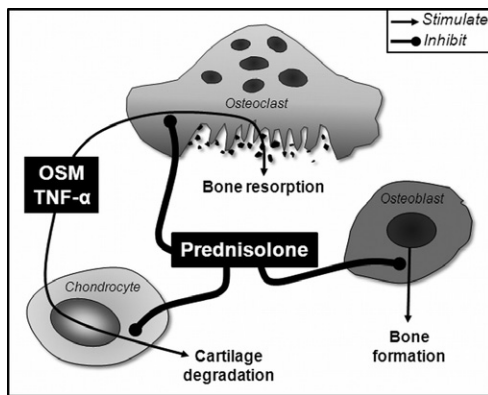


(catabolic stimulation), prednisolone + OSM + TNF- α , or IGF-I [100 nM] (anabolic stimulation). The conditioned medium from days 7 and 14 were measured and examined for biochemical markers of bone and cartilage degradation/formation.

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 ($P < 0.01$). 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 ($P < 0.05$). 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) compared 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 chondrocytes 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 hemicondyles 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-1 β (0.1 ng/ml), 3) IL-6 (3ng/ml), 4) IL-1 β (0.1 ng/ml) + IL-6 (3ng/ml), 5) IL-1 β (10 ng/ml), 6) IL-1 β (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), histological 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 mitochondrial 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 to pro-apoptotic insults and that exogenous administration of all of these peptides protects chondrocytes from NO induced apoptosis with Ucn I being the most potent. The purpose of this study was to elucidate potential 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 Dulbecco'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 α hCRH 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 CRFR1 α and CRFR2 β 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 with western blotting studies indicating a Ucn mediated increase in p42/44 MAPK activation, representing possible mechanisms for Ucn mediated chondroprotection.

Conclusions: Studies with α hCRH and RT-PCR analysis for the expression of CRFR receptors indicate the presence of two active forms of the CRF receptors, CRFR1 α and CRFR2 β on C-20/A4 chondrocytes. The presence of these receptors provides a putative cell surface binding site for Ucn family mem-