inflammation has the potential to reduce both formation of osteophytosis and alleviate synovitis. Osteophytes originate from a cartilage outgrowth that undergoes hypertrophic differentiation followed by ossification. Previously, we have identified an increased expression of Ephrin receptor A2 (EphA2), a tyrosine kinase associated with inflammation, in osteoarthritic cartilage and in hypertrophic cartilage of the growth plate. The aim of this study was to examine the effect of EphA2 inhibition on chondrocytes hypertrophy and inflammation *in vitro* and its potential to mitigate osteophytosis and synovitis *in vivo*.

Methods: *In vitro* target validation was performed using chondrocytes isolated from human OA donors (N=3) that underwent total knee arthroplasty. P3-expanded chondrocytes were encapsulated in alginate beads (4 million cells/mL) and redifferentiated for one week in medium containing 10 ng/mL Transforming growth factor beta-1. Pharmacologic inhibition of EphA2 was performed using 10 μM tyrosine kinase inhibitor ALW-II-41-27. Inflammatory stimulation was induced using 10 ng/mL Tumor necrosis factor (TNF) α. ALW-II-41-27 and TNFα stimulation were performed for 24 hours. mRNA expression of collagen type 10α1 (*COL10A1*) was determined by qPCR. Nitrite production was quantified in the medium though the Griess reaction as marker of inflammation.I*n vivo*, OA was induced in 12-week-old male C57BL/6 mice (N=16) by intra-articular injection of monoiodoacetate (60 μg / 6 μL) to the right knee joint. ALW-II-41-27 was delivered subcutaneously using Alzet micro-osmotic pumps (model 1002, delivery rates of 0.21 μL/ hour). Osmotic pumps were filled with dimethyl sulfoxide: polyethylenglicol alone (55:45 ratio, vehicle-treated group, N=8 mice) or containing 6 mg of ALW-II-41-27 dissolved in vehicle (treated group, N=8 mice). At day 0, osmotic pump implantation and intra-articular injection of monoiodoacetate was performed. Mice were euthanized at day 14 and knees were harvested for histological analysis. Statistical evaluation was performed using a two-tail unpaired t test for parametric data and a Mann-Whitney test for non-parametric data.

Results: EphA2 activity inhibitor ALW-II-41-27 reduced the expression of *COL10A1* in OA chondrocytes, suggesting a reduction in hypertrophy (Figure 1A). TNFα-induced upregulation of nitrite in the medium was attenuated by the addition of ALW-II-41-27 (Figure 1B). *In vivo*, treatment with ALW-II-41-27 led to smaller osteophytes compared to vehicle-treated mice (Figure 2A). Synovitis was reduced in ALW-II-41-27-treated mice according to the synovial membrane thickness and Krenn score (Figure 2B). Cartilage degeneration as observed by loss of proteoglycans, was present in all mice and not significantly influenced by the treatment.

Conclusions: ALW-II-41-27 attenuates hypertrophy and inflammation in human osteoarthritic chondrocytes *in vitro*. Subcutaneous delivery of ALW-II-41-27 in a mouse model mitigates osteophytosis and synovitis. These results suggest that this small kinase inhibitor may constitute a novel pharmacologic treatment for OA.

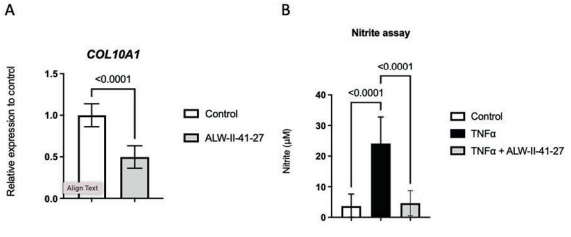

Figure 1. ALW-II-41-27 decreases hypertrophy and TNFα-induced inflammation of human OA chondrocytes *in vitro*. (A) Gene expression analysis of the hypertrophic marker *COL10A1*. (B) Quantification of the inflammatory mediator nitrite in the medium. Experiments were performed in 3 OA donors.

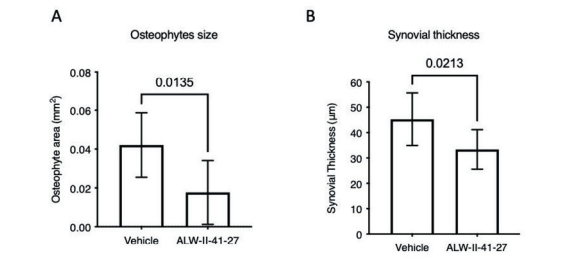

Figure 2. ALW-II-41-27 treatment attenuates osteophytosis and synovitis *in vivo*. (A) Osteophyte area and (B) synovial thickness quantified in the lateral side of the femoropatellar joint in Hematoxylin/Eosin staining's. The average of three measurements per mice, in three slides were used. Eight mice per group.

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THE REVERSION OF OSTEOARTHRITIS-LIKE ALTERATIONS BY RALOXIFENE AND PHLORETIN IN LIPOPOLYSACCHARIDE-INDUCED CHONDROGENIC CELL LINES

M. Paesa, C. Remírez de Ganuza, F. García-Álvarez, S. Irusta, M. Arruebo, G. Mendoza. *Univ. of Zaragoza, Zaragoza, Spain*

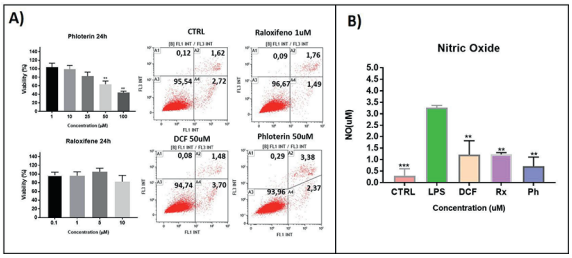
Purpose: Osteoarthritis (OA) is a chronic joint disease characterized by cartilage degradation, alterations in bone formation or subchondral bone remodeling and progressive synovial inflammation. Inflammatory signaling is involved in OA pathophysiology. The activation of the NF-κB pathway by pro-inflammatory cytokines is essential to induce various inflammation-related factors (MMPs, iNOS, IL-1β, TNF-α), which further activate the signaling cascade resulting in a major catabolic effect and subsequent OA progression. Thus, finding molecular inhibitors of chondrocyte inflammatory signaling is necessary for OA prevention and treatment. Raloxifene, a selective estrogen receptor modulator, is commonly used for its ability to reduce subchondral bone remodeling while increasing bone mineral density. Phloretin is a type of dihydrochalcone known for its antioxidant, anti-inflammatory, and immunomodulatory biological effects. However, the potential of these molecules for reverting an OA like-phonotype has not been widely explored. The aim of this study was to evaluate the anti-inflammatory effects of these compounds in a murine chondrogenic cell line (ATDC-5) with a particular focus on the inhibition of both nitric oxide (NO) production and gene expression of pro-inflammatory factors.

Methods: ATDC-5 cells were cultured in DMEM-Ham's F-12 medium supplemented with 5% FBS,10 μg/ml human transferrin, 3x10_-8M sodium selenite, 1% mixture of antibiotics and amphotericin and 1% stable glutamine. **Cell treatment**. Chondrocytes were cultured in 12-well plates, pre-incubated with Raloxifene Hydrochloride (Rx) and Phloretin (Ph) for 4h and then stimulated with 250 ng/mL of LPS from Escherichia coli O26:B6 for 24h. Subsequently, cell viability, cell apoptosis, NO production and RT-qPCR of common inflammation-related genes were evaluated.**Cell viability** was evaluated after treatment with Ph (10-100 μM) and Rx (0.1-1μM) for 24 h. Then, cells were washed twice with PBS and the Blue Cell Viability Assay reagent (10% w/w) was added to the supplemented medium. After 3h of incubation, viability was evaluated by fluorescence in a microplate reader at 530/590 nm wavelengths. Cell viability was calculated by linear interpolation of the fluorescence data from the treated cells vs the not treated samples.-Thereafter, **cell apoptosis** was studied after treatment for 24h with the mentioned compounds. After that, cells were washed with PBS, separated by centrifugation and collected at a final concentration of 10_6 cells/mL. Subsequently, cells were centrifuged and the pellet was resuspended in the Annexin-binding buffer. Then, cell suspension was stained with Annexin V-FITC and propidium iodide for 15 min at room temperature in the dark. After incubation, Annexin-binding buffer was added to each sample. In addition, **collagen type II and aggrecan markers** were evaluated using a commercial kit (i.e., Intracell) in order to elucidate the potential effects of the treatment with the proposed compounds in those cartilage biomarkers. In both cases, samples were analyzed by flow cytometry.**NO accumulation** was measured in the culture medium by the Griess reaction. Briefly, cell culture supernatant was incubated at room temperature with the Griess reagent (equal volumes of 1% (w/v) sulphanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphtylethylenediamine- HCl) and the absorbance at 540 nm was measured in a microplate reader. Nitrous oxide concentration was calculated from a standard curve.**Gene expression**. ATDC-5 cells were collected to evaluate their gene expression by quantitative RT-qPCR. Cells were washed with PBS and lysed with TRIzol, following the manufacturer's instructions. RNA was reverse transcribed with PrimeScript™ RT Master Mix and quantified with the NanoDrop 2000. The amplification was carried out using Premix Ex Taq Probe qPCR in a QuantStudioTM 5 Real Time PCR Instrument. Gene expression was normalized to the level of beta actin. The values obtained from tested genes were normalized to those obtained from control groups (non-stimulated cells).

Results: As shown in Fig. 1A, Rx in the concentration range of 1-10 μM did not alter chondrocyte viability, while Ph viability decreased in a dose-dependent manner. Consequently, Rx 1 μM was chosen, as indicated in literature, and Ph 50 μM was used in the subsequent experiments as they maintain viabilities higher than 70% at those concentrations. Results of cell apoptosis showed that these compounds displayed high viability percentages compared to the control sample;

diclofenac (DCF) 50 μM was used as positive control for the treatment of inflammation. COL II and ACAN were also analysed by flow cytometry after LPS stimulation. When both molecules were added, cartilage biomarkers expression was partially restored to the control levels. The anti-inflammatory activity of both molecules was tested evaluating LPS-induced NO production. As shown in Figure 1B, LPS led to a significant accumulation of NO in ATDC-5 cell culture supernatant while pre-treatment with both compounds strongly decreased NO production in LPS-activated chondrocytes. To further investigate their anti-inflammatory activity, the expression of several inflammation-related genes was analysed by RT-qPCR. Results showed the potential of both molecules in reducing the expression of iNOS mRNA as well as most of the inflammatory cytokines evaluated in LPS-activated chondrocytes.

Conclusions: Our study demonstrates the ability of Raloxifene and Phloretin to inhibit inflammatory and catabolic responses in LPS-activated chondrocytes indicating their potential therapeutic use in OA treatment.



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EFFECT OF ORAL SUPPLEMENTATION WITH SKATE FISH CARTILAGE HYDROLYSATE ON KNEE JOINT FUNCTIONALITY AND DISCOMFORT IN ADULT POPULATION

Y. Henrotin [1,2,3], J. Herman [2], C. Chapelle [4], S. Pietri [2], M. Uebelhoer [2], V. Mokam Fotso [2], M. Duquenne [3], E. Bouvret [5], A.-F. Donneau [1], J. Monseur [1], B. Costes [2]. [1] *Univ. of Liège, Liège, Belgium;* [2] *Artialis SA, Liège, Belgium;* [3] *Vivalia, Marche-en-Famenne, Belgium;* [4] *Ctr. Médical Chant d'oiseau, Woluwe-Saint-Pierre, Belgium;* [5] *Abyss Ingredients, Caudan, France*

Purpose: The tested product is a dried skate fish cartilage hydrolysate (SFCH) including collagen peptides together with glycosaminoglycans such as chondroitin sulfate and glucosamine sulfate . In a recent pre-clinical efficacy study, SFCH has shown a beneficial effect on pain and joint function in ACLT/pMMx osteoarthritis rat model (Henrotin et *al.,* 2021). The aim of this study was to determine if 3 months of oral administration of SFCH can contribute to improve knee functionality and discomfort in an adult population with knee pain or mobility issues.

Methods: This study is an exploratory, non-comparative, multi-centric trial including 33 subjects with moderate knee joint discomfort and loss of functionality. The eligible patients have taken 2 capsules of SFCH 500 mg, once daily in the morning for 3 months. The primary objective was to evaluate the effect of 3 months supplementation with SFCH on knee pain and function using the Knee injury and Osteoarthritis Outcome Score (KOOS) self-administered questionnaire The secondary objectives were to evaluate the effects of SFCH on knee pain (at rest or while walking), Patient Global Assessment (PGA) using Visual Analogue Scales (VAS), quality of life (SF-36) and product satisfaction by self-administered questionnaires, tolerability and finally compliance based on tablets accountability after 1-month and 3-month follow up. Patients' response to the treatment was calculated according the OMERACT-OARSI criteria. Statistical analysis was performed on full analysis set population.

Results: KOOS and its subscales improved significantly between baseline, 1-month and 3-months of supplementation with SFCH (figure 1a & b). Moreover, SFCH significantly reduced pain at rest and while walking and PGA after 1 and 3-months of supplementation compared to baseline. The SF-36 global score and physical functioning, pain, and general health subscores were significantly improved by SFCH at each time point, while the variables role limitations due to emotional problems, energy/fatigue and emotional well-being were not modified. At each time point, compliance was greater than 90% (100.0 (97.1-100.0) after 1 month follow-up and 99.2(92.8-100.4) after 3 months follow-up). After 1 month of follow-up, 11 (42.3%) patients were responders to the treatment according to OMEACT-OARSI criteria. At the end of the study, a total of 16 (61.5%) were responders.Only few minor adverse events were related to the investigational product (i.e. gastro intestinal, urinary or skin disorders). However, this supplementation did not significatively change pain killers' consumption during the 3-month period. Finally, the participants' feedback was good regarding product satisfaction.

Conclusions: The patients showed a large improvement of the KOOS after 3 months of supplementation. Interestingly, SFCH was already efficient after 1 month supplementation and its effect increased with intake duration. The VAS Effect Size (ES) at rest and at walking after 3 months of treatment with SFCH were superior to 1.30 which was over the reported placebo ES (0.66 (95% CI 0.56 to 0.76) (Zhang et *al.,* 2008). These data suggest that SFCH could be more efficient than placebo on pain. Although this study was exploratory and had some limitations including the small sample size and the absence of a control group, data indicate the potential of SFCH to manage joint discomfort. Of course, this positive trend needs to be confirmed in a larger randomized placebo controlled clinical trial.

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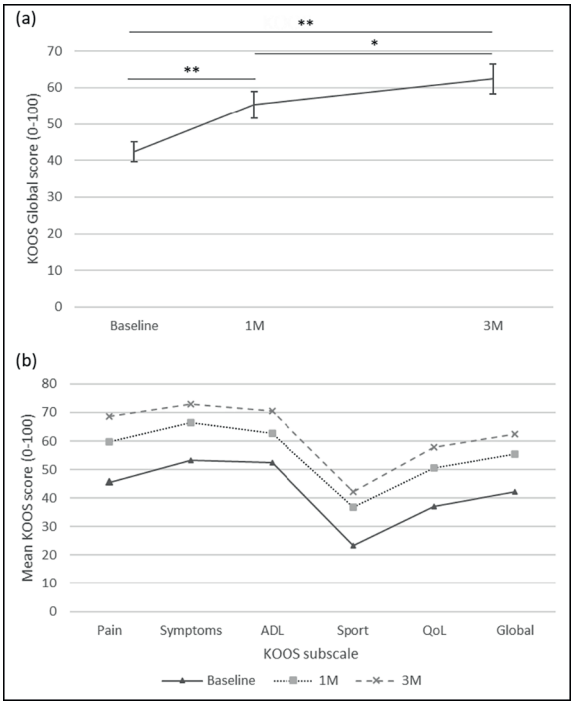

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CURCUMA LONGA AND BOSWELLIA SERRATA EXTRACTS MODULATE DIFFERENT AND COMPLEMENTARY PATHWAYS TO EXERT ANTI-INFLAMMATORY, ANTI-OXIDATIVE AND ANTI-CATABOLIC ACTIVITIES ON CHONDROCYTES: DECIPHERING OF A TRANSCRIPTOMIC STUDY

C. Sanchez [1], J. Zappia [1], C. Lambert [1], Y. Dierckxsens [2], J.-E. Dubuc [3], J.-P. Delcour [4], Y. Henrotin [1]. [1] *Univ. of Liège, Liège, Belgium;* [2] *Tilman SA,*