NBQX, An AMPA/Kainate Glutamate Receptor Antagonist, Alleviates Joint Disease In Models Of Inflammatory- And Osteo- Arthritis.

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Disclosures:

Introduction: Inflammation underlies pathology in osteoarthritis (OA) and rheumatoid arthritis (RA). Non-steroidal anti-inflammatory drugs, corticosteroids and anti-cytokine treatments that have revolutionised RA treatment, also relieve OA symptoms with varying success (1). Here, we investigate whether glutamate receptor (GluR) antagonists represent a new treatment targeting inflammatory stages of arthritis. Synovial fluid glutamate concentrations increase by more than 40 fold in human OA and RA patients (2). Glutamate signals in various musculoskeletal cells where it activates ionotropic (iGluRs) and metabotropic (mGluR) glutamate receptors to regulate peripheral pain, cytokine and matrix metalloproteinase (MMP) release, proliferation and immune responses. GluR antagonists represent potential peripheral treatments for inflammatory arthritis and inflammatory mechanisms that contribute to OA. Intra-articular injection of iGluR antagonists inhibit pain, but only two studies have investigated effects of GluR antagonists on arthritic pathology; one showed no effect of a single intra-articular treatment targeting all iGluRs on cartilage erosion (3), but the other revealed that continual systemic administration of memantine (NMDAR antagonist) alleviated synovitis and joint destruction (4). Long-term effects of single treatments of GluR antagonists on arthritic pain, inflammation and pathology are unknown, and no studies have investigated pathological effects of AMPA/KA GluR antagonists. Activation of kainate (KA) and AMPA glutamate receptors increase interleukin-6 (IL-6) release (5) and cause arthritic pain (6) respectively. We hypothesised that AMPA and KA GluRs are expressed in human arthritic joint tissues and that peripheral administration of NBQX (AMPA/KA GluR antagonist), would attenuate joint pathology in antigen-induced arthritis (AIA) and meniscal transection (MNX) osteoarthritis in vivo.

Methods: Joint degradation and bone remodelling were related to GluR immunohistochemistry in tibial plateaux samples from OA and RA patients. NBQX was applied to human primary osteoblasts derived from patients undergoing total knee replacement (TKR) for OA, and mineralisation assessed. NBQX was injected intra-articularly into the affected knees of AIA rats at the time of arthritis induction, and MNX rats immediately after and 7 days following surgery. Knee swelling and gait patterns of AIA (n=15), AIA+NBQX (n=15) and naive rats (n=6) were measured over 21 days. Knee swelling, rear limb weight bearing and paw withdrawal thresholds of MNX, MNX+NBQX, sham, sham+NBQX and naive rats (n=8 for all) were measured over 14 days. On day 21, AIA joint tissues were taken for QRT-PCR, x-ray, magnetic resonance imaging (MRI), histology and GluR immunohistochemistry, and MNX joint tissues were taken for GluR immunohistochemistry, and MNX joint tissues were taken for GluR immunohistochemistry.

Results: NBQX completely prevented mineralisation in human primary osteoblasts from all TKR patients. Both human OA and RA tissues showed extensive degradation and synovial inflammation with abundant GluR (AMPAR2, KA1) expression in pathological areas of bone/cartilage remodeling (Fig 1). Similar GluR expression was observed in AIA and MNX rats, with less extensive GluR expression after NBQX treatment. In AIA rats, NBQX treatment significantly reduced knee swelling (P<0.001, days 1-21), gait abnormalities (days 1-3), end-stage cartilage destruction (P<0.05), synovial inflammation (P<0.001), meniscal IL-6 and whole joint cathepsin K mRNA expression (P<0.05). Significant increases in mRNA markers of bone turnover (Cathepsin K, P<0.01; COL1A1, P<0.001; RANKL, P<0.05; RANKL:OPG, P<0.01) were prevented by NBQX treatment indicating a potential mechanism in the bone. X-ray and MRI revealed a smoother articular surface; fewer bone erosions and less inflammation after NBQX treatment (Fig 2). In MNX rats, NBQX treatment significantly reduced knee swelling (P<0.001) and differences in hind-limb weight bearing (P<0.01) 14 and 10 days after surgery respectively. Paw withdrawal thresholds were not affected in MNX rats.
Figure 2. Macroscopic joint pathology in AIA and AIA+NBQX inflamed and contralateral control rat knees. (A-C) Representative x-ray images show severe erosions in the tibial plateaux and femoral condyle in AIA rats (arrows, (B)). AIA+NBQX rats displayed a much smoother joint surface (C), resembling that seen in the contralateral control knee (A). (D-F) Representative MRIs confirm the erosions seen in x-rays (arrows), and also show the presence of severe synovial inflammation at day 21.
**Discussion:** To determine the role of glutamate signalling in local inflammatory processes underlying arthritic pathologies, we investigated synovial inflammation and AMPA/KA GluR expression in human OA, RA and rat AIA and MNX, and determined whether AMPA/KA GluR antagonists affect pathology. Synovial inflammation occurred in all arthritis patients. AMPA and KA GluRs were expressed in diseased areas of bone and cartilage in human arthritic tissue and rat models of inflammatory and osteoarthritis. This is the first demonstration of GluRs expression in human OA and RA tissue. A single intra-articular NBQX injection profoundly reduced joint pathology in AIA, reducing knee swelling by 33%, histological synovial inflammation scores by 34%, and degeneration scores by 27%, exceeding protective effects of etanercept, infliximab and methotrexate in the same model. This is the first study to show that a single intra-articular injection of any GluR antagonist achieves long-lasting anti-inflammatory effects and alleviates cartilage and bone destruction in arthritis. The relevance to human disease and attenuation of inflammation, pathology and pain in vivo by intra-articular NBQX treatment, reveals promise for NBQX as a new disease-modifying drug for inflammatory and osteoarthritis.

**Significance:** OA has no effective treatment and although treatment for RA has been revolutionised by anti-cytokine therapies, around 30% of patients remain unresponsive. NBQX alleviates pain and inflammation in both inflammatory and osteoarthritis and may be an effective treatment for both these diseases.

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**References:**

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