ALTERED ION CHANNEL FUNCTION IN AN *IN VITRO* MODEL OF INFLAMMATORY ARTHRITIS

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Purpose

Chondrocytes possess a complex complement of membrane ion channels, transporters and receptors, which have been shown to have a range of functions within these cells; including maintenance of membrane potential and cell volume regulation. Various transcript studies have identified changes in ion channel gene expression with osteoarthritis (OA), including aquaporin channels and calcium-selective TRP channels. *In vitro* studies have shown that pro-inflammatory cytokines can induce significant chondrocyte apoptosis, one of the hallmarks of OA. The aim of this study was to examine the function of aquaporins and calcium-selective ion channels in healthy and cytokine-stimulated cells.

Methods

Healthy cartilage was obtained from the stifle and elbow joints of young, skeletally mature bull terrier type dogs, euthanized for unrelated veterinary reasons (aged2-3years; weight 12-15kg). Chondrocytes were isolated overnight at 37^{o}C using a 0.1% collagenase II solution. The cell suspension was filtered and washed before being seeded into culture flasks and cultured until confluence was reached (37^{o}C, 21% O_2). Once cultures were established cells were split into two groups: healthy control (standard DMEM supplemented with 2% penicillin-streptomycin, 1% fungizone and 10% foetal calf serum) or stimulated cells (DMEM as above plus TNFα and IL-1β both at 10ng/ml). Chondroyctes were cytokine-stimulated for up to one week. Cells were used in experiments up to the 2nd passage. Two experimental protocols were used: a membrane water permeability assay and measurement of intracellular calcium using Fura-2AM.

Results

Using an established permeability assay, whereby a hypotonic solution is applied to the cells and cell volume recorded, aquaporin permeability was measured. In healthy cells permeability was found to be 31±3x10^{4}cm.s (*n*=5). Stimulated cells did not show a significant change in aquaporin permeability (*n*=12). Calcium measurements showed that healthy cells responded to the same hypotonic challenge with an intracellular calcium increase of 115±15nM (*n*=16). This calcium increase was inhibited by the TRP channel antagonist, PYR3 (*n*=69). When applied to cells from the *in vitro* model of arthritis the same osmotic challenge caused a significantly greater calcium increase of 328±45nM (*n*=11; p≤0.01).

Conclusions

We have investigated changes to two ion channels in healthy chondrocytes and those from an *in vitro* model of arthritis. Aquaporin function appeared unchanged in our cytokine arthritis model, possibly suggesting that this change in gene expression occurs as a result of OA, rather than contributing to OA development. Previous work has shown that intracellular calcium increases greater than 300nM can lead to cell apoptosis and therefore the changes we observe here could implicate important pathological changes in cytokine-stimulated chondrocytes. Further work is necessary to identify the exact mechanism by which this calcium increase occurs.