declined 2.3-fold, suggesting that metabolic suppression as well as cell losses contributed to the inhibitory effect of rotenone on overall biosynthetic activity. Although there was no change in proteoglycan content attributable to rotenone treatment, it is possible that proteoglycan depletion may have been detectable with longer culture times. These findings support the hypothesis that chronic suppression of mitochondrial activity inhibits protein synthesis. However, it is still unclear if these effects lead to cartilage matrix instability.

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INTRODUCING A NEW EX VIVO MODEL FOR OSTEOPOROSIS IN CLINICS DIFFERENT SUBPOPULATIONS OF ARTICULAR CHONDROCYTES: PHENOTYPE AS WELL AS CALCIFIED CARTILAGE AND SUBCHONDRAL BONE

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Purpose: The pathogenesis of osteoporosis (OA) is a chronic process, which include cartilage loss and abnormal differentiation of chondrocytes. The objective of this study was to develop and validate a novel ex vivo model for OA, which includes different chondrocyte phenotypes, as well as the cells of the calcified cartilage and the subchondral bone. We propose to use this model to investigate whole tissue pathology including cartilage turnover, cellular interactions between different subpopulations of chondrocytes, osteoblast and osteoclast upon stimulation with catabolic and anabolic factors (e.g. proinflammatory cytokines and potentially drug candidates).

Methods: Explant cultures Full depth cartilage explants were isolated from bovine femoral condyle or tibia plateau and cultured with or without anabolic or catabolic factors; without factors (W/O), with either Oncostatin M [10ng/ml] combined with TNF-α isolated from bovine femoral condyle or tibia plateau and cul- 

nats were collected and stored at -20°C for 21 days, with medium change three times a week. The supernatants were collected and stored at -20°C for 21 days. The supernatant was collected and stored at -20°C for 21 days. The supernatant was collected and stored at -20°C for 21 days.

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FOXO TRANSCRIPTION FACTORS IN ARTICULAR CHONDROCYTES

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Purpose: This study was undertaken to examine the expression of FoxO transcription factors in human osteoarthritic articular chondrocytes. Cellular localisation of these transcription factors is regulated by de PI3K/AKT signaling pathway. Activated AKT phosphorylated FoxO family members, which induces exclusion of FoxO from the nucleus. Unphosphorylated FoxO proteins remain active in the nucleus and promote the transcription of genes that regulate proliferation, apoptosis and stress resistance.

Methods: Human osteoarthritic articular chondrocytes were used in this study. Interleukin-1B was used to stimulate chondrocytes for 1 hour or 24 hours. The expression of FoxO family members was assessed by western blotting. FoxO1, FoxO3a and FoxO4 localisation was performed by confocal microscopy and immunohistochemistry.

Results: Each of the 3 family members, FoxO1, FoxO3a, and FoxO4, are expressed in human chondrocytes. FoxO3a expression was found to be more important than FoxO1, whereas FoxO4 expression was very weak. In control cells, all three proteins were found principally in the nucleus of the cell; with some proteins found in the cytoplasm. Interleukin-1B effects on FoxO family members localisation were observed after 24 hours of stimulation, whereas no obvious changes were observed after 1 hour. IL-1B treatment for 24 hours induces a cytoplasmic localisation of the FoxO transcription factors.

Conclusions: This study presents evidence that each of the 3 family members, FoxO1, FoxO3a, and FoxO4, are expressed in osteoarthritic chondrocytes. FoxO3a expression was found to be more important than FoxO1, whereas FoxO4 expression was very weak. In control cells, all three proteins were found principally in the nucleus of the cell; with some proteins found in the cytoplasm. Interleukin-1B effects on FoxO family members localisation were observed after 24 hours of stimulation, whereas no obvious changes were observed after 1 hour. IL-1B treatment for 24 hours induces a cytoplasmic localisation of the FoxO transcription factors.

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DIACERHEIN EFFECTS ON FOXO TRANSCRIPTION FACTORS LOCALISATION ON HUMAN CHONDROCYTES

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Purpose: The aim of this study was to investigate the effects of diacerein and rhein on FoxO transcription factors in human osteoarthritic chondrocytes. These transcription factors are involved in the control of cell cycle progression, cell proliferation, induction of apoptosis and protection from oxidative stress; and are regulated by the PI3K/AKT transduction pathway.
THE ROLE OF THE SWELLING-ACTIVATED Cl- CURRENT (ICl,swell) IN THE PROCESS OF REGULATORY VOLUME DECREASE (RVD) IN ISOLATED RABBIT ARTICULAR CHONDROCYTES

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Purpose: Articular chondrocytes are exposed in vivo to the continuously changing osmotic environment and thus require volume regulatory mechanisms. The present study was designed to investigate i) the functional role of the swelling-activated Cl- current (ICl,swell) in the regulatory volume decrease (RVD) and ii) the regulatory role of tyrosine phosphorylation in ICl,swell, in isolated rabbit articular chondrocytes.

Methods: Rabbit cartilages were collected from bilateral knee, hip and glenohumeral joints of male animals weighing 2.0 to 3.0 kg. The cartilage was dissected into slices and cultured in DMEM for 1-3 days. The day of experiments, chondrocytes were isolated by enzymatic digestion. Whole-cell membrane current was recorded during exposure to isosmotic (300 mOsm) and hyposmotic (210 mOsm) external solutions under conditions where Na+, K+ and Ca2+ currents were minimized. To measure cell volume, isolated chondrocytes were allowed to settle onto the experimental chamber mounted on an inverted microscope. The chamber was continuously perfused with bathing solutions at the rate of 2-3 ml min-1. Microscope images of chondrocytes were recorded with a CCD digital camera equipped with DS-L2 control unit at 2560×1592 resolution every 1 min, and the area of the cell image was measured using Image-J public domain software.

Results: The isolated chondrocytes exhibited a RVD during sustained exposure to hyposmotic solution, which was mostly inhibited by the ICl,swell blocker DCPIB (4-(2-buty1-6,7-dichloro-2-cyclopentyl-indan-1-on-5-y1) oxobutyric acid) at 20 μM. Exposure to a hyposmotic solution activated ICl,swell, which was also largely inhibited by 20 μM DCPIB. Activation of ICl,swell was significantly reduced by the protein tyrosine kinase (PTK) inhibitor genistein (30 μM) but was scarcely affected by its inactive analogue daidzein (30 μM). Intracellular application of protein tyrosine phosphatase (PTP) inhibitor sodium orthovanadate (250 and 500 μM) resulted in a gradual activation of a Cl- current even in isosmotic solutions. This Cl- current was almost completely inhibited by DIDS (4,4’-disothiocyanostilbene-2,2’-disulphonic acid, 500 μM) and was also largely suppressed by exposure to hyposmotic solution, thus indicating a close similarity to ICl,swell. Pretreatment of chondrocytes with genistein significantly prevented the activation of this Cl- current. It could explain the downregulated proliferation and the increased p27 expression observed on human osteoarthritic chondrocytes in the presence of Rhein recently found by other groups.

Conclusions: Results provide evidence to indicate that activation of ICl,swell is involved in RVD and is facilitated by tyrosine phosphorylation in isolated rabbit chondrocytes.

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NON-INVASIVE TREATMENT OF OSTEOARTHRITIS OF THE KNEE WITH QUANTUM MAGNETIC RESONANCE

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Objectives: To study the effects of Quantum Magnetic Resonance (QMR) beams on clinical and functional parameters and cartilage thickness of osteoarthritic knee joints.

Background of QMR Therapy: Quantum Magnetic Resonance Therapy™ utilizes highly complex quantum electromagnetic beams in the sub-radio and near-radio frequency spectrum. The beams can be precisely controlled and focused onto tissues therein generating streaming voltage potentials. In osteoarthritis, this flow in the joint causes forced movement of hydrogen protons in the extra cellular matrix (ECM) due to the alteration in QMR spin in the hydrogen atoms and stimulates the chondrocytes.

Methods: After the publication (2004) of the results of a pilot study on the effect of QMR on 35 patients with osteoarthritis, 300 more patients with osteoarthritis have been treated with QMR as a follow up study. The patients were assessed on the basis of well-established internationally recognized knee society rating system and scores prior to immediately after treatment and further after three months were computed. In addition, MRI of the knees was done using standard protocol before and after three months of treatment with a view to measure the changes in the cartilage thickness in the knee joints.

Results: By the end of the treatment the patients could walk up to five times more than before treatment without any difficulty. MRI showed a remarkable increase in the thickness of the cartilage in the knee joint at three months, from 0.67mm (±0.02) pre-treatment to 3.25mm (±0.74) in left knee, and 0.66mm (±0.02) to 2.71mm (±0.58) in the right knee joint (p<0.001).

Conclusions: QMR Therapy™ has now been successfully employed to induce mitotic activity in the fully differentiated chondrocytes. It is also seen that therapeutic exposure to quantum magnetic resonance beams is effective in ameliorating the signs and symptoms of OA, and inducing regenerative activity in the chondrocytes as evidenced by an increase in the cartilage thickness. QMR Therapy™ is a method for regeneration of cartilage and is effective for treatment of osteoarthritis of the knee joint.