P206

RHEIN, THE METABOLITE OF DIACERHEIN, MODULATES PROLIFERATION OF ARTICULAR CHONDROCYTES AND SYNOVIOCYTES

F. Legenda1, S. Leclercq2, F. Domagala3, J.-P. Pujo1, H. Ficheux4
1 Faculty of Medicine, Caen, France, 2 Clinic Saint-Martin, Caen, France, 3 NEGMA-LERADS Laboratories, 78771 Magny-Les-Hameaux, France

Purpose: To determine the effects of pharmacologically relevant concentrations of Rhein, the metabolite of Diacerein, on cell proliferation rate of both human chondrocytes and synoviocytes. Methods: Human articular chondrocytes and synoviocytes were obtained from the knee or hankle of osteoarthritic patients. They were isolated by enzymatic procedure and cultured as monolayers in DMEM + 10% FCS, either in normal atmosphere (21% O2 for synoviocytes) or in hypoxia (5% O2 for chondrocytes), with 10^{-6}, 10^{-5}, and 10^{-4} M Rhein or the corresponding concentrations of its vehicle DMSO. [3H] thymidine incorporation was used to determine Rhein proliferative effects at 24 h, 48 h and one week incubation times. Cytotoxicity of the drug was also assayed with a non radioactive assay kit. Nuclear extracts were prepared and used to detect variation of cell cycle proteins by Western blotting. Moreover, Rhein effects on apoptosis were investigated through measurement of caspase activity.

Results: Rhein inhibited the proliferative rate of both chondrocytes and synoviocytes in a dose-dependent manner, with significant decrease of thymidine labeling for 10^{-3} M Rhein (by approximately two-fold) and for 10^{-4} M Rhein (around 5-6 fold). In the same experimental conditions, no cytotoxicity of the drug was observed as compared to its vehicle DMSO. Western blots showed that Rhein modulates expression of cell cycle proteins: Rhein at 10^{-4} M decreased p27 expression, but not that p21, nor cyclin D1 expression.

Conclusions: Previous results indicated that Rhein could inhibit proliferation of both synoviocytes and chondrocytes. Thus, by its antiproliferative properties, this drug may decrease the development of the inflammatory synovial tissue that accompanies both OA and RA joint pathologies. Both its anti-catabolic and anti-proliferative effects may explain its value for the treatment of joint diseases.

P207

GLUCOSAMINE REDUCES CATABOLIC AND ANABOLIC PROCESSES IN CHONDROCYTES; INDICATIONS FOR INTERFERENCE WITH INTRACELLULAR ENERGY MANAGEMENT

Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Purpose: Glucosamine (GlcN) is increasingly being used for its claimed structure modifying effects in osteoarthritis. Despite this, the debate on its exact mechanism of action still continues. In vitro studies have reported conflicting results, probably due to different culture conditions in these various studies. The aim of our study was to determine the effects of GlcN under anabolic and catabolic culture conditions.

Methods: Chondrocytes from 6-12 month old calf MCP joints were seeded in alginate beads and cultured in low glucose D-MEM with 10% FCS and 25 μg/ml ascorbic acid 2-phosphate. To evaluate the effect under anabolic conditions 5 mM glucosamine hydrochloride (GlcN-HCl) or N-acetyl-glucosamine (N-Ac-GlcN) were added to the cultures for 14 days after a 3-day pre-culture period. For the evaluation of the effect of GlcN under catabolic conditions, alginate beads were treated with 10 ng/ml recombinant human IL-1β on day 8-10, in combination with GlcN-HCl 5 mM from day 6-10. Following this treatment, culturing was continued for 7 more days. A no supplement condition and GlcN-HCl pre-treatment only condition were used as controls. Beads were harvested for analyses of DNA and GAG content at various time points.

Results: The no supplements control and 5 mM N-Ac-GlcN showed an increase of GAG content with time during the first 17 days of culture. Addition of 5 mM GlcN-HCl showed a statistically significant reduced GAG production. The amount of DNA per bead showed a similar trend. As expected, addition of IL-1β also led to inhibition of GAG content. Pre-treatment with GlcN-HCl, reduced the effect of IL-1β.

Conclusions: This study indicates that GlcN-HCl inhibits anabolic and the catabolic process. A possible explanation might be a direct competition between glucose and GlcN-HCl. At first on entering the cell, utilizing the same transporter system and secondly intracellular, since glucose and GlcN are both phosphorylated by glucokinase. Phosphorylated GlcN in turn is an allosteric inhibitor of glucokinase, resulting in extra negative feedback. The result of this competition might be that less glucose-6-P enters the glycolysis, leading to a depletion of intracellular ATP. This eventually results in less energy for all kinds of intracellular processes, like extracellular matrix production and breakdown. This competition hypothesis was tested by adding more glucose to the medium and that resulted in normalization of GAG synthesis. An explanation why N-Ac-GlcN does not inhibit matrix synthesis, may be that it is phosphorylated by a different enzyme and thus no competition with glucose occurs, while the phosphorylated product can be used for GAG synthesis as well. Based on our results, we propose that the claimed structure modifying effects of glucosamine might be based on interference with the energy household of chondrocytes.

P208

ACTIVATION BY IL-1 OF BOVINE CHONDROCYTES IN CULTURE WITHIN A 3D COLLAGEN-BASED SCAFFOLD AS AN IN VITRO MODEL TO STUDY THE EFFECT OF AVOCADO/SOYBEAN UNSAPONIFIABLES

D. Cortial1, C. Rousseau1, M.-C. Ronziere1, N. Piccardi2, P. Misika2, F. Malein-Gerin1, A.-M. Freyria1, D. Herbage1
1 IBCP UMR 5086, Lyon, France, 2 Laboratoires Expanscience, Epernon, France

Purpose: To determine the best protocol for the preparation of a tissue-engineered cartilage to investigate the potential antiarthritic and/or anti-osteoarthritic effects of drugs such as Avocado/Soybean Unsaponifiables (ASU).

Methods: Calf articular chondrocytes, seeded in collagen sponges, were grown in culture for up to 1 month. Cultures received interleukin (IL)-1β (ranging from 0.1 to 20 ng/ml) at day 14 for 1 to 3 days and/or ASU (ranging from 1 to 10 μg/ml) from day 2 every day or from day 14 for 1 to 3 days. Analyses of gene expression for extracellular matrix proteins, collagen-binding integrins, matrix metalloproteinases (MMPs), aggrecanases, TIMPs, IL-1Ra and IκBα were carried out using real-time polymerase chain reaction (PCR). Metalloproteinase activities were analysed in the culture medium using both zymography and fluorogenic peptide substrates.

Results: We selected a culture for 15 to 18 days with collagen sponges seeded with 107 chondrocytes showing a minimal cell proliferation, a maximal sulphated glycosaminoglycan (sGAG)
deposition and a high expression of COL2A1, aggrecan and the a10 integrin sub-unit and low expression of COL1A2 and the a11 integrin sub-unit. After treatment at day 14 with 1 ng/ml IL-1b, we observed at day 15 up-regulations of 450-fold for MMP-1, 60-fold for MMP-13, 54-fold for ADAMTS-4 and MMP-3 and 10-fold for ADAMTS-5 and IL-1Ra. Down-regulations of 2.5-fold for COL2A1 and aggrecan were observed only at day 17. At the protein level a dose-dependent increase of total MMP-1 and MMP-13 was noted with less than 15% in the active form. The effect of a 1 day treatment with 1 ng/ml IL-1b (day 14/15) disappears completely after 3 days (day 18).

In this culture model, in the absence of IL-1b, addition of ASU was without effect on the chondrocyte metabolism. However, treatment with ASU (10 μg/ml) from day 14 decreased significantly at day 16 the level of expression of MMP-3 and MMP-13 activated by a 1 day treatment (day 14/15) with 1 ng/ml IL-1b.

Conclusions: This in vitro model of chondrocyte culture in three dimensional (3D) seems well adapted to investigate the responses of these cells to inflammatory cytokines and to evaluate the potential anti-inflammatory effects of drugs. Under the conditions selected, chondrocytes maintained a cartilage phenotype and expressed a catabolic profile when stimulated by IL-1b. ASU were able to partially counteract this effect demonstrating their potential to reduce the deleterious effect of IL-1 reported in OA.

P209
EXPOSURE OF HUMAN CARTILAGE TISSUE TO LOW CONCENTRATIONS OF BLOOD FOR A SHORT PERIOD OF TIME LEADS TO PROLONGED CARTILAGE DAMAGE; AN IN VITRO STUDY
N.W. Jansen1, G. Roosendaal1, J.W. Bijlsma1, J. DeGroot2, F.P. Lafeber1
1UMC Utrecht, Utrecht, The Netherlands, 2TNO, Leiden, The Netherlands

Purpose: Exposure of cartilage to blood as a consequence of joint bleeds leads to cartilage damage. In the present study we determined the thresholds for the exposure time and concentration that lead to prolonged cartilage damage caused by exposure of cartilage to blood. This information may be particularly useful in the discussion whether aspiration of blood from a joint after a haemarthrosis is indicated or not.

Methods: Healthy human articular cartilage tissue explants were cultured in the presence or absence of 50% v/v blood for 1, 2, 3 or 4 days or for four days in the presence of 0, 5, 10, 20, 30 or 50% v/v blood, both followed by a recovery period of 12 days. The effect of blood exposure on cartilage was determined by measuring matrix synthesis, release, and content, as well as MMP-activity.

Results: Exposure of cartilage to 50% v/v blood led to direct cartilage damage largely independent of the exposure time; a complete inhibition of matrix synthesis, an approximate doubling of the release, and increased MMP activity and a decreased proteoglycan content were observed. These effects persisted during recovery in the absence of blood but only after an initial exposure equal to or exceeding 2 days. Exposure of cartilage for 4 days to varying doses of blood led to concentration dependent cartilage damage. These effects were long lasting when the concentration equalled or exceeded 10% v/v blood. When a blood load of 10% v/v for 2 days was evaluated, the adverse effects on cartilage were also persisting. All data are statistically significant.

Conclusions: This study demonstrates that a 2-day exposure of cartilage to 10% v/v blood (in vitro) leads to prolonged impairment of joint cartilage. This suggests that aspiration of blood from a joint within 2 days should be considered in clinical practice to prevent blood induced joint damage.

P210
DEGENERATED CARTILAGE IS SLIGHTLY MORE PRONE TO BLOOD INDUCED CARTILAGE DAMAGE
N.W. Jansen, G. Roosendaal, J.W. Bijlsma, F.P. Lafeber
UMC Utrecht, Utrecht, The Netherlands

Purpose: Joint bleeds can occur after a joint trauma and are frequently seen in patients suffering from haemophilia. Even a limited number of these joint bleeds lead to severe joint destruction in later years. Currently the general opinion amongst physicians is that a few joint bleeds are acceptable. However, it could well be that degenerated cartilage that is impaired by previous joint bleeds or mechanical stress (e.g. osteoarthritic cartilage) is more susceptible to blood induced damage. When this holds true, this would implicate that specifically in case of degenerated cartilage, joint bleeds should be prevented to avoid joint destruction in later years. We therefore investigated whether degenerative and osteoarthritic cartilage are more susceptible to blood induced cartilage damage than healthy cartilage.

Methods: Healthy, degenerative and osteoarthritic human articular cartilage tissue explants were cultured in the presence or absence of 10% v/v blood for 2 days (a minimal blood load compared to a clinical joint haemorrhage), followed by a recovery period of 12 days. The effect of blood exposure to cartilage was determined by its effect on matrix synthesis, release, and content. Both the direct effects and the effects after the recovery period were determined.

Results: When healthy cartilage was exposed to blood, this resulted in a direct decrease of the matrix synthesis (-73%), and an increase in release of matrix components (+148%). These effects were still present after the recovery period and at that time proteoglycan content of the matrix was decreased (-18%). The degenerative and osteoarthritic cartilage had the characteristic decreased synthesis, increased release, and decreased content of proteoglycans. Release of proteoglycans after the recovery period was slightly but statistically significantly elevated for the degenerated cartilage compared to healthy cartilage (p<0.05). The other parameters were not significantly changed in degenerated cartilage compared to healthy cartilage.

Conclusions: This study demonstrates that degenerated and osteoarthritic cartilage are slightly more susceptible to the adverse effects of blood exposure than healthy cartilage. It might therefore be of importance to prevent joint bleeds in patients with degenerated cartilage due to e.g. osteoarthritis. Moreover, aspiration of blood in case of a joint haemorrhage is indicated under these conditions.

P211
A ROLE FOR APOPTOSIS IN THE ETIOPATHOGENESIS OF OSTEochondritis DISSEcans of the ANKle CARTILAGE
B. Grigolo1, L. De Franceschi1, G. Lisignoli1, L. Roseti1, R. Buda2, F. Vananni1, S. Giannini1, A. Facchin1
1Istituti Ortopedici Rizzoli, Bologna, Italy, 2Università degli Studi di Bologna, Bologna, Italy

Purpose: Osteochondritis dissecans (OCD) is a common disease with unknown aetiology which particularly affects young people. It is characterized by a separation of a cartilage fragment from the subchondral bone. Many hypotheses about its aetopathogenesis have been proposed, one of the most considerate, the start of a catabolic program following an initial traumatic event. The aim of the study was to highlight these mechanisms and to look for an apoptosis activity as one of a probable cause of the pathology development.