HUMAN AMNIOTIC MEMBRANE TO REPAIR
OSTEOARTHRITIC CARTILAGE

S.M. Díaz-Prado1, E. Muñoz2, E. Rendal3, T. Hermida-Gómez2, I.M. Fuentes-Boquete1, M.C. Arufe-Gondal1, P. Figueras2, M.J. Sánchez-Dopico1, F.J. De Toro1, F. Galdó1, F.J. Blanco1, 1Osteoarthritis and
Aging Research Laboratory, Biomedical Research Center. CIBER-BBN, Cellular Therapy Area. INIBIC – Hospital Universitario Juan Canalejo. University of A Coruña, Coruña, SPAIN, 2Osteoarticular and Aging Research Laboratory. Biomedical Research Center. INIBIC-Hospital Universitario Juan Canalejo., Coruña, SPAIN, 3CIBER-BBN, Cellular Therapy Area. Coruña, SPAIN, 4Osteoarticular and Aging Research Laboratory. Biomedical Research Center. CIBER-BBN, Cellular Therapy Area. INIBIC – Hospital Universitario Juan Canalejo., Coruña, SPAIN

Purpose: Human amniotic membrane is a highly abundant and easily accessible tissue that may potentially be an important chondrocyte carrier for cartilage regeneration in vivo.

Objective: Study the feasibility of cryopreserved human amniotic membrane as a chondrocyte carrier for in vitro repair human articular cartilage injuries.

Methods: Patches (6-6 cm) of cryopreserved human amniotic membrane were extended into a culture dish. Then human chondrocytes (5×10^6 cells) were cultured over the basal face of the amniotic membrane for 3-4 weeks until 80-90% confluence. These membranes were cut into pieces of 0.7 × 0.7 cm, and each of them was placed over a chondral defect (6 mm in diameter) of osteoarthritic cartilage with the basal layer facing the defect. The implants were cultured for 16 weeks. Evaluation of in vitro chondrocytes culture over the human amniotic membrane and repair, in in vitro models, of injured articular cartilage was carried out using histological (hematoxylin-eosin) and immunohistochemical (collagen type I and II) stainings.

Results: Chondrocytes culture over the human amniotic membrane showed that cells grew over the basal layer, but did not penetrate on the epithelial side. Histological techniques demonstrated that collagen type II but not type I was expressed in the chondrocytes culture on amniotic membrane. Valuation of repair, in 13 in vitro repair models, showed the formation of a new tissue on OA cartilage. Integration of new tissue with OA cartilage was excellent. Repair tissue expressed type II collagen in 100% of experiments, however collagen type I was present in 54% of cases.

Conclusions: These results indicated that cryopreserved amniotic membrane can be used as support for chondrocytes proliferation in cell therapy to repair OA cartilage.


GLUCOSAMINE PREVENTS AND NF-κB INHIBITORS REDUCE THE CYTOKINE-INDUCED EXPRESSION OF IL-1 IN NORMAL HUMAN ARTICULAR CHONDROCYTES IN VITRO

C.H. Lee, M. Glaysho, H.I. Roach. University of Southampton, Southampton, UNITED KINGDOM

Purpose: Glucosamine (GlcN) supplements are taken by millions of osteoarthritic (OA) patients to alleviate their symptoms and several large-scale studies have shown a benefit in slowing the progression of the disease. However, the molecular bases for the beneficial effect of GlcN are only beginning to be unravelled. Our aims were to determine whether glucosamine, added to the medium of cultured non-OA chondrocytes, affected the cytokine-induced expression of IL-1β, and to identify the potential mechanisms involved in the cytokine-induced expression of IL-1β.

Methods: Non-OA chondrocytes were isolated by sequential trypsin, hyaluronidase and collagenase digestion from osteoporotic patients who had sustained a fracture of the neck of femur. Previous studies had shown that these primary chondrocytes are suitable cell culture models, because the cells express chondrocytic genes, such as collagen types II, IX and XI, but do not express IL-1β. Cells were maintained in MEM with 5%FCS. Passage 2 cells were divided into the following experimental groups (n = 4 for each group): (i) control culture with no addition; (ii) culture with GlcN alone (2 mM); (iii) culture with TNF-α/OSM (10 ng/ml and 10 μM) and (iv) TNF-α/OSM + GlcN. In a further serie, non-OA chondrocytes were cultured with TNF-α/OSM in presence of two compounds that inhibited the translocation of NF-κB to the nucleus. The first was NF-kB p65 (Ser 276) inhibitory peptide (50 μM) from Imgenex, the second was (E)-2-Fluoro-4'-methoxystilbene (0.2 μM), an analogue of Resveratrol from Merck. Media were changed twice-weekly, when the relevant factors were also added to II, II and IV. Cells were cultured until confluence (2–3 weeks) and semi-quantitative RT-PCR was carried out.

Results: The expression of IL-1β was found in control cultures, with GlcN alone or with the NF-kB inhibitor alone. As expected, TNF-α/OSM induced expression of IL-1β in all cultures. However, the presence of GlcN completely prevented the cytokine-induced expression of IL-1β, as indicated by absence of the PCR band. Presence of the NF-kB transcription inhibitors reduced IL-1β expression, but did not abolish it completely, as suggested by reduced intensity of the PCR band.

Conclusions: The present studies demonstrate that glucosamine has the capacity to inhibit the cytokine-induced expression of IL-1β. The glucosamine effect was greater than the effect of interfering with the NF-kB pathway of signal transduction. GlcN is a basic structural unit of chondroitin sulphate and hyaluronan of cartilage proteoglycan aggregates. Hence its beneficial effects were initially suggested to be due to stimulation of GAG synthesis, but the evidence for this mechanism is so far limited. Other studies have shown chondroprotective effects in vitro that GlcN promoted the chondrogenic phenotype and inhibited MMP-13 expression. IL-1β is thought to play a major role in the progression of osteoarthritis, since IL-1β increases the expression of cartilage-degrading proteases. The present study suggests that GlcN treatment may have beneficial effects through prevention of IL-1β expression, which, in turn, might also reduce expression if its down-stream target genes. However, these are in vitro observations and need to be confirmed by in vivo studies.

THE SYNERGISTIC DRUG COMBINATION CRX-102 INHIBITS MATRIX METALLOPROTEINASE EXPRESSION IN MACROPHAGES AND HUMAN OSTEOARTHRITIC CHONDROCYTES

C.C. Fraser1, Y. Wang1, J. Lehar1, E. Lee2, K. McCabe2, S. O'Brien2, S. Kilfeather2, A. Boris1, G.R. Zimmermann2, CombinatoRx Pharmaceuticals, Cambridge, MA, USA, 2Aeirtec Ltd, Newcastle Upon Tyne, UNITED KINGDOM

Purpose: Glucocorticoids are therapeutic anti-inflammatory drugs, but are not routinely used to treat osteoarthritis (OA) due to potential side effects. In a proof-of-concept hand OA clinical trial, oral dosing with CRX-102, which is a combination of the cardiovascular drug dipyridamole (Dp) and very low-dose prednisolone (Pd), resulted in a significant reduction in tender and swollen joint counts, suggesting that the combination may modify disease progression. We have previously shown that Dp synergizes with Pd to inhibit inflammation in vitro and in vivo. This synergy allows a much lower dose of Pd to be used to inhibit inflammation, without the side effects associated with glucocorticoids. To determine if Dp and Pd synergize to inhibit matrix metalloproteinase (MMP) expression, we studied LPS-stimulated macrophages and IL-1α stimulated normal human chondrocytes. Additionally, we evaluated the activity of the combination on chondrocytes from human osteoarthritic joints ex vivo. The purpose of this research was to characterize the effect of the combination of Dp and Pd on cells and molecules relevant to the progression of OA.

Methods and Results: Transcriptional profiling (TP) of LPS stimulated macrophages revealed that ADAMTS4 was up-regulated (~20-fold) in response to LPS. Dp (10 μM) in combination with Pd (0.03 μM) inhibited ADAMTS4 expression (67%), more than Dp (47%) or Pd (30%) alone. MMPs 3, 10, 12, 13 and 14 were up-regulated in response to LPS, and like ADAMTS4, were maximally inhibited by the combination of Dp and Pd. Interestingly, analysis of supernatants from stimulated macrophages cultured in serially diluted combinations of Pd and Dp identified strong synergistic inhibition of MMP2 and MMP9. The MAP kinase pathway was therefore played a role in MMP expression. Western blot analysis showed the combination of Pd and Dp inhibited phosphorylation of p38 and JNK but not ERK, while Pd or Dp alone did not. To further characterize the combination on OA-relevant cell types, inhibition of MMPs and other inflammatory mediators by Dp and Pd was measured in II-1α stimulated macrophages. Pd demonstrated differential partial inhibition of MMP-3, II-6, MCP-1 and GRO-α. Dp demonstrated complete inhibition of MMP-3 and MCP-1, partial inhibition of GRO-α, and no inhibition of II-6. OA chondrocytes were isolated from osteoarthritic joint cartilage obtained from total knee replacement surgery, and the ability of Dp and Pd to suppress II-1 induced production of pro-inflammatory mediators including MMPs was assessed. Dp and Pd alone and in combination suppressed production of TNFα, MCP-1, IL-1β, MIP-1β, RANTES, and MIP-3. To study the potential of the combination to inhibit MPP production in macrophages and decrease expression in human