AB0083

LOCAL ICE CRYOTHERAPY DECREASES PROSTAGLANDIN-E2, NF-KB AND IL-6 SYNOVIAL LEVELS IN ARTHRITIC KNEES COMPARED TO CONTRALATERAL NON-TREATED JOINTS

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Background: Cryotherapy is widely used in rheumatic diseases, with a low level of evidence. Tissue mild hypothermia was reported to inhibit pivotal pro-inflammatory enzyme pathways such as COX-2/PGE₂ and NF-κB.²

Objectives: We hypothesised that local cryotherapy (LC) might reduce joint inflammation through PGE₂ and NF-κB repression.

Methods: 47 patients suffering from non-septic knee arthritis were included (17 gout, 11 calcium pyrophosphate dehydrate crystal deposition diseases, 6 spondyloarthritis, 13 rheumatoid arthritis), taking no concurrent anti-inflammatory drug/DMARD. They were first randomised to receive local ice (30 min-N=16) or cold pulsed CO₂ (2 min – Cryo+ Cryonic-N=16) at 9 A.M and 5 P.M. Synovial fluid was collected just before the first cold application then 24 hours later (9 A.M). Synovial fluid IL-6, IL-1β, TNF-α, IL-17A, VEGF (Multiplex flow cytometry), NF-κB P65 (total/phosphorylated) and PGE₂ (ELISA) were measured and compared before and after 2 LC applications. Contralateral non-treated knees were then used as paired controls (in 16 other ice-treated patients).

Results: Synovial IL-6 significantly decreased after 2 LC applications (n=43 paired Wilcoxon: p=0.00015). This decrease was also significant in ice-treated subgroup (n=16 p<0.005) but not in CO₂-treated patients (n=14; p=0.1). IL-1β and VEGF levels also decreased after treatment (n=44-p=0.02 and n=47-p=0.03) but not significantly in treatment subgroups. By contrast, IL-6 (n=13), IL-1β (n=15) and VEGF (n=15) didn't change in contralateral non-treated knees. LC had no significant effect on IL-17A nor TNF-α synovial levels. LC significantly reduced synovial NF-κB (n=38-p=0.04) and NF-κB-P levels (n=38-p=0.004). These levels were also significantly decreased in ice-treated patients (n=26-p=0.03 and 0.003) but not in CO₂-treated patients (n=13). We observed no effect on the NF-κB levels in contralateral non-treated knees (n=12). NF-κB variations correlated significantly with IL-6 (Pearson's r=0.48-N=32 p<0.01) and VEGF (r=0.34-N=36-p=0.038) variations, and with the maximal skin temperature drop induced by LC (r=0.32-N=36-p=0.047). LC significantly reduced synovial PGE₂ (n=38-p=0.04), which was also significantly decreased in ice-treated patients (n=26-p=0.02) but not in CO₂-treated patients (n=13). Conversely, we observed a significant increase in PGE₂ levels in contralateral non-treated knees (n=12-p=0.04), with a significant inter-class effect-size (weighted mean difference –1329 [–2232;–426] pg/mL in 12 bi-articular patients).

Conclusions: Local ice cryotherapy applied twice during one day showed superior anti-inflammatory effects compared to local CO₂, notably by reducing synovial IL-6 levels in arthritic knees. We also showed for the first time that this inhibitory effect on cytokine levels might be PGE₂ and NF-κB dependent, notably through significant inhibitory effects on the PGE₂ and (to a lesser extent) NF-κB pathways.

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AB0084

ELEVATED LEVELS OF IL-37 ARE ASSOCIATED WITH TOPHUS AND SUPPRESSED THE PRODUCTION OF INFLAMMATORY CYTOKINES IN PATIENTS WITH GOUT

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Background: IL-37 has been identified as a natural inhibitor of innate immunity. Although increasing evidence shows elevated IL-37 expression in various autoimmune diseases, its correlation with clinical symptoms in gout is still unclear.

Objectives: This study aims to determine the correlation between the levels of IL-37 and clinical indexes in gout patients, and to examine the inhibitory effect of IL-37 on pro-inflammatory cytokine including IL-1β, IL-6 and IL-18 from peripheral blood mononuclear cells (PBMC) of gout patients in culture.

Methods: Levels of serum IL-37 and concentrations of IL-1β, IL-6, IL-18 in cell culture from 42 patients with gout and 40 healthy controls (HCs) were measured by enzyme-linked immunoassay (ELISA). Moreover, the relative mRNA

expression of these cytokines in PBMCs was detected by real-time PCR (RT-PCR). The correlations between serum IL-37 levels and clinical values in gout patients were analysed by Spearman correlation test.

Results: Both protein and mRNA levels of IL-37 were higher in gout patients than healthy controls, especially in patients with tophus. Serum IL-37 levels of gout patients were positively correlated with C-reactive protein and uric acid. Furthermore, the expression of IL-1β, IL-6 and IL-18 in PBMCs from gout patients was significantly increased, which were markedly suppressed by IL-37 in culture.

Abstract AB0084 – Figure 1. IL-37 suppresses the expression of pro-inflammatory cytokines in PBMCs from gout patients

Conclusions: Serum levels of IL-37 are closely associated with clinical symptoms in gout patients and may represent a potential biomarker for the disease activity.

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AB0085

THE MECHANISM OF TOTAL GLUCOSIDE OF PAEONY AND SINOMENINE ON THE PREVENTION OF ACUTE GOUT FLARES

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Background: The compliance of drug prevention for acute gout flares (GFs) during urate-lowering treatment (ULT) is very low. New preventive drugs of GFs during ULT is required. Our previous study found that the total glucoside of paeony (TGP), a traditional Chinese drug's extracts, has a good effect on the prevention and treatment for urate induced acute gouty arthritis (AGA) in rat.

Objectives: To explore the possible mechanism of TGP and Sinomenine (SIN) in the prevention and treatment of acute gouty arthritis.

Methods: Logarithmic growth phase of RAW264.7 cells were seeded into 96 well plate at a density of 5×10^4 /mL. Cultured overnight in a humidified atmosphere of 5% CO₂ at 37°C. Then, the medium was changed to serum-free DMEM. Then divided into Control group (DMEM), Model group (DMEM +50 mg/L MSU), Colchicine group (DMEM +50 mg/L MSU +1 μmol/L Colchicine), TGP group (DMEM +50 mg/L MSU +40 mg/L TGP), SIN group (DMEM +300 mmol/L SIN), SIN +TGP group (DMEM +40 mg/L TGP +300 mmol/L SIN). The final volume of solution was 2 ml in each well, and each group had 3 wells, culturing for 24 hour in a humidified atmosphere of 5% CO₂ at 37°C. Then, culture supernatant was collected. NO level were detected by method of nitrate reductase, the level of IL-1β,

TNF- α , IL-8, MCP-1, MIF were detected by ELISA method. Western blot method was used to detect the expression of MyD88, NF- κ B, ASC, and Caspase-1 in macrophages.

Results: The expression level of MyD88, NF- κ B, ASC and Caspase-1 protein in macrophages of model group was significantly up-regulated, and the levels of IL-1 β , TNF- α , IL-8, MCP-1, MIF and NO in the supernatant is increased significantly. TGP, SIN, TGP +SIN and Colchicine group compared with the model group, the expression of NF- κ B, MyD88, ASC, Caspase-1 protein in macrophage and the level of IL-1 β , TNF- α , IL-8, MCP-1, MIF and NO in supernatant were significantly decreased, the difference was statistically significant ($p < 0.05$). TGP +SIN group compared with Colchicine group, the expression of inflammatory pathways proteins and the levels of inflammatory cytokines were no difference ($p > 0.05$) (table 1).

Abstract AB0085 – Table 1. Expression of major inflammatory cytokines and signalling pathway proteins(X $\pm$ S)

Group	N	IL-1 β (pg/ml)	TNF- α (pg/ml)	MCP-1(pg/ml)	N	MyD88	NF- κ B
Control	9	60.258 ± 5.159	330.962 ± 15.046	196.222 ± 17.696	3	0.374 ± 0.017	0.413 ± 0.059
Model	9	115.360 $\pm 8.027^*$	638.118 $\pm 31.297^*$	568.332 $\pm 41.052^*$	3	0.831 $\pm 0.020^*$	0.871 $\pm 0.053^*$
Colch	9	78.561 $\pm 4.645^{\#}$	391.539 $\pm 21.616^{\#}$	339.777 $\pm 27.326^{\#}$	3	0.573 $\pm 0.024^{\#}$	0.568 $\pm 0.012^{\#}$
SIN	9	86.842 $\pm 5.758^{\# \Delta}$	461.174 $\pm 33.014^{\# \Delta}$	383.103 $\pm 26.296^{\# \Delta}$	3	0.674 $\pm 0.029^{\# \Delta}$	0.674 $\pm 0.012^{\# \Delta}$
TGP	9	93.200 $\pm 4.717^{\# \Delta \circ}$	466.507 $\pm 28.218^{\# \Delta \circ}$	421.988 $\pm 39.226^{\# \Delta \circ}$	3	0.699 $\pm 0.042^{\# \Delta \circ}$	0.777 $\pm 0.015^{\# \Delta \circ}$
SIN	9	79.902 $\pm 5.499^{\#}$	405.834 $\pm 20.824^{\#}$	343.728 $\pm 26.328^{\#}$	3	0.613 $\pm 0.026^{\#}$	0.575 $\pm 0.014^{\#}$

Note: * $P < 0.05$ vs Control; $^{\#}$ $p < 0.05$ vs Model; $^{\Delta}$ $p < 0.05$ vs Colchicine; $^{\circ}$ $p < 0.05$ vs SIN

Conclusions: The anti-inflammatory effect of Chinese herbal extracts (TGP and SIN) on the multiple targets of the inflammatory model may be one of its mechanisms of action for the prevention and control of acute gout flares.

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Cartilage, synovium and bone

AB0086

TYPE II COLLAGEN AND GLYCOSAMINOGLYCAN TURNOVER IN BOVINE ARTICULAR CARTILAGE IS MODULATED BY LONG-TERM DYNAMIC COMPRESSION

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Background: Osteoarthritis (OA) patients suffer from progressive degradation of articular cartilage, to which there is no available treatment to block or reverse the catabolic mechanisms. Articular cartilage confers a biomechanical function in the joints, and in concordance chondrocytes have shown to be mechanosensitive. The effect of dynamic compression on cartilage extracellular matrix (ECM) could prove to be crucial for translational research in development and screening of new OA drug candidates.

Objectives: This study investigates the effect of long-term dynamic compression of a bovine articular cartilage *ex vivo* model through quantification of cartilage associated biomarkers.

Methods: Full depth bovine cartilage explants were cultured for 2 weeks. The explants were treated 3 times a week with either OSM [10 ng/mL] and TNF- α [2 ng/mL] (O+T), or TGF- β 1 [50 ng/mL]. Untreated samples were included as negative controls (w/o). For each condition two groups were established; an unloaded group and a group compressed 3 times a week. Compression was applied using Electroforce 5500 (TA Instruments), in a sine wave with a maximum load per cycle of 1 MPa, at 1 Hz frequency for 1200 cycles. Metabolic activity was measured once a week using AlamarBlue. Biomarkers released to the supernatant were assessed using the following well-described ELISAs: formation of type II collagen was measured by ProC2, and MMP-degradation of type II collagen was

measured by C2M. Sulfated glycosaminoglycans (sGAG) released to media, and sGAG extracted from the explants on the last day using a 4 mM GuHCl solution were quantified using a 1,9-dimethylmethylene blue (DMB) assay.

Results: Compression of bovine explants significantly decreased release of C2M compared to unloaded samples in O+T and w/o groups with 42% and 48% respectively. ProC2 release was not affected by compression. Compression of w/o or TGF- β treated explants increased sGAG release with 195% and 152% relative to their unloaded controls. However, the total amount of sGAG extracted from the explants on the last day remained unchanged. Compression of the explants did not affect the metabolic activity.

Abstract AB0086 – Figure 1

Conclusions: Compression significantly reduced the degradation of type II collagen, which in combination with unaltered formation as measured by ProC2, may suggest suppression of the involved catabolic processes and increased deposition of type II collagen in the cartilage matrix. Furthermore, compression elevated the sGAG release without reduction in the explant GAG content, indicating an increased turnover of GAG. In conclusion, the cartilage ECM turnover is significantly modulated by dynamic compression. This method adds essential translational value to the continuous research addressing OA.

Disclosure of Interest: None declared

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AB0087

SYNOVIAL TISSUE MACROPHAGES POLARISATION (M1, M2) IN PATIENTS WITH UNDIFFERENTIATED ARTHRITIS MEETING DIAGNOSTIC CRITERIA FOR RHEUMATOID ARTHRITIS OR PSORIATIC ARTHRITIS ALONG THE FOLLOW UP

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Background: Undifferentiated arthritis (UA) is defined as an inflammatory oligo- or polyarthritis that does not fulfil criteria for a definitive diagnosis. Earlier diagnosis would permit a better functional prognosis. Synovial tissue (ST) macrophages have been associated with disease activity, radiographic erosion and response to therapy in RA. Furthermore, it has been reported that M1 polarised macrophages predominate in RA synovitis, whereas M2 predominate in SpA synovitis.¹

Objectives: To analyse polarised macrophages (M1 proinflammatory and M2 anti-inflammatory) in ST of patients with UA which evolved to RA or PsA, after a long follow-up, to explore their diagnostic value.

Methods: To determine the polarisation state of macrophages in ST obtained by arthroscopy from patients with UA that evolved to RA (UA-RA=8) or PsA (UA-PsA=9), the expression of proteins associated to GM-CSF-driven polarisation M1 (INHBA, TNF α and MMP12) and M-CSF-driven polarisation M2 (CD209) was assessed in ST CD163⁺ macrophages. Patients with established RA (n=12) or PsA (n=10) were included as control. 4 μ m cryosections of ST in OCT were blocked for 10 min with 1% human immunoglobulins and incubated with primary (1–5 μ g/ml) and secondary antibodies. Imaging was performed with an inverted confocal microscope (SPE, Leica Microsystems) and glycerol immersion objective (ACS-APO 20x/NA 0.60). Single cell mean fluorescence intensity (MFI) was