sulting from lack of homogentisate 1,2 dioxygenase (HGD), the enzyme responsible for the breakdown of homogentisic acid (HGA). HGA accumulates in body tissues resulting in ochronosis, the deposition of pigmented polymers in collagenous tissues, primarily the articular cartilage of the weight bearing joints. Over time, ochronosis leads to severe, early onset joint degeneration presenting clinically as osteoarthritis. The aim of this study was to use light and scanning electron microscopy (SEM) to elucidate the initiation and progression of pigmentation and the consequent osteoarthropathy in AKU.

Methods: Tissues were collected from patients undergoing joint replacement surgery for alkaptonuric osteoarthropathy (n=14). Samples were processed as follows:- histology to identify pigment deposition, topographical 3D SEM to observe the trabecular bone network and quantitative back scattered electron SEM (qBSE-SEM) to determine the mineral content of cartilage and bone.

Results: Histological examination of articular cartilage revealed significant variation in the extent of pigment between samples, and regionally within samples. Initial pigmentation was associated with single chondrocytes near the tidemark and was present intracellularly, and in the lacunae and territorial matrix. From there, deposition of pigment progressed to the interterritorial matrix in the deep zones and then towards the articular surface. Once the hyaline cartilage became extensively pigmented, there was aggressive remodeling of the calcified cartilage and underlying bone. Eventually this led to complete loss of the subchondral plate leaving non-calcified, pigmented hyaline cartilage in contact with the trabecular bone network. In addition, poorly or non-mineralised bone was present in the trabecular network in closest proximity to the pigmented hyaline articular cartilage. Pigmentation was not detected in mineralised bone matrix, however osteocytes, osteoclasts and osteoblasts all displayed intracellular pigmentation, along with the canalicular network and osteocyte lacunae. Osteoclasts were also seen phagocytosing pigmented osteocytes.

Conclusions: Calcified cartilage and subchondral bone appear to play key roles in the pathogenesis of osteoarthropathy in AKU. Our findings indicate that cartilage matrix is initially protected from ochronosis. Early pigmentation occurs deep in the cartilage, possibly in response to mechanical factors and biochemical injury. HGA thus appears to be an endogenous marker of osteoarthritic changes. Once the initial pigment is deposited, the biomechanical and functional properties of cartilage are further altered leading to additional damage, more pigmentation and a downstream spiral of tissue destruction producing a more rapid, severe and earlier onset of osteoarthropathy than is seen in typical OA. Extreme phenotypes in monogenic diseases can help elucidate the molecular pathogenesis of more common disorders. We believe that the initiation and rapid progression of ochronosis and in osteoarthropathy in AKU may also help elucidate the molecular pathology of OA.

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EXPRESSSION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID (TRPV) CHANNELS IN DIFFERENT PASSAGES OF EQUINE ARTICULAR CHONDROCYTES

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Purpose: Chondrocytes are highly mechanosensitive cells responsible for the production and maintenance of the extracellular matrix (ECM) in articular cartilage. ECM turnover is influenced by the mechanical and osmotic factors and calcium signalling is a key component of mechanical responsiveness in these cells. The mammalian Transient Receptor Potential Vanilloid (TRPV) subfamily consists of six members. TRPV1-4 are temperature sensitive calcium-permeable, non-selective cation channels whereas TRPV5 and TRPV6 show high selectivity for calcium over other cations. Recent studies have demonstrated the presence of functional TRPV channels in porcine articular cartilage. The purpose of this study was to investigate the effect of time in culture and passage on expression of TRPV4, TRPV5 and TRPV6 in equine articular chondrocytes.

Methods: Artricular cartilage was obtained from weight-bearing regions of equine metacarpophalangeal. Chondrocytes were enzymatically isolated and cells were cultivated in low glucose Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 2% penicillin-streptomycin and 10% Fetal Cali Serum (FCS). Polyclonal antibodies raised against TRPV4, TRPV5 and TRPV6 was used to compare the expression of these channels in lysates from first expansion cells (P0) and cells from passages 1-3 (P1, P2 and P3) by western blotting. Densitometry was carried out using Image J (Image Processing and Analysis in Java; http://rsb.info.nih.gov/ij/).

Results: Western blotting confirmed TRPV4, TRPV5 and TRPV6 expression in all passages of equine chondrocytes. The corresponding immuno-reactive bands were calculated to be approximately 98 kDa for TRPV4 and 83 kDa for both TRPV5 and TRPV6. TRPV5 and TRPV6 were upregulated with time and cell passage in culture (Figure 1).

Conclusions: This study confirmed the presence of TRPV4, TRPV5 and TRPV6 in equine articular chondrocytes up to passage 3. Furthermore, the TRPV5 and TRPV6 were upregulated with time and cell passage in culture suggesting that a shift in the phenotype of the cells in monolayer culture can alter the expression of these channels. The results presented suggest that several TRPV channels may be involved in calcium signalling and homeostasis in chondrocytes. A better understanding of the interactions between calcium, TRPV channels and mechanical or osmotic changes in the joint will hopefully provide new insights into cartilage mechanotransduction and the pathophysiology of osteoarthritis.

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PREDNISOLONE INDUCES A CATABOLIC EFFECT ON BONE FORMATION; BUT INHIBITS CYTOKINE-INDUCED CARTILAGE DEGRADATION AND SUBCHONDRAL BONE RESORPTION, INDICATING ANTI-CATABOLIC EFFECTS

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Purpose: Glucocorticoids are beneficial in the treatment of inflammatory and immune disorders, but have also been shown to protect cartilage when treating for osteoarthritis. Unfortunately, prolonged glucocorticoid therapy, in high doses, has been associated with bone loss, resulting in severe osteoporosis. These conflicting effects by glucocorticoids on bone and cartilage may result from a miscommunication between the bone and cartilage cells. We investigated the effect of the glucocorticoid, prednisolone, in an ex vivo murine femoral head model, which allowed the interactions between osteoblasts, osteoclasts and chondrocytes.

Methods: Femoral heads from 12-week-old female mice were isolated and cultured for 21 days in serum-free media in the absence or presence of prednisolone [10 μM], OSM [10 ng/ml] + TNF-α [20 ng/ml]
(catabolic stimulation), prednisolone + OSM + TNF-α, or IGF-I [100 nM]
(anabolic stimulation). The conditioned medium from days 7 and 14 were
measured and examined for biochemical markers of bone and cartilage
degradation/formation.

**Results:** The cartilage degradation increased ~25-fold (day 7) and ~20-fold
(day 14) when stimulated with the cytokines, OSM + TNF-α, compared
with the non-stimulated vehicles (P<0.001). Additional stimulation with
prednisolone inhibited the catabolic effects by OSM + TNF-α at both days
(P<0.001). The bone resorption also increased ~5.5-fold (day 7) when
stimulated with OSM + TNF-α, compared with the vehicle (P<0.001). Again,
additional stimulation with prednisolone inhibited the catabolic effect on
bone resorption by OSM + TNF-α (P<0.005). No resorption was detected for
any treatments at day 14.

Prednisolone stimulation did not affect the cartilage formation. However,
IGF-I stimulation increased cartilage formation by ~2.7-fold (day 14) com-
pared with the vehicle (P<0.001). OSM + TNF-α stimulation inhibited all
cartilage formation (P<0.001).

The bone formation was not affected by prednisolone stimulation at day 7.
However, at day 14, prednisolone decreased the bone formation by ~8.9
fold compared with vehicle (P<0.001). IGF-I stimulation increased the bone
formation ~2.5-fold (day 7) and ~2.6-fold (day 14), compared with the
vehicles (P<0.001). OSM + TNF-α stimulation decreased bone formation by
~3.5-fold (day 7) compared with vehicle, and completely inhibited the
formation at day 14 (P<0.001).

**Conclusions:** Prednisolone inhibits cytokine-induced cartilage degradation
and bone resorption, indicating an anti-catabolic effect on chondrocytes
and osteoclasts. Cartilage formation is not affected by prednisolone, which
supports the literature suggesting that glucocorticoids protect cartilage.
However, bone formation decreased when stimulated with prednisolone,
suggesting that prednisolone catabolically affects the osteoblasts. This
overall indicates that prednisolone decreases bone turnover and protects
cartilage against degradation.

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**HOW GOOD IS ALLOGRAFT CARTILAGE?**

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**Purpose:** The purpose of this study was to evaluate the effect of pro-
inflammatory cytokines on the metabolism and survival of chondrocytes
obtained from osteochondral allografts (OA) as compared to fresh chondro-
cytes obtained from human organ donors.

**Methods:** Nine OA specimens (hemicondyle), previously refrigerated for
14-28 days, were collected at the time of surgery and six fresh hemi-
condytes were obtained from normal donors within 24 hours of death
through the Gift of Hope Organ and Tissue Donor Network. 4mm tissue
explants from OA and fresh cartilage were cultured in media containing
10% fetal bovine serum and divided into the following treatment groups:
1) culture control (serum only), 2) IL-1β (0.1 ng/ml), 3) IL-6 (3ng/ml), 4)
IL-1β (0.1 ng/ml) + IL-6 (3ng/ml), 5) IL-1β (10 ng/ml), 6) IL-1β (10 ng/ml)
+ IL-6 (3ng/ml). IL-6 soluble receptor (5ng/ml) was added to all cultures
containing IL-6. Doses of cytokines were determined based on synovial
fluid levels in patients undergoing allograft transplantation. Treatment was
administered every other day. Tissue and media were collected on days 0,
2, 7, and 14. Cell viability (live/dead assay), apoptosis (Tunel assay), his-
tological appearance with Safranin O staining, proteoglycan (PG) synthesis
and content (normalized to wet weight) were used to analyze cartilage
survival and metabolism.

**Results:** At day zero, the viability of OA chondrocytes was 24% lower and
they contained 29% more apoptotic cells than fresh chondrocytes (p<0.05).
Treatment with cytokines did not further induce cell death or apoptosis
in OA cartilage. However, in fresh cartilage, treatment with high dose IL-1
(10ng/ml) alone or in combination with IL-6 showed a significant decrease
in chondrocyte viability by day 14 (p<0.05 and p<0.05) correspondingly
as well as a significant increase in the number of apoptotic cells (p<0.05
and p<0.05) when compared to day 0 control. Fresh chondrocytes showed
2.5 times greater PG synthesis (p<0.05) and only half as much release of
PGs into the media (p<0.01) when compared to OA chondrocytes at day 0.
However, fresh chondrocytes were more sensitive to cytokine treatments:
by day 14 high dose IL-1 alone or combined with IL-6 inhibited PG
synthesis by more than 8-fold (p<0.02) vs 4-fold in OA cells (p<0.02)
and induced higher PG release, which resulted in higher Mankin score for fresh
cartilage (1.3 vs 3.0, p<0.04).

**Conclusions:** The viability and metabolism of OA cartilage is significantly
lower than fresh cartilage; however, OA cartilage is more resistant to
cytokine treatment. The reduced metabolism and sensitivity of OA cartilage
might explain allograft survival in an acute inflammatory environment (at
least short-term) suggesting that OA cartilage may act more like a scaffold
rather than an active tissue. Further long-term studies are warranted in
order to understand the performance of OA cartilage.

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**MECHANISMS OF UROCORTIN FAMILY PEPTIDE MEDIATED
CHONDROPROTECTION**

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**Purpose:** It is increasingly recognised that the overproduction of mediators
such as Nitric Oxide (NO) in Osteoarthritic cartilage contributes to the
disease pathology partly by inducing chondrocyte apoptosis via the mi-
tochondrial pathway. Therefore, agents which protect against NO-induced
mitochondrial injury may have therapeutic potential in Osteoarthritis (OA).
We have previously shown that the human chondrocyte cell line C-20/A4
produces members of the Urocortin (Ucn) family of peptides in response
pro-apoptotic insults and that exogenous administration of all of these
peptides protects chondrocytes from NO induced apoptosis with Ucn 1 being
the most potent. The purpose of this study was to elucidate poten-
tial mechanisms of action and signalling pathways through which these
peptides may exert their effects.

**Methods:** C-20/A4 cells were maintained in monolayer culture in a Dul-
becco’s MEM (DMEM) - based medium containing 10% foetal calf serum
(FCS) at 37 °C and 5% CO2. Prior to treatment, cells were transferred to
T25 tissue culture flasks, allowed to reach approx. 80% confluency and then
serum-starved for 24 hours in DMEM based medium containing 1% FCS and
then treated with 1mM SNAP (NO donor) for 6 hours. CRH receptor
and KATP channel subunit expression were analysed by RT-PCR, p42/44 MAPK
activation was studied by western blotting with antibodies specific for total
and phosphorylated p42/44. Apoptotic cell death was assessed by Annexin
V/PI binding and TUNEL assay with necrosis assessed by LDH release

**Results:** The addition of Ucn1 to SNAP treated C-20/A4 cells provides
protection against apoptosis which is abrogated by the addition of the
CRFR antagonist aUcHR suggesting the presence of CRH receptors on these
cells the expression of which was confirmed by RT-PCR demonstrating
the expression of both corticotropin releasing factor receptors, CRFR1 and
CRFR2 mRNA, specifically the CRFR1α and CRFR2β splice variants. Further
RT-PCR studies demonstrate the expression of both the Kir and SUR
subunits of the mitochondrial ATP sensitive inwardly rectifying potassium
channel (Kir and SUR) which were western blotting studies indicating an ucn mediated increase
in p42/44 MAPK activation, representing possible mechanisms for Ucn
mediated chondroprotection.

**Conclusions:** Studies with aUcHR and RT-PCR analysis for the expression of
CRF receptors indicate the presence of two active forms of the CRF recep-
tors, CRFR1α and CRFR2β in C-20/A4 chondrocytes. The presence of these
receptors provides a putative cell surface binding site for Ucn family mem-