Genetic Loss Of PKCδ Induces Knee Joint Hyperalgesia In OA

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Introduction: Pain is the most prominent symptom of osteoarthritis (OA) and a key reason why patients seek medical help. Available therapies (NSAIDs, steroids, viscosupplementation such as intrarticular injection of hyaluronic acid) can alleviate mild to moderate joint OA pain. However, relief from severe chronic OA pain remains an unmet medical need and the major reason for seeking surgical intervention. One fundamental issue in OA pain field is that there is lack of a correlation between the degree of structural change in the joint and the degree of pain sensation. It is not clear what causes pain in OA, and currently there is no effective way to relieve pain caused by OA. Protein kinase Cδ (PKCδ) is expressed ubiquitously among cells and tissues. The aim of this study was to assess the role of the PKCδ pathways in knee joint OA pain.

Methods:
Experimental Animals: PKCδ gene knockout (KO) and WT mice were subjected to the surgical destabilization of the medial meniscus (DMM) model. A calibrated set of von Frey filaments for mechanical allodynia and spontaneous activity were measured.

Gross Microscopic and μ-CT Analyses: Specimens were immediately fixed in 10% formalin followed by μCT imaging analyses. Microscopic analyses were performed to examine structural alterations in anterior and inferior articular cartilage using a Nikon SMZ1000.

Histopathological Analyses: Specimens were harvested at 4 and 8 weeks post-DMM surgery, fixed and decalcified followed by paraffin-embedding. Serial knee sections of 5 μm were prepared for Safranin-O Fast Green and immunofluorescence staining.

RNA analysis and Real-time PCR: RNA fractions from the whole knee joint, lumbar section of DRGs and the dorsal horn of the spinal cords were extracted. Relative messenger RNA (mRNA) expression was determined using the ΔΔCT method (Bio-Rad).

Results:
Inhibition of PKCδ signals protects joints from pathological changes. The OA-like pathology progresses slowly and in defined stages over 16 weeks after surgery. Joint pathology scores for PKCδ KO mice are 4 weeks post DMM = 4.2 ± 1.5, 8 weeks post DMM = 4.5 ± 1.3 and 16 weeks post DMM = 5.4 ± 2.2. On the other hand, joint pathology scores for WT mice are 8.9±1.7 (4 wks), 12.2±1.3 (8 wks) and 23.4±2.1 (16 wks) respectively.

PKCδ KO mice manifest knee joint OA-associated hypersensitivity compared to WT littermate. Mechanical allodynia progressed up to 4 weeks after DMM but not sham surgery, and this mechanical sensitivity maintained throughout the 16 weeks follow-up period where PKCδ KO mice developed significantly lower pain threshold (higher sensitivity) compared to WT; In WT mice, changes in spontaneous locomotive activity indicative of chronic pain or pain triggered by movement, including decreases in distance traveled (ambulation) and rearing (frequency of stand-up position assessed by vertical photo-beam counts) do not appear until 8 weeks post DMM surgery. On the other hand, in PKCδ KO mice, spontaneous activity was changed as early as 4 weeks post DMM surgery and this sensitivity maintained throughout the follow-up period, suggesting manifestation of hypersensitivity in knee joint.

Sensory neural distribution is detected in the painful knee OA joint synovium both in an experimental OA animal model and human, and this neural distribution is strikingly abundant in PKCδ KO mice after DMM. Sensory nerve terminals marker PGP-9.5 in knee joint synovium of animals experiencing pain were increased in the mouse joints after OA induction by DMM surgery, and this induction was strikingly prominent in PKCδ KO mice compared with WT littermates at 8 weeks post-DMM surgery. We validated this sensory neuronal distribution by use of human knee joint tissues comparing normal (Collin’s G:0, no history of OA pain) and patients with chronic OA knee joint pain (surgically removed). We found a striking increase in distribution of sensory neurons in OA synovium tissues reflected by a positive immunostaining for PGP9.5.

Genetic loss of PKCδ in mice stimulates MCP-1/CCR2 and NGF/TrkA axis compared with wild type littermates during the course of OA progression. Multiple catabolic / inflammatory molecules, known to be increased in OA, are significantly reduced in PKCδ KO mice after OA induction by DMM surgery. These include IL-1β, TNFα, IL-6, IL-8 and TLR-2/-4, and consequently, their downstream target cartilage degrading enzymes (e.g., MMP-3/-13 and ADAMTS-4/-5) were significantly reduced in the PKCδ KO mice compared to the littermates after DMM surgery, providing molecular bases of potent resistance to cartilage degeneration in the absence of PKCδ. Surprisingly, inflammatory chemokine MCP-1 and neuropeptide NGF as well as their cognate receptors (CCR2 and TrkA) were significantly increased in the PKCδ KO mouse joints during the course of OA. We verified these animal studies by use of human primary chondrocytes and fibroblast-like synovial cells in vitro. Synovial cells transfected with siRNA targeting PKCδ sufficiently upregulated MCP-1/CCR2 (2-fold and 2.5-fold, respectively) and NGF/TrkA (10-fold and 8-fold, respectively), and theses inductions were further enhanced when combined with inflammatory cytokines, whereas, only a
limited impact was observed in chondrocytes on the expression of any of these factors.
NGF/TrkA axis is increased in the sensory neurons in the DRG after DMM surgery, and this increase is further enhanced in the PKCδ KO mice.
Total RNA were harvested from ipsilateral L3/L5 DRGs after 4- and 8-weeks post-DMM preparation and analyzed by qPCR targeting axonal growth promoting factors and their cognate receptors (NGF/TrkA, MCP-1/CCR2, NT-3/TrkC, IGF-1/IGF-1R and TRPV4). Among them, the NGF/TrkA axis was the most prominently increased axonal promoting growth factor by DMM surgery. Consistent results were observed by the use immunofluorescence staining using innervating L3/L5 DRG at 8 week post-DMM surgery.

**Discussion:** Our findings suggest that genetic loss of PKCδ-dependent nociceptive pathways are pathologically linked to manifestation of OA-induced hyperalgesia, and these behavioral changes are closely correlated to abundance of sensory neuronal distribution in the joints and axonal growth promoting factors in the joints and sensory neurons.

**Significance:** The present study address a fundamental question: “Why is there no close correlation between the degree of cartilage degeneration and the degree of pain sensation?” and elucidates key molecular components that control OA-induced specific algesic pathways by determining how genetic loss of PKCδ impacts OA-related pain signals.

**Acknowledgments:**

**References:**