AB0088

MECHANICAL STIMULUS INDUCED BY CHIROPRACTIC MANIPULATION REDUCES CARTILAGE, SUBCHONDRAL BONE DAMAGE AND SYNOVIAL INFLAMMATION IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS

Francisco Miguel Conesa-Buendía[1,2], Aránzazu Mediero-Muñoz[2], Ricardo Fujikawa[1], Paula Gratal Viñuales[2], Francisca Mulero[3], Juan Pablo Medina Giménez[2], Gabriel Herrero-Beaumont[2,4], Raquel Largo-Carazo[2,4], Arantxa Ortega DE Mues[1]. *[1]MAdrid College of Chiropractic/RCU Maria Cristina El Escorial, Madrid, Spain*; *[2]IIS-Fundación Jiménez Díaz, Rheumatology, Madrid, Spain*; *[3]Centro Nacional De Investigaciones Oncologicas (CNIO), Madrid, Spain*; *[4]Universidad Autónoma De Madrid, Madrid, Spain*

Background: Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage degradation, although both subchondral bone deterioration and synovial inflammation are also hallmarks of the disease. Chiropractic manipulation (CM) is a therapeutic approach focused on the diagnosis, treatment and prevention of musculoskeletal disorders. It is essentially manual, allowing the chiropractor to restore the normal range of motion and function of the joints, muscles, and ligaments. Clinical evidences suggest that CM might exert positive effects in OA patients.
Objectives: The aim of this study was to evaluate the effects of CM on cartilage, subchondral bone and synovitis state in an OA rabbit model.
Methods: Ten (4 months old) male New Zealand rabbits underwent knee surgery to induce OA by transection of anterior cruciate ligament. One week after the surgery, CM was performed using the chiropractic adjusting instrument ActivatorV as follows: Force 2 setting was applied onto the tibial tubercle of the right hind limb (true manipulation, TM-OA group), at an angle of approximately 90°, from medial to lateral, whereas the corresponding left hind limb received a false manipulation (FM-OA group) consisting of ActivatorV firing in the air and slightly touching the tibial tubercle. These procedures were repeated 3 times a week for 8 weeks. Three healthy animals were used as control. Following sacrifice, µCT and histological damage evaluation (Mankin score) were done in femur and tibiae. RANKL/OPG protein expressions were studied by immunohistochemistry in tibia samples. Sinovitis was assessed by Krenn score and immunohistochemistry for macrophages (RAM11) and angiogenesis (CD31) were done. Protein expression of VEGF, COX2, TNFa, IL-1b and MMP3 were determinated by Western Blot.
Results: In the OA rabbits, subchondral BMD decreased in relation to control, been partially reversed in the tibiae of TM-OA group. When subchondral trabecular bone structural parameters were analyzed by microCT, a significant decrease of bone volume/trabecular volume (BV/TV), trabecular number (Tb.N) and trabecular thickness (Tb.Th) was observed in the OA rabbits, while trabecular separation (Tb.S) increased compared to control animals. TM-OA group showed a significant improvement of these parameters compare to FM-OA group. FM-OA joints had higher Mankin score (cartilage damage) than control joints, and TM decreased Mankin score compare to FM-OA ($p<0.05$). The study of RANKL and OPG in cartilage and subchondral bone showed that the significant increase in the RANKL/OPG ratio observed in both tissues respect to controls, was partially reversed in TM-OA group. OA synovial membranes presented a total Krenn synovitis score higher than healthy animals; however, TM-OA rabbits exhibited scores lower than FM-OA ($p<0.05$). RAM11 immunostaining revealed lower expression in TM-OA synovial membranes compared to FM-OA ($p<0.05$). Finally, the significant increase in proinflammatory cytokines (COX2, TNFa and IL-1b) and MMP3 were observed in the synovial membranes of OA rabbits respect to controls, being partially reversed in TM-OA group. Likewise, VEGF and CD31 expression was higher in FM-OA synovium compared to TM-OA.
Conclusion: These results suggest that mechanical stimulus induced by CM may retard the pathologic progression of OA. The beneficial effects of CM might be associated with an improvement in bone and cartilage damage and also inflammatory state.
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2019-eular.1193

AB0089

IN VITRO EFFECT OF FASCIOLA HEPATICA EXTRACT ON SYNOVIAL FIBROBLAST OF RHEUMATOID ARTHRITIS PATIENTS

Suelen Dalmolin[1,2], Renata Ternus Pedo[1,2], Mirian Farinon[1,2], Jordana Miranda de Souza Silva[1,2], Vanessa Hax[3], Martin Cancela[4], Henrique Bunselmeyer Ferreira[4], Rafaela Cavalheiro Do Espírito Santo[1,2], Fabiany Gonçalves[5], Ricardo Xavier[2,3]on behalf of Laboratório de Doenças Autoimunes. *[1]Laboratório de Doenças Autoimunes, Porto Alegre, Brazil*; *[2]Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*; *[3]Hospital de Clínicas de Porto Alegre, Serviço de Reumatologia, Porto Alegre, Brazil*; *[4]Universidade Federal do Rio Grande do Sul, Departamento de Biologia Molecular e Biotecnologia, Porto Alegre, Brazil*; *[5]Erasmus University Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*

Background: Synovial fibroblasts (FLS) have an aggressive and invasive profile and play a major role in RA[1]. Available therapeutic options are effective for induction and maintenance of disease remission[2], but not all patients respond to treatment and there is no cure. So new therapeutic options need to be explored. Studies have shown that products secreted by helminths contain components with anti-inflammatory properties[3 4], capable of suppressing Th1 immune response[5] and the production of inflammatory cytokines[6]. One example is the immunomodulating properties[7] of the extract of *Fasciola hepatica*.
Objectives: To evaluate the effects of *F. hepatica* extract in FLS from RA patients.
Methods: Firstly, the cultures of FLS collected from the synovial fluid of RA patients were exposed at different concentration of *F. hepatica* extract (60µg/mL, 80µg/mL and 100µg/mL) and analyzed after 24h, 48h and 72h by MTT cell proliferation assay. FLS controls were exposed to standard culture medium. The effect of extract was also evaluated through adhesion and wound healing assay, and TNF-α production after IFN*g* stimulation. Statistical analysis was performed with ANOVA or T Test and the $p<0.05$ were considered statistically significant.
Results: *F. hepatica* extract decreased the cell proliferation of FLS at concentration of 100µg/ml after 48h (83.8% ± 5.0 extract *vs* 100.0% ± 0.0 control; $p<0.05$), and at concentrations of 80 µg/ml (88.4% ± 3.0 extract *vs* 100.0% ± 0.0 control; $p<0.05$) and 100 µg/ml (89.8% extract ± 3.8 extract *vs* 100.0% ± 0.0 control; $p<0.05$) after 72h, when compared with control group. Based on these results, the concentration of 100µg/ml and the time of 48h were chosen for the following tests. The treatment with extract showed a decreased of FLS adhesion (92.0 cells ± 5.8 extract *vs* 116.3 cells ± 7.9 control; $p<0.05$) and migratory potential (40.2 inch/h ± 13.9 extract *vs* 58.7 inch/h ± 6.5 control). Moreover, after treatment there was a trend of decreased TNF levels (7.0 pg/mL ± 0.5 extract *vs* 8.1 pg/mL ± 0.7 control).
Conclusion: Taken together, our results suggest that extract of *F. hepatica* is able to reduce the aggressive and invasive profile of FLS. However, further analyses are needed for a better understanding the mechanisms of the effect of *F. hepatica* extract on FLS and its different components, such as cystatin.

REFERENCES

[1] Bottini N, et al. Nat Rev Rheumatol. 2013;9(1):24–33.
[2] Tanaka Y. Korean J Intern Med. 2016;31(2): 210–218.
[3] Hamilton C, et al. Infect. Immun. 2009;(77):2488–98.
[4] Wang S, et al. Parasites & Vetors. 2016;(9):6.
[5] Donnelly S. Infection and immunity. 2005;(73):166–73.
[6] Ravidà A, et al. Molecular & cellular proteomics: MCP. 2016;15(3139–3153).
[7] Cancela M, et al. Parasitology. 2017;144(13):1695–707.

Acknowledgement: FIPE/HCPA, Serviço de Reumatologia/HCPA
Disclosure of Interests: Suelen Dalmolin: None declared, Renata Ternus Pedo: None declared, Mirian Farinon: None declared, Jordana Miranda de Souza Silva: None declared, Vanessa Hax: None declared, Martin Cancela: None declared, Henrique Bunselmeyer Ferreira: None declared, Rafaela Cavalheiro do Espírito Santo: None declared, Fabiany Gonçalves: None declared, Ricardo Xavier Consultant for: Abbvie, Pfizer, Novartis, Janssen, Lilly, Roche
DOI: 10.1136/annrheumdis-2019-eular.5907

AB0090

INHERITED DEFICIT OF PROTEOGLYCAN MIMICKING SEPTIC ARTHRITIS

Angelo Florio[1], Riccardo Papa[1], Roberta Caorsi[1], Alessandro Consolaro[1], Roberto Gastaldi[2], Angelo Ravelli[2], Marco Gattorno[1], Paolo Picco[1], Tuula Rinne[3]. *[1]IRCCS Istituto Giannina Gaslini, Clinica Pediatrica e Reumatologia, Genova, Italy*; *[2]IRCCS Istituto Giannina Gaslini, Clinica Pediatrica ed Endocrinologia, Genova, Italy*; *[3]Radboud university medical center, Genome Diagnostics Nijmegen – Department of Human Genetics, Nijmegen, Netherlands*

Background: Aggrecanopathies (AP) are a heterogeneous group of skeletal disorders caused by *ACAN* gene mutations leading to dysfunction of a proteoglycan called aggrecan that plays a pivotal role in the organization of the extracellular matrix of the growth-plate cartilage. Clinically, patients with AP display bone dysplasias ranging from severe spondyloepimetaphyseal dysplasia to familial cases of osteochondritis dissecans (OCD) associated with short stature and typical facial dysmorphisms[1].
Objectives: Herein we report a patient with AP referred to our Institute for an inflammatory articular involvement mimicking a septic arthritis.
Methods: Molecular analysis of the *ACAN* gene was performed using Sanger sequencing method.
Results: A 14-year-old boy displayed pain and swelling at the right elbow. Echoscan revealed an effusion in both coronoid and olecranon recess. Acute phase reactants were negative. A month later, since non-steroidal anti-inflammatory drugs were administered without benefit, the patient was admitted in our Institute. On physical examination, acute arthritis at the right elbow was noted: it appeared painful, warm, and not erythematous. Laboratory test showed slight elevation of acute phase reactant (C reactive protein 1.7 mg/dl, normal value <0.5).

Arthrocentesis was performed and sterile synovial fluid was found. Magnetic resonance images displayed a bone fragment detachment from the humeral condyle of the right elbow with synovium thickening and persistent effusion: the diagnosis of OCD was pointed out.
The patient showed minor dysmorphisms (i.e. dolicocephaly, hypotelorism, arched palate and brachydactyly of the IV finger of both hands) and parents reported a previous episode of OCD when he was 12: at that time, symptoms resolved with non-weightbearing and non-steroidal anti-inflammatory therapy after few days. Furthermore, the patient went under regular endocrinologist follow-up for short stature since he was 8. At the age of 10, his height was 123 cm, SDS 2.4, and growth hormone (GH) stimulation tests showed partial response to insulin tolerance test (GH peak 6.27 ng/mL). Bone age at the X-Ray of right hand and wrist was delayed of 12 months. Human recombinant GH replacement therapy was administered without significant growth-velocity improvement. Although the patient came to observation because of suspected elbow septic arthritis, we re-considered the diagnosis: namely, i. recurrent episodes of OCD; ii. short stature that was poorly responsive to the human recombinant GH treatment, iii. mild skeletal and facial dysmorphisms, led us to hypothesize a form of aggrecanopathy. Molecular analysis of the *ACAN* gene revealed the novel missense variant c.6970T>C, p.Trp2324Arg in the G3 domain of the protein. Notably, another mutation of the G3 domain (c.7249G>A) has been previously related to aggrecanopathy2. Intra-familial molecular analysis allowed us to detect the same gene variant in other three subjects (the mother and 2 siblings) affected only by brachydactily and short stature.
Conclusion: A patient carrying a novel mutation of the ACAN gene presented an atypical form of aggrecanopathy mimicking inflammatory and/or septic arthritis associated with slight short stature and bone dysmorphisms. Further studies are needed to investigate a possible role of this novel ACAN gene variant in the inflammatory articular involvement.

REFERENCES

[1] [Gibson BG, Briggs MD.The aggrecanopathies; an evolving phenotypic spectrum of human genetic skeletal diseases Orphanet J Rare Dis. 2016; 11: 86.]
[2] [E.L. Stattin, F. Wiklund, K. Lindblom, et al.A missense mutation in the aggrecan C-type lectin domain disrupts extracellular matrix interactions and causes dominant familial osteochondritis dissecans. Am. J. Hum. Genet., 86 (2010), pp. 126-137]

Disclosure of Interests: Angelo Florio: None declared, Riccardo Papa: None declared, Roberta Caorsi: None declared, Alessandro Consolaro Grant/research support from: AbbVie, Pfizer, Roberto Gastaldi : None declared, Angelo Ravelli Grant/research support from: Angelini, AbbVie, Bristol-Myers Squibb, Johnson & Johnson, Novartis, Pfizer, Reckitt Benkiser, and Roche, Consultant for: Angelini, AbbVie, Bristol-Myers Squibb, Johnson & Johnson, Novartis, Pfizer, Reckitt Benkiser, and Roche, Speakers bureau: Angelini, AbbVie, Bristol-Myers Squibb, Johnson & Johnson, Novartis, Pfizer, Reckitt Benkiser, and Roche, Marco Gattorno Grant/research support from: MG has received unrestricted grants from Sobi and Novartis, Paolo Picco: None declared, Tuula Rinne: None declared
DOI: 10.1136/annrheumdis-2019-eular.3617

AB0091 SYNDECAN-4 IS INCREASED IN OSTEOARTHRITIC KNEE, BUT NOT HIP OR SHOULDER, ARTICULAR HYPERTROPHIC CHONDROCYTES

Christelle Sanchez[1], Cécile Lambert[1], Jean-Emile Dubuc[2], Jessica Bertrand[3], Thomas Pap[4], Yves Henrotin[1]. [1]*University of Liège, Bone and Cartilage Research Unit, Liège, Belgium*; [2]*Cliniques universitaires Saint-Luc, Brussels, Belgium*; [3]*University Hospital Magdeburg, Magdeburg, Germany*; [4]*University Hospital Munster, Munster, Germany*

Background: Syndecan-4 plays a critical role in cartilage degradation during osteoarthritis (OA).
Objectives: To investigate the expression and localization of syndecan-4 in different OA joint tissues.
Methods: Syndecan-4 mRNA levels were quantified by RT-PCR in human OA primary cells to compare non-hypertrophic vs hypertrophic articular chondrocytes, non-sclerotic vs sclerotic subchondral osteoblasts and normal/reactive vs inflamed fibroblasts-like synoviocytes. Syndecan-4 was localized by immunohistochemistry in knee, hip or shoulder OA bone/cartilage biopsies. Syndecan-4 was quantified by immmunoassay in chondrocytes culture supernatant and cell fraction.
Results: By immunohistochemistry, syndecan-4 was observed in chondrocytes clusters in the superficial zone of OA knee, but not in OA hip or shoulder cartilage. No staining was observed in the deep zone of cartilage and in subchondral bone. No difference between syndecan-4 expression level in sclerotic and non-sclerotic osteoblasts was observed. Syndecan-4 tended to be increased in inflamed synoviocytes compared to normal/reactive ones but difference was not significant. Differentiated hypertrophic chondrocytes from knee, but not from hip cartilage, expressed more syndecan-4 than non-hypertrophic cells. Using an immunoassay for the extracellular domain of syndecan-4, we found 68% of the syndecan-4 in the culture supernatant of OA chondrocytes culture, suggesting that a large majority of the syndecan-4 is shed and released in the extracellular medium. The shedding rate was not affected by hypertrophic differentiation state of the chondrocytes or their joint origin.
Conclusion: Syndecan-4 could be related to the hypertrophic differentiation of the OA chondrocytes, but the pathway seems to be knee specific. Even if chondrocytes clusters are seen in OA knee, hip and shoulder cartilage and hypertrophic differentiation appears in knee and hip OA articular chondrocytes, syndecan-4 synthesis only increased in knee. These findings suggests the presence of biochemical difference between articular cartilage according to their location and that syndecan-4 could be a biochemical marker specific for knee OA.
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2019-eular.1454

AB0092 SERUM LEVELS OF COLL2–1, A SPECIFIC BIOMARKER OF CARTILAGE DEGRADATION, ARE NOT AFFECTED BY SAMPLING CONDITIONS, CIRCADIAN RHYTHM, SEASONABILITY AND PHYSICAL ACTIVITY

Yves Henrotin[1], Berenice Costes[2], Elisabeth Cobraiville[3], Sebastien Pirson[4], Anne-Christine Hick[5]. [1]*University of Liège, Motricity Sciences, Liège, Belgium*; [2]*Artialis, Clinical CRO, Liège, Belgium*; [3]*Artialis SA, Clinical CRO, Liège, Belgium*; [3]*Artialis SA, Clinical CRO, Liège, Belgium*; [5]*Artialis SA, Biotesting, Liège, Belgium*

Background: Coll2-1 is a nine amino acid sequence (HRGYPGLDG) specific of type II collagen which is released during cartilage degradation. This peptide is located in the triple helicoidal part of type II collagen molecule(1,2,3).
Objectives: This study aims to assess intra-individual biological variability of serum cartilage specific biomarker Coll2-1 and define the best standardized conditions for blood sampling in clinical trials.
Methods: Blood samples were taken from 122 subjects with diagnosed knee osteoarthritis (KOA) at a single timepoint as well as from 15 healthy subjects under various conditions, including fasting condition (before or after breakfast and lunch), sampling time (at 8am, 9am, 12pm, 2pm and 5pm), sampling season (at baseline and after 2, 16, 52 and 68 weeks), physical activity (after a resting weekend versus working day), blood treatment (blood clotting from 1h to 24h at room temperature or 4°C; centrifugation at room temperature or 4°C) and type of blood collection tube (dry tube versus dry tube with gel separator). Type II collagen-specific biomarker Coll2-1 was measured using ELISA (Artialis Groups SA, Liège, Belgium) on all collected samples.
Results: There was no significant difference on Coll2-1 values between samples collected at any of the five sampling times (p=0.85) or at any of the sampling days measured (p=0.58). None of the sampling parameters tested had a significant impact on Coll2-1 value (clotting time, clotting temperature and temperature of blood centrifugation (p=0.93), type of tube: p=0.38) when all data from healthy subjects were pooled (n=631 samples). On the contrary,subjects with knee OA had a significantly higher Coll2-1 concentration then healthy subjects (OA: 481+/-144nM vs Healthy 192+/-32nM, p<0,001).
Conclusion: Coll2-1 assay is sufficiently robust for use during OA clinical trials. Coll2-1 measurement is not affected by subject specific conditions such as fasting, resting state, circadian rhythm, seasonality, nor by sampling process factors such as type of dry tube, clotting patterns and centrifugation temperature.

REFERENCES

[1] Henrotin Y, Addison S, Kraus V, Deberg M: Type II collagen markers in osteoarthritis: what do they indicate? Curr Opin Rheumatol 2007, 19:444–450.
[2] Henrotin Y, Deberg M, Dubuc JE, Quettier E, Christgau S, Reginster JY: Type II collagen peptides for measuring cartilage degradation. Biorheology 2004, 41:543–547.
[3] Henrotin Y, Sanchez C, Cornet A, Van de Put J, Douette P, Gharbi M : Soluble biomarkers development in osteoarthritis: from discovery to personalized medicine. Biomarkers 2016, 20:8, 540-546.

Disclosure of Interests: Yves Henrotin Shareholder of: Artialis SA, Berenice Costes Employee of: Artialis SA, Elisabeth Cobraiville Employee of: Artialis SA, Sebastien Pirson Employee of: Artialis SA, Anne-Christine Hick Employee of: Artialis SA
DOI: 10.1136/annrheumdis-2019-eular.1490