

**Conclusions:** We have established a whole tissue model for osteoarthritis comprising both cartilage and bone, which is highly responsive to both catabolic and anabolic stimulation. This model responded both anabolic and catabolic to PTH treatment, demonstrating the importance of cell-cell communication. The dual effect on cartilage may be divided in direct and indirect effects from PTH on bone and cartilage, which had not been possible to detect in a single-tissue model system. This model may be useful for testing potential disease-modifying osteoarthritis drugs (DMOADs) interfering with more than one aspect of the pathological situation. Furthermore, it presents a unique opportunity for investigating the role of osteoclast-, osteoblast- and chondrocyte-interactions in the pathogenesis of OA and other joint degenerative diseases.

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##### HUMAN OSTEOARTHRITIC (OA) AND RHEUMATOID ARTHRITIS (RA) TISSUES EXPRESS THE NOVEL HISTAMINE H4 RECEPTOR (H4R)

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**Purpose:** To determine whether the H4 receptor is pharmacologically expressed in human OA and RA tissues, and the different cell types that express this receptor.

**Methods:** Human synovial tissues from OA and RA tissues were collected with the consent from patients undergoing total knee replacement. These tissues were investigated using RT-PCR, immunohistochemistry and by competitive radiolabeled antagonist binding assays. The expression of H4R RNA and protein were investigated using RT-PCR and immunohistochemical methods with human H4R antibodies. Synovial membranes were evaluated histologically following Hematoxyline Eosine staining. To determine the phenotype of the cells expressing H4R, mast cells were revealed using anti tryptase and c-kit antibodies. Dendritic cells, macrophage and endothelial cells were revealed using S100, CD68 and the Von Willebrand factor respectively. CD4 was used as a marker for mature helper T cells, and CD8 as a marker for mature Cytotoxic T cells. To ascertain whether the H4 receptor is pharmacologically expressed, human OA synoviocytes from 5 patients were evaluated in competitive radiolabeled antagonist binding assays. The effect of Thioperamide on metalloproteinases was studied zymography.

**Results:** The H4 receptor was detected at RNA and protein levels in both OA and RA synovial tissues, with a significantly greater number of cells staining positive in inflammatory RA tissues. In synovial membranes from patients with RA, H4 receptor was detected in mast and dendritic cells as well as in the macrophage-like type B synoviocytes infiltrating within the vascular wall, in the diffuse cellular infiltrate and in the superficial lining cell layer and in the pannus. When vessels from rheumatoid synovial membranes or OA showed features of vasculitis, a positive signal for H4 receptor was detected within the media and adventitia of the vascular wall but not in the internal tunic endothelial cells (intima, which stains positive for the von Willebrand factor). In newly dissociated and cultured human OA synoviocytes, specific [3H] radiolabeled antagonist binding was displaced by both Thioperamide and a selective H4 receptor antagonist indicating expression of pharmacologically active H4R in these diseased tissues. The zymographies revealed that MMP-9 activity is upregulated by IL-1b and this effect is greatly diminished by Thioperamide.

**Conclusions:** This study shows the *in situ* localization of H4R in human OA and RA tissues in both infiltrating immune cells and synoviocytes associated with disease pathogenesis. We provide

also first pharmacological evidence of H4R expression by radio-labeled binding. Hence, H4R may be an attractive target in the development of new approaches for therapeutic treatment of OA and RA and possibly other diseases where histamine plays a crucial event in mediating leukocyte trafficking and tissue degradation.

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##### EVIDENCE FOR REACTIVE OXYGEN SPECIES INDUCED APOPTOSIS IN ANTEROMEDIAL GONARTHROSIS

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**Purpose:** Anteromedial gonarthrosis (AMG) is a distinct phenotype of osteoarthritis (OA), with a specific pattern of disease. There is full thickness cartilage loss anteromedially, progressing to an area of damaged cartilage, and then to an area of macroscopically and histologically normal cartilage posteriorly. It can be considered to be a spatial model of OA progression. Apoptosis, or chondrocyte cell death, has been shown to be a feature of OA cartilage, however the triggers are poorly understood; similarly, reactive oxygen species (ROS) have been implicated in OA. They have never been studied in a replicable topographical model of OA.

This study characterises the regional levels of cell death and implicated ROS in AMG using a number of immunohistochemical studies.

**Methods:** Ten tibial resection specimens were obtained from patients undergoing unicompartmental knee arthroplasty. Eight above knee amputations (from patients with peripheral vascular disease) were used as age matched controls.

Cross sections taken through all regions were paraffin embedded. Routine histology was performed and immunohistochemical studies were conducted for Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), Active Caspase 3, Cytochrome C, Active Bax, Bim, 3-Nitrotyrosine and Forkhead box O3A (FOXO3A).

**Results:** Cell death, as detected by TUNEL appeared predominantly in the surface layer of chondrocytes of damaged cartilage ( $p < 0.001$ ). Median values were 23% in superficial cartilage (range 0 - 51) compared to 0% in deeper cartilage (range 0 - 15). There was a significant difference in TUNEL staining between regions ( $p = 0.001$ ). This ranged from 26% (most damaged) to 4% (undamaged). There was a good correlation with degree of cartilage damage ( $\rho = 0.66$ ,  $p < 0.001$ ) as defined by histological grade and TUNEL was significantly higher ( $p < 0.001$ ) in AMG compared to the control samples which showed an average of 2% TUNEL overall. Upstream markers of apoptosis (Active Caspase 3, Cytochrome C, Active Bax), assessed qualitatively, were present in a similar distribution to that of TUNEL staining.

3-Nitrotyrosine was also shown to be a predominantly surface phenomenon. There was a significant difference ( $p < 0.001$ ) between regions, ranging from 58% (most damaged) to 10% (undamaged). Again, this was significantly higher than the control samples ( $p < 0.001$ ). In line with indicators of ROS mediated damage, Bim and FOXO3A were also detected.

**Conclusions:** The mechanism of apoptosis in OA cartilage has not been studied in depth, and understanding the biochemical and molecular responses of 'stressed' chondrocytes may provide invaluable information about the specific causes of cell death. Such cellular responses may provide targets for disease modification, thus delaying or preventing the need for joint arthroplasty.

We conclude that AMG is a phenotype demonstrating cartilage at progressive stages of disease. Apoptosis involves the intrinsic mitochondrial pathway and ROS appear to be implicated. Further work is needed to provide evidence of what lies further upstream of markers demonstrated in this study.