Expression of Glutamate Receptors and Transporters in Human Subchondral Bone in Osteoarthritis

1Mason, DJ; 1Brakspear, K; 2Wilson, C; 3Williams, R; +2Kotwal, RS
1Cardiff University, Cardiff, UK. +2Llandough University Hospital, Cardiff, UK.
Senior author rahktwl@aol.com

ABSTRACT INTRODUCTION:

Pain is the major indication determining the need for joint replacement in osteoarthritis. The extent of pain experienced by patients, however, does not correlate with the extent of joint damage. The neurotransmitter glutamate is known to mediate both central and peripheral nociception via activation of various classes of glutamate receptors. Glutamatergic receptors and transporters have been shown to be expressed in osteoblasts, osteocytes and osteoclasts and modulate the phenotype of each bone cell type in vitro (Mason 2004). To our knowledge, glutamatergic signalling has not previously been investigated in human arthritis in vivo. We hypothesize that glutamatergic signalling in the subchondral bone represents a molecular mechanism underlying differing in patients’ pain perception. We therefore investigated differential mRNA expression of glutamate signalling components across the subchondral bone of patients with osteoarthritis to determine whether they are expressed, whether this varies in different anatomical regions of the bone and whether it is influenced by severity of disease.

METHODS:

Informed consent was obtained from patients to collect surgical waste tissue (approved by the University) for our study. Subchondral bone samples were obtained from defined sites across the tibial cuts of two patients undergoing total knee arthroplasty (Kellgren Lawrence grade 3) and from the tibial drill hole sites of two patients undergoing high tibial osteotomy (KL grades 2 and 3) for osteoarthritis. Multiple samples were obtained from both the medial and lateral sides. Samples were homogenised in liquid nitrogen, RNA extracted in Trizol (Invitrogen) and contaminating genomic DNA removed (DNA free, Ambion). RNA purity and concentration was analysed prior to reverse transcription (1-3 micrograms, Superscript II). Real time-PCR was performed for the housekeeping gene GAPDH, the glutamate transporter EAAT-1, the NMDA receptor subunit NR2A and the kainate receptor subunit KA1 using standard conditions. Relative quantitative real time-PCR with SYBR green was used to determine differences in the expression of EAAT-1ex9skip, a dominant negative splice variant of EAAT-1 called EAAT-1ex9skip and osteocalcin after normalisation to GAPDH. Statistical analysis was performed using Statistical package for the Social Sciences version 12.0 (SPSS Inc, Chicago, Illinos).

RESULTS:

We found that good quality and quantity of RNA was obtained, even from small bone cores removed from drill holes during HTO surgery. The housekeeping gene GAPDH was expressed in all samples. EAAT-1 and ionotropic glutamate receptor subunits (NR2A and KA1) expression was also detected. Relative quantitative RT-PCR revealed high levels of expression of osteocalcin indicating that the RNA was largely derived from osteoblasts or osteocytes. Osteocalcin expression when normalised to GAPDH did not vary significantly with anatomical site (ANOVA, p = 0.478) or disease status (ANOVA, p = 0.296). Interestingly, standard RT-PCR suggested a differential expression of EAAT-1 between medial and lateral bone samples in the total knee arthroplasty patients, however these differences were not significant when analysed by quantitative RT-PCR. On further analysis of samples from the lateral side in one patient (from which we had more samples), EAAT-1 expression was significantly influenced by anatomical location in the subchondral bone, with lower expression in samples from the anterior zone versus the middle or posterior zones (ANOVA, p<0.001). The dominant negative splice variant EAAT-1ex9skip represented a significant proportion of the total EAAT-1 mRNA expression in bone from late OA patients, but appeared less abundant in early OA samples derived from HTO surgery. In contrast to the full-length EAAT-1, the expression of EAAT-1ex9skip was not significantly affected by anatomical zone (ANOVA, p=0.547).

DISCUSSION:

We have shown for the first time that glutamate transporters and receptors are highly expressed in subchondral bone of patients with early and late osteoarthritis. Glutamate concentrations are increased in the synovial fluid of patients with rheumatoid (McNearney 2000) and osteoarthritis (Jean 2005) and activation of glutamate receptors in the subchondral bone may contribute to both pathological changes and nociception. Our preliminary data indicates that EAAT-1 and EAAT-1ex9skip expression in the subchondral bone may vary with anatomical location and extent of pathology. Our future work would involve analysis of a large cohort of samples from patients with different grades of degenerative joint disease to profile expression of glutamatergic signalling components at both the RNA and protein level.

REFERENCE:

