of pro-angiogenic factors by SC. In IL-1beta treated SC, CS increased the mRNA expression of the anti-angiogenic factors TSP-1 and VEGI ($p < 0.01$). TSP-2 was not affected by CS. The mRNA of anti-angiogenic factors Ang-2 and PF-4 were not detected in our culture conditions.

Conclusions: Synovium inflammation is associated with an imbalance between pro- and anti-angiogenic factors production. IL-1beta is a key inflammatory mediator capable of inducing this pro-angiogenic imbalance. CS trends to normalize the IL-1beta-induced angiogenic response in OA SC. This could constitute a new mechanism of action of this drug, modulating the molecular mechanisms underlying the synovium angiogenesis in OA. These results also contribute to understand the molecular mechanism of angiogenesis in OA, leading, in the future, to the development of new promising therapeutic agents.

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EXPLORATION OF POSSIBLE CATABOLIC FACTORS FOR CARTILAGE MATRIX IN OSTEOARTHRITIC SYNOVIAL FLUID

N. Fukui[1], Y. Ikeda[1], N. Tanaka[1], M. Wake[1], M. Ohmori[1], T. Yamaguchi[1], H. Furukawa[1], S. Touma[1], Y. Miyamoto[2], T. Tashiro[3], Y. Katsuragawa[4]
[1]Clinical Resarch Ctr., Natl. Hosp. Organization Sagamihara Hosp., Sagamihara, Kanagawa Pref, Japan; [2]Tokyo Metropolitan Geriatric Hosp., Itabashi, Tokyo, Japan; [3]JR Tokyo Gen. Hosp., Shinjyuku, Tokyo, Japan; [4]Natl. Ctr. for Global Hlth.and Med., Shinjyuku, Tokyo, Japan

Purpose: The exact mechanism for cartilage degeneration in osteoarthritis (OA) is not yet fully known. Although chondrocytes are considered to be most responsible, synovium could also play a certain role in the loss of cartilage. If synovium is indeed involved in cartilage degeneration, a catabolic factor(s) released from synovium may be found within the synovial fluid. In this study, we attempted to find a possible catabolic factor(s) for cartilage matrix in synovial fluid from knee OA patients.

Methods: *Synovial fluid analysis.* The institutional review boards approved the study project. Synovial fluid was collected from OA knees in the early stages of the disease (K/L grade I-III) (**Early OA knees**; n = 53), and those in the late stage (K/L grade IV) (Advanced OA knees; n = 32). Concentrations of type II collagen neo-epitope (CIINE) and aggrecan core protein (ACP), keratin sulfate (KS), and sulfated glucosaminoglycan (sGAG) were determined respectively to estimate the rate of matrix degeneration. To find possible catabolic factors for cartilage matrix, concentrations of 57 factors that could induce matrix degradation, such as MMPs, cytokines and chemokines, were determined in those samples by Luminex and ELISA. Correlation of concentration was investigated between those factors and the above four cartilage degenerative products.

Determination of the source of possible catabolic factors. Synovial tissues were obtained from 18 Advanced OA knees with medial involvement for gene expression analysis by qPCR. Control synovium and cartilage were obtained from 8 disease-free knees of age-matched donors. In those knees, synovium was harvetsted at medial, lateral, and patellofemoral compartment, respectively. Synovial tissues from another 8 OA knees were used to obtain synovial cells. The cells were isolated by enzymic digestion, and were separated into CD14+ and CD14- cells by magnetic sorting, and gene expression was analyzed respectively. Some synovial tissues were used for immunohistochemistry.

Measurement of collagenolytic activity of OA synovial fluid. Synovial fluid samples from 8 Early OA knees were incubated respectively with bovine type II collagen with or without MMP activation by APMA. After incubation at 37°C for 48 hours, the increase in CIINE concentration was determined by ELISA.

Results: Among the 57 factors evaluated, the concentrations of MMP-1 and 3 were significantly correlated with those of the cartilage degenerative products in both sample groups (Table 1).

The synovial fluid from OA knees contained considerable amounts of MMP-1 and MMP-3, and their concentrations were closely correlated. Consistently, the expression of *MMP-1* and *MMP-3* was highly enhanced in OA synovium compared with the control, and their expression levels were strongly correlated there. Although the tissues were obtained from medially involved knees, these MMPs were expressed at similar levels across the three compartments. The expression of those MMPs was more enhanced in CD14- synoviocytes than in CD14+ cells, which was consistent with the result of immunohistochemistry.

In the last experiment, the concentration of CIINE increased little when the synovial fluid was incubated with bovine type II collagen. However, when the MMPs in the fluid were activated by APMA, the CIINE concentration increased dramatically by the incubation in all 8 samples. This collagenolysis could be ascribed to MMP-1, as the synovial fluid contained little MMP-8 or 13.

Type II collagen degenerative product:

Table 1A. Correlation between CIINE and MMPs

	Early OA	Advanced OA
MMP-1	$p<0.01$	NS
MMP-3	$p<0.05$	$p<0.01$

Aggrecan degenerative products:

Table 1B. Correlation between ACP and MMPs

	Early OA	Advanced OA
MMP-1	$p<0.001$	NS
MMP-3	$p<0.01$	$p<0.05$

Conclusions: In OA joints, fibroblast-like synoviocytes may release MMP-1 and MMP-3 into synovial fluid, which could play a certain role in cartilage degeneration. MMPs (most likely MMP-1) in synovial fluid can degrade type II collagen rather efficiently when activated, even in the presence of TIMPs and α2-macroglobulin in the fluid.

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MITOCHONDRIAL DYSFUNCTION PROMOTES PRO-INFLAMMATORY RESPONSES IN CULTURED NORMAL HUMAN SYNOVIOCYTES

M.N. Valcárcel-Ares[1], R.R. Riveiro-Naveira[1], C. Vaamonde-Garcia[1], F.J. Blanco[2], M.J. López-Armada[1]
[1]Inflammation and Aging Unit - INIBIC - CHUAC, A Coruña, Spain; [2]Osteoarticular and Aging Res. Lab. - INIBIC - CHUAC, A Coruña, Spain

Purpose: Inflammation hypothesis of aging suggests that molecular inflammation could be an underpinning of aging and age-related diseases, as Rheumatoid Arthritis (RA). Besides, mitochondrial alterations may contribute to the progression of RA. In this study, we investigated the relationship between mitochondrial dysfunction and the in vitro expression of cyclooxigenase-2 (COX-2), prostaglandin E2 (PGE2), and interleukin-8 (IL-8) in normal human synoviocytes.

Method: Commonly used inhibitors to induce mitochondrial dysfunction were employed [Antimycin A (AA) and Oligomycin (OLI), inhibitors of complexes III and V of mitochondrial respiratory chain (MRC), respectively] in synoviocytes. IL-1β and TNF-α were used as inflammatory mediators. To identify possible pathways, we used N-acetylcysteine as ROS scavenger; the natural antioxidant resveratrol; and BAY as an inhibitor of NF-kB activation. COX-2 protein and mRNA expression and both PGE2 and IL-8 levels were analyzed by flow cytometry, RT-PCR and ELISA, respectively.

Results: First, we found that exposure of synoviocytes to AA and OLI significantly increased COX-2 protein expression in a time- and dose-dependent manner. The maximal response was observed at 6h with 20μg/ml AA and 25μg/ml OLI (3.0±0.3, n=4, $p<0.001$ and 6.5±1.9, n=9 $p<0.001$, respectively vs. basal 1) while the positive control, 1ng/ml IL-1β, expression was 12.6±3.4. Quantification of COX-2 mRNA expression at 4h showed similar results. PGE2 levels were also significantly increased when cells were stimulated for 9h with OLI. Second, we tested if mitochondrial dysfunction could modulate the response induced by sub-optimal doses of IL-1β (0.1ng/ml) on COX-2 protein expression and PGE2 production. We found that pre-treatment of synoviocytes with 10μg/ml OLI for 30 minutes significantly increases the IL-1β-induced COX-2 protein expression (32.5±2.5 OLI+IL-1β vs. 6.5±2.4 IL-1β and 4.5±1.7 OLI, n=3, $p<0.001$) and COX-2 mRNA expression. Similar results were obtained when PGE2 production was assessed (277.0±67.6 OLI+IL-1β vs. 15.4±2.6 IL-1β and 97.5±45.6 OLI, expressed as pg/50,000cells, n=3 duplicate, $p<0.005$). Equivalent results were observed when TNF-α or AA was employed. Besides, we also explored that OLI together with IL-1β significantly potentiates the expression of the pro-inflammatory chemokine IL-8 (2886.6±272.6 OLI+IL-1β vs. 1313.1±201.2 IL-1β and 62.4±15.4 OLI, expressed as pg/50,000cells, n=4, $p<0.001$).

Finally, we observed that this inflammatory response was counteracted by the addition of N-acetylcysteine, resveratrol or BAY, demonstrating the involvement of ROS and NF-kB in this process.

Conclusion: Besides of inducing a slight inflammatory response, mitochondrial respiratory activity dysfunction significantly potentiates the cytokine-induced inflammatory response in synoviocytes in relation with PGE2 and IL-8 release, via ROS production and NF-kB activation, contributing to chronic inflammation of synovial tissue in RA and aging joint. Resveratrol, a natural antioxidant, must be taken into account as a potential preventive or therapeutic agent in such conditions.

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PTH IMPROVES SYNOVITIS IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS PRECEDED BY OSTEOPOROSIS

L. Lugo[1], M. Bellido[1], O. Sánchez-Pernaute[1], J. Roman-Blas[1], S. Castañeda[2], R. Largo[1], G. Herrero-Beaumont[1]
[1]*Fundacion Jimenez Diaz, Madrid, Spain;* [2]*Hosp. de la Princesa, Madrid, Spain*

Purpose: Synovitis is a major component of OA, contributing to both patient symptoms and progression of cartilage damage. Intermittent PTH administration is associated with a decrease in musculoskeletal pain in osteoporosis patients. We have already observed that PTH prevents progression of cartilage damage by improving subchondral bone quality in experimental osteoarthritis preceded by osteoporosis in rabbits. In this study, we aimed to characterize the main histopathological features of synovitis compromise in our combined animal model, as well as to determine whether intermittent administration of PTH could decrease the synovial inflammatory response.
Methods: OP was induced in 14 (8 month-old; 3.5-4.8 kg body weight), skeletally mature female NZ rabbits, by ovariectomy (OVX) and intramuscular injections of methylprednisolone 1 mg/kg/day for 4 weeks (OP group). Ten age and gender-matched additional animals were used as controls (Healthy group). Surgical OA was simultaneously induced in the left knees of all the rabbits (OA and OPOA groups). Twelve weeks after OVX, iPTH (1-34) was s.c. administered to 6 OP rabbits (40 μg/day, 5 days/week) for 10 wks; right knees: OP+PTH group; left knees: OPOA+PTH group). All the animals were sacrificed 22 weeks after OVX. The synovium was analyzed by Krenn grading of chronic synovitis and immunohistochemically for RAM11 and T lymphocytes. ARN was isolated to perform quantitative RT-PCR for type I collagen. Articular cartilage damage was assessed by Mankin'score. Nonpair-wise statistical comparisons were done using Mann-Whitney test for the different variables studied and the correlations were performed with Spearman test (SPSS v. 15).
Results: There was a positive and significant correlation between the Mankin and the Krenn scores (r=0,473, p=0,035). The synovitis score was similar for OA or OPOA groups, however it was significant reduced in OPOA+PTH vs OPOA group ($p<0.05$). Regarding the histopathology, both, OA and OPOA showed an increase of hyperplasia, synovial stroma activation and inflammation ($p<0.05$). But, there was no difference between OA and OPOA groups in any outcome. Nevertheless, PTH treatment reduced significantly hyperplasia and synovial activation. Respect to RAM11 expression, we observed a tendency to rise in OA and OPOA vs Healthy, but PTH treatment does not affect the expression of RAM11. No presence for T limphocytes was detected in any case. Type I collagen expression in synovium was increased in OA and OPOA ($p<0.05$) and reduced after PTH treatment ($p<0.05$).
Conclusions: In our model of OA aggravated by previous OP, synovitis correlated with OA cartilage damage. OPOA synovitis is characterized by hyperplasia and synovial stroma activation and in a lesser extent by inflammatory infiltrates. Intermittent administration of PTH succeeded in improving the synovitis, by ameliorating both hyperplasia and fibrosis.

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IF1 AND BETA SUBUNITS ARE PRESENT ON THE EXTERNAL SURFACE OF CULTURED HUMAN MENISCI AND CARTILAGE CELLS

R. Aguilar-Gaytán[1], C. Jiménez-Díaz[1], M. Tuena de Gómez-Puyou[2], C. Ibarra-Ponce de León[1]
[1]*Natl. Inst. of Rehabilitation, Mexico, City, Mexico;* [2]*Cellular Physiology Inst., UNAM., Mexico, City, Mexico*

Purpose: The F_1-F_0-ATP synthase is an enzyme complex responsible for ATP synthesis in the mitochondrial inner membrane and is driven by oxidative phosphorylation. In the last years several reports have shown that some subunits as inhibitory protein (IF1) localize in the plasma membrane of neoplasic, endothelial, tumor cells, keratinocytes and hepatic. It has been shown that α and β subunits bind angiostatin on cell surface of endothelial cells (EC) and mediate down-regulation of endothelial cell proliferation and migration. Some data suggests that angiostatin inhibits vascularization by suppression of endothelial ATP metabolism, which, in turn, may regulate vascular physiology. The angiostatin blocks synthesis and hydrolysis of ATP on the surface of endothelial cells as well as EC differentiation to form tubes and IF1 did not. IF1 does not have the anti-angiogenic effect of angiostatin, but it may attenuate the anti-angiogenic response to angiostatin. Osteoarthritis (OA) is a disorder of articular cartilage characterized by degeneration, increased cell proliferation, proteinases, growth factors, cytokines, inflammatory mediators and vascularization known as vascular pathology. At this moment, no evidence about the ectopic presence of these subunits of ATP synthase on the surface of osteoarthritic chondrocytes. Therefore we studied the dentification of the ectopic presence of IF1 and β subunits on the membrane surface of human menisci and cartilage cells.
Methods: The identification of the IF1 and β subunits of ATP synthase were using flow cytometry and immunofluorescence detection on cultured human menisci (vascular region) and cartilage (avascular) cells, using specific antibodies. The tissues were obtained during surgery of the patients and digested with collagenase II and cultured under 5% CO_2 and 37°C.
Results: Human menisci and cartilage cells were incubated with specific antibodies against IF1 and β subunits to show a plasma membrane location. Histograms of flow cytometry data show the increase fluorescence relative to that observed when only the secondary antibody was used. These results were corroborated by fluorescent microscopy using the same antibodies.
Conclusions: We showed that the surface membranes of cultured human chondrocytes and fibrochondrocytes contain the IF1 and β subunits of ATP synthase. We have to define if angiostatin is able to bind on ATP synthase and modulate angiogenesis.

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ANGIOGENESIS IN SUBCHONDRAL BONES OF OSTEOARTHRITIC KNEES

R. Akagi, M. Saito, N. Ikegawa, S. Yamaguchi, K. Matsuki, S. Sato, K. Nakagawa, K. Takahashi, T. Sasho
Chiba Univ. Hosp., Chiba, Japan

Purpose: Angiogenesis is implicated as a cause of many diseases as well as inevitable repair process. In osteoarthritic (OA) knees, pathological changes in the subchondral bone were considered to be related to disease initiation, progression, and a potent source of knee pain. Our previous study revealed positive immunoreactivity of Substance P, Cox-2, TNF-α, and TUJ1 in cystic lesions formed in the subchondral bone of affected compartments. These cystic lesions consist of several types of cells including CD-34 positive vascular endothelial cells. This implicates up-regulated angiogenetic activities of affected compartments. Using specimens derived at the time of total knee arthropasty (TKA) for medial-type OA knees, histological examination revealed elevated vascularity in the subcondral bone of the medial femoral condyle (MFC) compared with that of the lateral femoral condyle (LFC). In this study we investigated the relationship between angiogenetic activities of subchondral bone in OA knees and the extent of degeneration overlaying the subchondral bone.
Methods: Among patients who received total knee arthroplasty (TKA) in our institution, 7 patients with medial-type OA knees (one male, six female) with minimum involvement of lateral compartment confirmed with lack of bone marrow edema on Magnetic Resonance Imaging as well as macroscopic examination were selected. The mean age was 73.5 years, and every patient was classified as grade IV according to the Kellgren and Lawrence scale.Subchondral bone of the weight bearing compartment of MFC, LFC, and the anterior non-weight bearing compartment of distal femur (AF) were obtained at the time of TKA. Specimens were cultured in Dulbecco's Modified Eagle Medium with 7% Fetal Bovine Serum for 2 weeks. The culture supernatant was then added to the angiogenesis kit (KURABO, Osaka Japan) and cultured for another 11 days until vessel lumens were formed. Primary antibody against CD 31 and secondary antibody of Goat anti-mouse IgG AlkP conjugate were used to stain the vessels. The length, area, number of joints and paths of the vessels were analyzed automatically by Angiogenesis Image Analyzer (KURABO, Osaka Japan) for each compartment.
Results: The degrees of angiogenesis assessed by the four parameters have a tendency to be higher in the MFC compared to the LFC and the AF, but it did not reach statistical significance (Figs. 1–4).