# NBQX, an AMPA-kainate glutamate receptor antagonist, alleviates inflammation, degeneration and pain related behaviour in two models of osteoarthritis.

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**Background and Aims:** Synovial fluid glutamate concentrations increase in various arthritides (1). Activation of kainate (KA) and AMPA glutamate receptors (GluRs) increase interleukin-6 (IL-6) release and cause arthritic pain respectively (2). GluR antagonists represent potential peripheral treatments for inflammatory arthritis and inflammatory mechanisms that contribute to osteoarthritis (OA). We previously found that AMPA and KA GluRs localise to bone, cartilage and synovial tissue from osteoarthritic patients and that the AMPA/KA GluR antagonist, NBQX, reduced knee swelling, gait abnormalities and joint destruction in a rat model of inflammatory arthritis (2). Here, we determined whether NBQX influenced inflammation, joint degeneration and pain in a surgical model of OA (medial meniscal transection (MNX)) and a non-invasive model of post traumatic OA (ACL rupture model).

**Methods:** Right knees of male Sprague-Dawley rats received MNX or SHAM surgery (n=8) and NBQX (25mM) or sterile water (vehicle control) was administered by intra-articular injection immediately after surgery (after suturing) and 7 days later. Five naïve rats received no surgery or treatment. To determine dose response, MNX rats received 2 injections (as above) of 2.5mM, 12.5mM, 25mM NBQX or sterile water (n=3 for each group). Three naïve rats served as controls. Over 21 days, knee swelling (digital calliper) and rear limb loading (Linton incapacitance meter) were measured on days 0, 1, 2, 3, 7, 8, 10, 14 and 21. For ACL rupture, custom built cups were used to hold the knee in flexion and a 12N load at 4Hz (ElectroForce® 3200, BOSE) was applied to the right knees of anaesthetised 12-week-old C57Bl6 mice. Ligament rupture occurred on load application, revealed by a continued increase in displacement following release of the applied compressive force during the loading cycle. A single intra-articular injection of NBQX (20mM) was administered to 10 animals immediately following ACL rupture, whilst another 15 received an intra-articular injection of vehicle (sterile water) immediately following ACL rupture. Contralateral knees (intact ACL, n=15) were used as controls. A second experiment tested different dosing regimens of 20mM NBQX. Following ACL rupture mice received either a single intra-articular injection (day 0) of 20mM NBQX (or sterile water control), two injections (NBQX or vehicle, day 0, day 1) or 3 injections (NBQX or vehicle, day 0, day 1, day 7) (n=5 for each group). Contralateral knees (intact ACL with no load, n=5) and mice who received intra-articular 20mM NBQX without load (intact ACL, to assess effects of NBQX in normal joint) were used as controls. Over 21 days, knee swelling (digital calliper) was measured on days 0, 1, 2, 3, 7, 16 and 21. On day 21, all animals were culled and knees taken for histology.

**Results:** In the MNX model, 25mM NBQX significantly reduced knee swelling on days 7 and 8 compared to vehicle ($p<0.05$, $p<0.01$, GLM). On all days (except day 0), vehicle treated knees show significantly greater knee swelling compared to naïve knees ($p<0.01$, GLM), whereas NBQX reduces swelling to similar levels as naïve. At day 21, histological inflammation was significantly reduced in 25mM NBQX treated MNX rats compared to vehicle controls ($p<0.05$). Histological inflammation score is also significantly lower in 25mM treated rats compared to 2.5mM and shows no significant difference to naïve scores. In the ACL rupture model, NBQX significantly reduced knee swelling on days 1 and 2 compared to vehicle ($p<0.05$, $p<0.01$, GLM). On days 1, 2, 3 and 7, knee swelling in vehicle controls was significantly greater than day 0 swelling ($p<0.001$, GLM), whereas NBQX treated knees showed no significant increase compared to day 0. At day 21, no significant difference in histological inflammation was seen between NBQX and vehicle treated ACL rupture mice. In the MNX model, on days 7, 8 and 14, vehicle treated rats had a greater difference in weight bearing ($p<0.05$, GLM) compared to naïve rats. 25mM NBQX treatment reduced weight bearing differences to levels similar to naïve rats. In the MNX model, both 12.5mM and 25mM NBQX reduce joint severity scores to those of naïve animals. 2.5mM NBQX and vehicle control joint severity scores are significantly higher compared to naïve animals. In the ACL rupture model, single NBQX treatment reduced cartilage and bone pathology ($p<0.001$). ACL rupture caused substantial loss of cartilage and subchondral bone remodelling, whereas NBQX treated knees showed less severe cartilage and bone changes. NBQX reduced ACL rupture severity score by 29% ($p<0.001$), although not to naïve values. Two intra-articular doses of NBQX (at time of rupture and 24 hours later) significantly reduced knee severity score compared to a single dose ($p<0.05$), three doses ($p<0.01$) and also two doses of vehicle control ($p<0.05$). Knee severity scores following two doses of NBQX was reduced to control knees, whereas all other treatments remained significantly higher.

**Conclusion:** This study provides new evidence that NBQX treatment is effective at relieving inflammation, degeneration and pain in OA. Combined with our previous data from an inflammatory model of arthritis (2), NBQX shows promise as a new disease-modifying drug for prevention of post-traumatic osteoarthritis.

**Significance:** An estimated 52.5 million adults in the United States are reported to have some form of arthritis, with OA (approximately 27 million) being the most prevalent (statistic from the Centers for Disease Control and Prevention website). There is currently no cure for OA and where pain/disability is intolerable, joint replacement is performed. Surgery is costly, carries significant risks, and up to 20% of knee replacements are unsuccessful (3). Our novel data reveal that NBQX has exciting potential as a new disease modifying drug to tackle the shortfall in OA treatment. Combined with the beneficial effects on inflammatory forms of arthritis (2), NBQX shows great promise as a new disease modifying drug for many forms of arthritis.

**References:**

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