Concurrent alterations in peripheral and central compartments of pain pathways in experimental osteoarthritis pain model: is OA pain neuropathic?

INTRODUCTION: Despite many years of intensive research, the precise pathogenesis of osteoarthritis (OA) and the etiology of OA symptom still remain elusive. Knowledge of involvement of concurrent cellular and structural alterations in peripheral knee joint components and neuronal, spinal responses due to osteoarthritic pain could be essential for therapeutic drug development. By using established animal model for knee joint OA pain, involving an intra-articular knee injection of mononiodoacetate (MIA), we were able to generate predictable, and sustained chronic knee joint pain that are correlated with alterations in cellular and structural parameters of knee joint tissues and of subchondral bone structure [1]. In this study, we assessed the sensory neuronal reactions in dorsal root ganglion (DRGs) and dorsal horn of the spinal cord in response to the knee joint OA pain to explore how the pain pathway from peripheral tissues in the symptomatic knee joint OA legion can be transmitted into central nerve system.

METHODS: Tissue preparation. At 2 and 4 weeks post-MIA or saline injection (n=8 rats per group) were euthanized in halothane anaesthesia. The entire knee joints were then dissected for histology. Lumbar section of DRGs and the dorsal horn of the spinal cords were harvested for the preparation of either total RNA for real-time PCR or protein extract for cytokine antibody array or western blotting analysis. Cytokine Antibody Array Quantification. An array for cytokine proteins (Cytokine Array, RayBio) was used to determine relative alterations in the level of cytokines by following manufacturer’s instruction. Densitometric measurement was performed by calculating the integrated density values for each spot (area times relative intensity) by using the Molecular Imager Versadoc MP 4000 System and Quantity One-4.5.0 Basic 1-D Analysis Software (Bio-Rad). Positive control signals on each molecular image were used for normalization. Histology. The decalcified knee was cut in the mid-sagittal plane, washed in running tap water, and paraffin-embedded. The synovium was stained with hematoxylin and eosin (H&E) stain to assess general morphology, and neovascularization. RESULTS: Synovial angiogenesis is accompanied with inflammatory feature in OA model. H&E staining demonstrates neovascularization in the MIA-injected knee joint synovium along with moderate degree of infiltration of immune cells, reflecting inflammatory events (Fig.1) which are seen in human OA (Fig.2). Furthermore, we observed that significantly increased sensory innervation represented by increased act ivity of peripheral nociceptors by neuromflament-M (NF-M) immunoreactivity in MIA-injected synovium tissues which is absent in saline-injected or contralateral knee joint tissues. Alterations in sensory neurons in DRGs and spinal cords in an experimental OA pain model. Alterations of the peripheral sensory neurons in response to MIA-induced joint discomfort was investigated by assessing the levels of cytokines and pain mediators in L3-L5 DRGs. TNFα and IL-1 are important pain mediators influencing the neuropathic and nociceptive pain pathways. TNFα (Fig.3) and IL-1 as well as other pain mediators (e.g.,neuropeptide Y or galanin) are markedly upregulated compared to contralateral DRGs. Table 1. Comparison of gene expression in the sensory neurons in DRGs between neuropathic and MIA-induced knee OA pain model. PAIN: MODEL: NEUROPATHIC (SNL) [3,4] OA KNEE PAIN (MIA-induced) DREAM ↑ [2,3] ↑ TNFα ↑ ↑ CGRP ↓ ↓ Substance P ↓ ↓ Galanin ↑ [5] ↑ Neuropeptide Y ↑ [5] ↑ IL-1α,β ↑ ↑ CONCLUSION: Intra-articular injection of MIA into the knee joint features athophysiological, behavioural indices [1], and cellular alterations in peripheral joint tissues (e.g., angiogenesis) aligned with the human pain experience of OA. In the MIA model the induction of pro-inflammatory cytokines and simultaneous reduction of anti-inflammatory cytokines in spinal cord may facilitate low-threshold mechanical responses contributing to secondary hyperalgiesia. Interestingly, our gene analysis in the sensory neuronal response to the MIA-induced knee joint OA pain demonstrates similar gene expression pattern that have been seen in neuropathic pain models [2-5]. Our data suggest that the OA pain pathways may share the neuropathic pain cascades.


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