Vagus nerve stimulation suppresses local and systemic inflammation and alleviates the post-traumatic osteoarthritis pain in young and old mice

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INTRODUCTION Osteoarthritis (OA) is a degenerative joint disease characterized by joint pain, stiffness, swelling, and loss of functional mobility. Post-traumatic osteoarthritis (PTOA) occurs due to acute joint injury, such as anterior cruciate ligament (ACL) tears or meniscal damage, leading to joint inflammation, cartilage damage, and pain sensitization. PTOA due to mechanical injury to cartilage, bone, ligaments, and synovium triggers innate immune responses in the synovium and surrounding tissues characterized by the release of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-17A), driving chronic low-grade inflammation. Neuroimmune modulation by vagus nerve stimulation (VNS), activating the cholinergic anti-inflammatory pathway, may offer a multi-faceted approach to treat the PTOA pain and limit joint damage. In this study, we investigated the role of transcutaneous VNS (tVNS) in PTOA pain and inflammation in young adults and old mice.

METHODS Three cohorts of mice were used to test if tVNS, delivered via the tragus nerve of the ear, improved pain behaviors in acute and chronic PTOA pain, in young 4 month (mo) or 13 mo old mice. Cohorts 1 and 2 included 4 mo old female C57BL/6J mice (n=40) subjected to forced tibial compression to induce ACL rupture (ACLR) in the right limb. Twenty mice were randomized to tVNS or sham stimulation beginning on post-injury day 1, and the other 20 mice began stimulation on post-injury day 30. Mice received tVNS (2mA current) for 10 minutes, five days a week, for two weeks under isoflurane sedation. Mice receiving sham stimulation underwent the same procedure, but the electrodes were not activated. A third cohort included 13 mo old female C57BL/6J mice (n=20) with ACLR that were treated with tVNS or sham stimulation beginning post-injury day 1 for two weeks. After two weeks of tVNS or sham treatment, mechanical hyperalgesia by a digital pressure algometer, spontaneous pain with weight-bearing, and allodynia by digital Von Frey were assessed in a blinded fashion. Serum samples were collected for cytokine and chemokine analysis using a 23-plex assay. Splenic and joint CD45+ cells were isolated for gene expression of inflammatory cytokines and macrophage activation markers using qPCR. Knee joints were harvested for histological examination using OARSI and zone-based OA grading methods.

RESULTS Two weeks of tVNS treatment significantly improved mechanical allodynia and hyperalgesia compared to sham stimulation in 4 mo (Fig. 1A) and 13 mo (Fig. 1B) old female mice but did not significantly improve weight bearing on the ACLR limb. Mice treated with tVNS beginning on day 30 after ACLR, when PTOA is fully established, also had significant improvements in allodynia and hyperalgesia but not weight bearing (Fig. 1C). tVNS significantly reduced synovial CD45+ cells expressing CD86 compared to sham stimulation treatment (Fig. 1D). These mice also showed decreased serum pro-inflammatory cytokines (IFN-γ, Il-1β, MIP-1β, and IL-12p70). Splenic CD45+ cells expressing CD86 or Il-1β were reduced compared to sham-stimulated splenic CD45+ cells. (Fig. 1E-F)

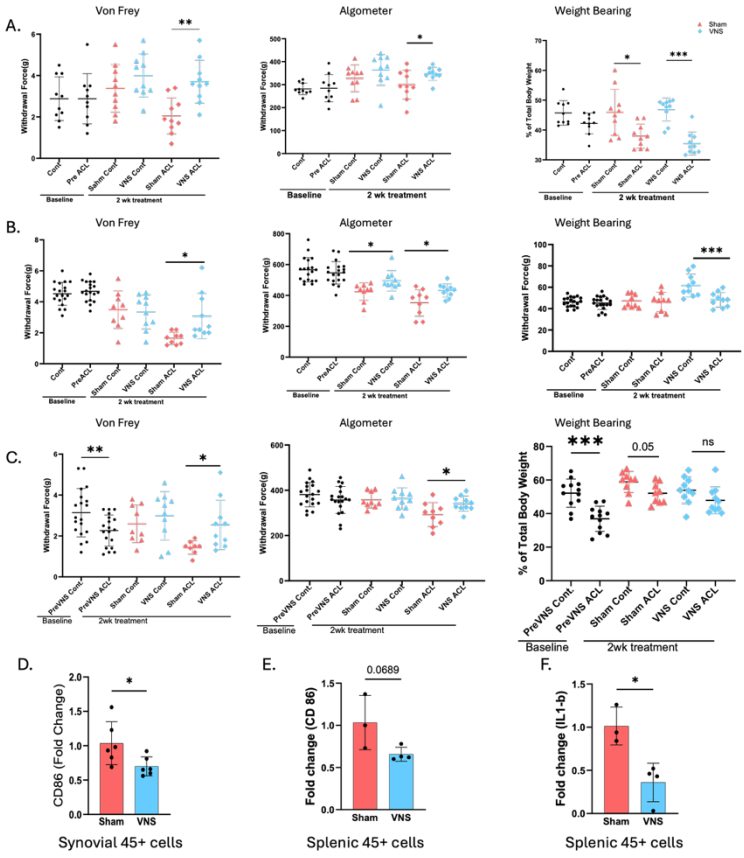

Figure 1. Transcutaneous VNS improves PTOA pain behaviors and reduces inflammation in the synovium and spleen. (A) In 4-month-old female mice, 2wk VNS treatment alleviated mechanical allodynia and hyperalgesia. (B) In 13-month-old mice, 2wk VNS treatment improved mechanical allodynia and hyperalgesia. (C) In 4-month-old female mice, 30 days post ACL injury, 2 wk VNS treatment alleviated mechanical allodynia and hyperalgesia. (D) 2 wk VNS decreased CD86 expression in synovial CD45+ cells in 4-month mice. (E) 2wk VNS, initiated 31-day post-ACLR, decreased CD86 expression in splenic CD45+ cells of 4-month-old mice.(F) 2-week VNS, initiated 1-day post-ACLR, reduced IL-1β expression in splenic CD45+ cells of 13-month-old mice

CONCLUSIONS Using the preclinical ACLR model of PTOA, our findings demonstrate that tVNS is a promising treatment for reducing acute (days 1-15 post-ACLR) and chronic (days 31-46 post-ACLR) PTOA pain. Mechanistically, VNS suppresses local and systemic inflammation by reducing the expression of CD86, likely on activated M1 macrophages, B cells, and dendritic cells, in the synovium and spleen.

CLINICAL RELEVANCE Our results indicate that tVNS may serve as an effective therapeutic strategy for PTOA pain by attenuating inflammation and alleviating hyperalgesia and allodynia. These findings warrant further investigation and validation in human clinical trials.