Expression of Glutamate Receptors and Transporters in Human Meniscus: Correlation with Anatomical Location, Pain or Pathology.

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ABSTRACT INTRODUCTION:

Injury to the meniscus often results in pain, which is one of the commonest symptoms of a degenerate meniscal tear. It is said that the inner two-thirds of the meniscus has no nerve fibres (Day, 1985), and pain is due to mechanical interference of the torn meniscus with the normal joint mechanics and also because of swelling and injury to the surrounding tissues. However many a times, it is found that the tears are relatively small to have caused altered mechanics or injury to surrounding structures. Clinical studies have shown that the pain usually disappears after surgical excision of a degenerate torn meniscus. Hence it is difficult to correlate and explain why the pain disappears after surgical excision. The neurotransmitter glutamate is known to be involved in both central and peripheral nociception via activation of various classes of glutamate receptors. Glutamatergic receptors and transporters have been shown to be expressed in various musculoskeletal tissues, including the meniscus in rat, and can mediate release of cytokines and degradative enzymes (Mason 2004, Flood et al. 2007). To our knowledge, glutamatergic signalling has not previously been investigated in human meniscus in vivo. We hypothesize that glutamatergic signalling in meniscus represents a molecular mechanism that influences pain perception. The aim of this study was to investigate mRNA expression of glutamate signalling components across different parts of the meniscus in patients with degenerate painful meniscal tears and also in asymptomatic normal meniscus samples to determine whether they are expressed, whether this varies in different anatomical regions of the meniscus and whether it is influenced by pain or degeneration.

METHODS:

Informed consent was obtained from patients to collect surgical waste tissues (approved by the University) for our study. Meniscus samples were obtained from two patients undergoing arthroscopic partial meniscal resection for chronic degenerate painful meniscal tears, from one patient with a torn painless meniscus that was incidentally found during Anterior cruciate ligament reconstruction and from the less affected compartment of the knee joint of three patients undergoing total knee arthroplasty. Samples were further classified according to anatomical regions of the meniscus (anterior horn, body or posterior horn) and whether they were from the inner vascular or outer avascular portion of the meniscus. Samples were cryosectioned and RNA extracted using RNAasy Mini Kit (Qiagen) and contaminating genomic DNA removed using DNA-Free DNA Purification Kit (Ambion). RNA purity and concentration was analysed prior to reverse transcription of 500ng (Superscript II). Real-time PCR was performed for the housekeeping gene GAPDH, the NMDA receptor subunit NR2A, the AMPA receptor subunit GluR3 and the kainate receptor subunit KA1 using standard conditions. Absolute quantitative real-time-PCR with SYBR green was used to quantify differences in the glutamate transporter EAAT-1, a dominant negative splice variant of EAAT-1 called EAAT-1ex9skip and type I collagen after normalisation to GAPDH or total RNA. Statistical analysis was performed using Statistical package for the Social Sciences version 12.0 (SSPS Inc, Chicago, Illinois).

RESULTS:

We found that good quality and quantity of RNA was obtained from all the human meniscus samples. The housekeeping gene GAPDH was expressed in all samples. EAAT-1, EAAT-1ex9skip and ionotropic glutamate receptor subunits (NR2A, AMPA GluR3 and KA1) expression was also detected. Quantitative RT-PCR revealed high levels of expression of type I collagen indicating that the RNA was largely derived from meniscal tissue. Further analysis of the menisci from the less-affected compartment of the arthritic knee by absolute quantification and RT-PCR revealed that levels of EAAT-1 expression normalised to GAPDH showed no significant differences between the inner and outer halves of the meniscus or in the anterior, middle or posterior regions of the meniscus (2-way ANOVA, p=0.405 and p=0.445, respectively).

With the small number of samples that we had of the pathological meniscus, it appeared that EAAT-1 expression was higher in painful than in the non-painful meniscus. Interestingly, standard RT-PCR analysis of EAAT-1ex9skip showed that detection of this splice variant was significantly more common within the outer zones (ANOVA, P=0.040) and in the posterior horns of the menisci (ANOVA, P=0.038).

DISCUSSION:

We have shown for the first time that glutamate receptors and transporters are expressed in human meniscus and it could possibly explain the pathophysiology of pain associated with a degenerate torn meniscus. Preliminary data indicates that EAAT-1 and EAAT-1ex9skip expression in the human meniscus may vary with extent of damage to the meniscus and anatomical location, although it is difficult to accurately demarcate the various zones and regions of the meniscus macroscopically. Glutamate concentrations are increased in the synovial fluid of patients with degenerate joint disease (Jean 2005) and activation of glutamate receptors in the meniscus may contribute to nociception.

REFERENCES:


