Iba1, and SubP as the strongest predictors of pain, surpassing IVD measures, reinforcing the SC as a central therapeutic target for IVDD-related pain. Therefore, therapies aimed at modifying IVDD may be more effective if applied in less severe stages of degeneration, delivered locally, or initiated earlier systemically. Future studies should also evaluate longer treatment durations to determine whether chronic IVDD eventually reestablishes pain. We conclude that this combined systemic pharmacological approach has potential to address both neurochemical and inflammatory pathways in chronic discogenic pain yet may be limited for treatment of severe IVDD, as induced in this model. Female cohort SC and IVD assays are ongoing to determine sex-specific mechanisms of action.

SIGNIFICANCE: Combination Duloxetine and Etanercept alleviated discogenic pain in both male and female rats and reduced SC sensitization and neuroinflammation in males, supporting its potential as a multimodal therapeutic approach for chronic discogenic pain.

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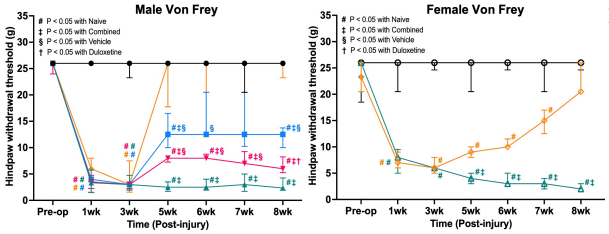

Figure 1. Combination treatment restored VF thresholds in males and females, outperforming monotherapies.

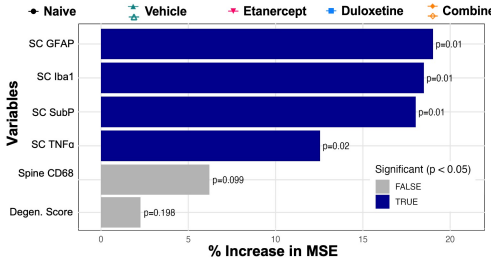

Figure 3. Random forest identified SC markers as strongest predictors of pain behaviors over IVD measures in males.

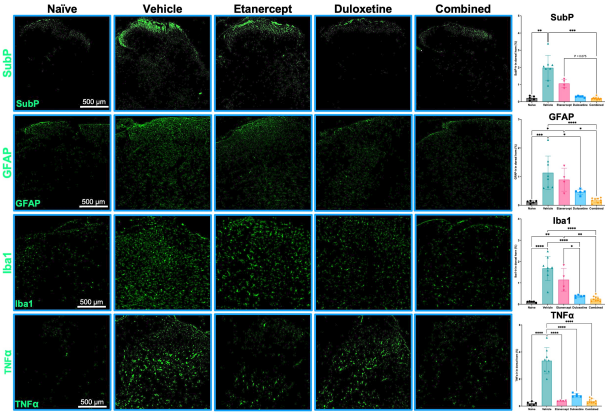

Figure 2. Combination treatment broadly suppressed SC SubP, GFAP, Iba1, and TNFα in males.