AB0072 RATIONAL DESIGNED GOLD NANOPARTICLE SUPPRESSES RANKL-INDUCED OSTEOCLASTOGENESIS IN RAW264.7 CELLS VIA NF-KAPPA B PATHWAY AND MAPK PATHWAYS

Q. Yuan^{1,2}, X. Gao^{1,2}, ¹Key Laboratory for Biological Effects of Nanomaterial and Nanosafety, Institute of High Energy Physics, CAS; ²Department of Chemistry and Chemical Engineering, Beijing University of Technology, Beijing, China

Background: Bone erosion in joint is the most deleterious effect induced by rheumatoid arthritis (RA) and is the major cause of disability. New therapies are urgently required to prevent RA-associated bone destruction. The excessive bone resorption in RA was proved to be mediated by osteoclasts which are derived from the monocyte/macrophage lineage and induced by RANKL. Therefore, RANKL could be a potential target for the preservation of bone mass in RA.

Objectives: Chrysotherapy showed potential effects in reducing of joint destruction but with serious side-effects in RA therapy. A rational designed gold nanoparticle named GN was prepared to provide a choice for suppressing bone loss in RA therapy with more safety.

Methods: Mice macrophage cell line RAW264.7 was activated by RANKL to evaluate the potential osteoclastogenesis suppressing effects of GN. The differentiation, fusion and function of osteoclast were assessed by tartrate-resistant acid (TRAP) staining, actin ring formation assay and osteologic discs detection respectively. Relative expressions of osteoclast-specific genes were evaluated by RT-PCR and the activity of NF- κ B pathway as well as MAPK pathways were analyzed by immunoblotting.

Results: Osteoclast differentiation, fusion and bone resorption were activated by RANKL in RAW264.7 cell, while GN significantly attenuates this process in a dose-dependent manner. The expressions of osteoclast-specific genes including TRAP and OSCAR were increased by RANKL stimulation but were effectively suppressed by GN. The activation of NF-kappa B pathway and MAPK pathways induced by RANKL was also suppressed by GN treatment.

Conclusions: Our findings revealed that treatment with GN could prevent RANKL-induced osteoclastogenesis and reduce increased expression of osteoclast-specific genes which may through the suppression of NF- κ B and MAPK activation in macrophage. GN may provide a basis for the design of chrysotherapy drugs that effect in progression of RA-associated articular erosions.

Disclosure of Interest: None declared

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AB0073 ACCUMULATION OF ADVANCED GLYCATION END PRODUCTS (AGES) IN OSTEOARTHRITIC CARTILAGE IS RELATED TO AN IMPAIRMENT OF THE ADAPTATIVE MECHANISM OF GLYOXALASE-1

S. Trellu¹, A. Courties¹, S. Jaisson², B. Friguet³, X. Houard⁴, F.-P. Ehkirch⁵, C. Jacques⁴, F. Berenbaum¹, J. Sellam¹, ¹UPMC Univ Paris 06, Inserm UMRS_938, Rheumatology department, AP- HP, Saint-Antoine Hospital, Paris; ²University of Reims Champagne-Ardenne, UMR CNRS 7369, Laboratory of Biochemistry and Molecular Biology, Reims; ³UPMC Univ Paris 06, UMR 8256, Biological Adaptation and Ageing-IBPS; ⁴UPMC Univ Paris 06, Inserm UMRS_938, Saint-Antoine Hospital; ⁵Groupe Maussins, Clinique des Maussins-Ramsay, Général de Santé, Paris, France

Background: Advanced glycation end-products (AGEs) that result from a non-enzymatic reaction between a sugar and a protein, are generated during tissular ageing. Accumulation of AGEs could also be part of the osteoarthritis (OA) process by modifying the biomechanic properties of cartilage and by inducing chondrocyte activation. Glyoxalase-1 (Glo-1) is the main enzyme involved in the removal of AGEs precursors, especially the AGE carboxymethyllysine (CML).

Objectives: We aimed to quantify CML in human osteoarthritic cartilage, to investigate Glo-1 expression in chondrocytes and to study chondrocytic Glo-1 regulation in an inflammatory context.

Methods: 1) *Ex vivo*: Osteoarthritic cartilages from patients undergoing knee replacement were collected, dissected and incubated for 24h with or without IL-1 β (5ng/ml). We quantified CML in these cartilage explants using liquid chromatography and mass spectrometry, Glo-1 protein expression (Western Blot and immunohistochemistry) and Glo-1 enzymatic activity by measuring the kinetic of formation of the Glo-1 product (S-D lactoylglutathione) using spectrophotometry at 240nm during 10 minutes. 2) *In vitro*: Primary cultured murine chondrocytes (C57B6) were stimulated 72 h with increasing IL-1 β doses (0 to 10ng/mL). Glo-1 expression was assessed by quantitative RT-PCR, Western blot and enzymatic activity. To analyze whether oxidative stress is involved in Glo-1 regulation induced by IL-1 β , cells were treated with inhibitors of mitochondrial oxidative stress (MitoTEMPO) or of nitric oxide (NO) synthase (L-NAME).

Results: 1) *Ex vivo*, CML was found in all human OA cartilage samples and its level increased according to age (correlation coefficient CML/age: $r=0.78$, $p<0.01$). In parallel to CML increase, Glo-1 protein was expressed in chondrocytes on all layers of cartilage. A positive correlation was found between Glo-1 enzymatic activity and the age of the patients ($r=0.45$, $p<0.05$) but was lost in case of incubation with IL-1 β ($r=-0.09$, p non significant). 2) *In vitro*, in murine chondrocytes cultures, we observed a dose-dependent decrease of IL1 β -induced Glo-1 mRNA (0.67 fold, $p<0.05$), protein quantity (0.56 fold, $p<0.05$) and enzymatic activity (0.7 fold, $p<0.05$) ($n=5$). The blockade of NO by L-NAME, and of mitochondrial

oxidative stress by MitoTEMPO counteracted the downregulation of IL1 β -induced Glo-1 expression and enzymatic activity ($n=5$).

Conclusions: We show here that the age-dependent accumulation of AGEs in OA cartilage could be due to an impairment of the adaptative mechanism of Glo-1, mediated by oxidative stress. Further studies aiming at targeting Glo-1 restoration as a therapeutic strategy for ageing-related OA are needed.

Disclosure of Interest: None declared

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AB0074 INFLUENCE OF COBALT AND CHROMIUM IONS ON TGF-BETA EXPRESSION AND MINERALIZATION OF BONE FORMING CELLS IN-VITRO

S. Drynda^{1,2}, A. Drynda¹, J. Kekow², C.H. Lohmann¹, ¹Department of Orthopaedic Surgery, Magdeburg; ²Clinic of Rheumatology, Vogelsang, Germany

Background: Bone homeostasis is regulated at different levels. Transforming growth factor-beta (TGF- β) which exists in three isoforms in human and is stored in the latent form in the extracellular matrix is a major player in the regulation of osteoblast and osteoclast maturation, development, and function. The balance of bone formation and resorption is affected by numerous influences, such as hormone status, inflammation, and mechanical load.

In the aging population, supplied increasingly with artificial joint replacements, release of particles or ions from arthroplasty may exert local effects in the periprosthetic tissue. Cobalt and chromium ions are released through corrosion from metal-on-metal arthroplasty and modulate the gene expression level of several cytokines, chemokines and other mediators in bone cells.

Objectives: It was the aim of the study to analyse the effect of Co²⁺ and Cr³⁺ ions on the expression of TGF- β isoforms in bone forming cells and their impact on mineralization.

Methods: The study investigated the effect of Co²⁺ and Cr³⁺ ions on the expression of TGF- β 1-3 in the human osteosarcoma cell lines (MG63 and SaOs2) and primary human osteoblasts.

Cells (3×10^5) were seeded in 2 ml DMEM (10%FCS) into 12 well plates and stimulated with CoCl₂ and CrCl₃ in concentrations between 50–250 μ M for 24 hours. Total RNA was extracted and changes of expression levels of TGF- β 1-3 were analysed by real-time PCR using sequence-specific primers and probes.

For mineralization cells were cultivated for up to 4 weeks in DMEM (10% FCS) supplemented with 0.2mM ascorbic acid, 10nm dexamethasone and 10mM glycerophosphate and Co²⁺ and Cr³⁺. Calcium deposits were detected by 1% Alizarin Red S (pH 4.1) staining.

Results: The osteosarcoma cell lines as well as primary human osteoblasts isolated from bone explants expressed all three TGF- β isoforms, with TGF- β 1 as most abundantly expressed isoform. A dose dependent reduction of all TGF- β isoforms by Co²⁺ ions was observed, the strongest effect was found for TGF- β 2. In MG63 cells and primary osteoblasts the mRNA levels of TGF- β 2 decreased to 15 $\pm$ 4% and 17 $\pm$ 15% compared to the unstimulated control. The effect was lesser in SaOs2 cells with a reduction to 61 $\pm$ 7% compared to control. In contrast to bivalent Co ions, the trivalent Cr ions had no significant effect on the expression of all TGF- β isoforms.

While Co²⁺ in a concentration range of 50–250 μ M did not impair the mineralization, Cr³⁺ exerted a strong inhibitory effect on the mineralization in osteosarcoma cells and primary osteoblasts. In a dose dependent fashion mineralization was influenced already at concentrations of 50 μ M CrCl₃ and showed the strongest effect at 250 μ M.

Conclusions: The results of our study show that bivalent cobalt ions and trivalent chromium ions have different effects on bone forming cells. While Cr²⁺ affects the expression of the different TGF- β isoforms in osteoblast-like cells and primary osteoblasts, no inhibitory effect on mineralization in the tested concentrations was seen. Cr³⁺ however, did not influence the expression of TGF- β but strongly inhibited the mineralization in vitro.

Our data implicate that the inhibitory effect of metal ions such as Co and Cr ions on the transcription of the bone regulating cytokines TGF- β 1-3 and on bone forming activity may influence bone homeostasis.

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AB0075 TARGETING IMMUNE AND NON-IMMUNO SYNOVITIS BY 1,3,5-TRIAZINE-THIAZOLE VIA DUAL INHIBITION OF NF-KB AND EGFR-TKS FOR POSSIBLE BENEFIT IN RHEUMATOID ARTHRITIS

U.P. Singh¹, J.K. Shrivastava¹, A. Verma¹, H.R. Bhat², ¹Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad; ²Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, India

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease which is easily recognised by unrelenting inflammation, joint erosion, joint immobility and severe debilitation. The cause of the disease suggests that, T cells, B cells, macrophages, and dendritic cells gain access to the inflamed synovium causing progression of disease via generation of cytokines and autoantibodies.

The current available therapies to treat RA target the immune response which in turn adversely affects the immunity of the patients.

Objectives: The presents study deals with the discovery of 1,3,5-triazine-thiazoles targeting immune and non-immune synovitis by via dual inhibition of NF- κ B and EGFR-TKs for possible benefit in rheumatoid arthritis.

Methods: The 1,3,5-triazine-thiazole hybrid derivatives were synthesized via cascade of nucleophilic and cyclo-condensation reaction. These inhibitors was screened for NF- κ B transcriptional activity in RAW264.7 macrophages, whereas, EGFR-TKs inhibitor activity was assessed via kinase inhibition assay kit. The docking study was carried out with 3D-crystal structure of NF- κ B and EGFR-TK to explicate the inhibitory action.

Results: The designed hybrid analogues showed excellent inhibitory activity against both NF- κ B and EGFR-TK. Particularly, against NF- κ B, methyl (5c) containing molecule showed most significant activity (respectively with relative NF- κ B activity: 1.82 $\pm$ 1.87). Docking study suggests that, 5c was deeply buried in the DNA binding domain of NF- κ B interacting with Tyr57, Val58, Cys59, His141, and Val142. In EGFR-TK inhibitory assay, the synthesized molecules showed IC₅₀ ranging from 4.23–39.32 μ M via interacting with Leu788, Met766, Lys745, Glu762 with 3D crystal structure of EGFR-TK.

Conclusions: These results demonstrate the feasibility of 1,3,5-triazine-thiazole as dual inhibitor of NF- κ B and EGFR-TKs for the treatment of inflammatory disorders such as rheumatoid arthritis in more efficient way.

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Rheumatoid arthritis - etiology, pathogenesis and animal models

AB0076 PERIODONTAL MICROBIOTA IN EGYPTIAN RA PATIENTS AND THEIR RELATION TO SERUM AND GINGIVAL ANTI-CITRULLINATED PROTEIN ANTIBODIES AND OTHER DISEASE PARAMETERS

A.A. Abdelati. *Internal Medicine, Rheumatology Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt*

Background: A possible infectious trigger for RA is suspected at the gingival site. Emerging data implicates the microbiome in RA pathogenesis. Mucosal sites exposed to a high load of bacterial antigens, such as the periodontium, may represent the initial site of autoimmune generation. If validated, these findings could lead to the discovery of potential biomarkers and therapeutic approaches in the pre-clinical and clinical phases of RA⁽¹⁾.

Objectives: To determine the organisms causing periodontitis in Egyptian RA patients and their relation to serum and gingival ACPA level and other disease parameters.

Methods: This study was carried out on 100 Egyptian RA patients fulfilling the 2010 ACR/EULAR classification criteria for RA and of less than 5 years disease duration, recruited from Rheumatology Unit, outpatient clinic and Dental clinic at Alexandria Main University Hospital. RA disease activity was assessed by applying DAS28 and functional state of the patients was assessed by applying HAQ score. Dental examination, serum RF, and ACPA in serum and GCF were done for all patients. X-ray of both hands to detect erosions and severity of the disease. Gingival Crevicular Fluid (GCF) culture was performed for all cases with periodontitis for the three micro-organisms most reported in the literature to produce periodontitis (Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Prevotella intermedia).

Results: Of the 100 patient, 66 patient had periodontitis, for them, GCF culture was performed and Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Prevotella intermedia were found in 60.6%, 15.2%, and 30.3% of RA patients with periodontitis respectively. Gingival ACPA was detected in the 3 studied organisms, being of significant higher level with P.gingivalis than P.intermedia positive cases (p=0.047). No statistical significant difference detected on comparing P.gingivalis with A.actinomycetemcomitans or A.actinomycetemcomitans with P.intermedia. A. actinomycetemcomitans positive cases were associated with significantly higher level of CRP than P. intermedia positive cases (p=0.029), while no statistical significant difference was detected between P.gingivalis and A. actinomycetemcomitans or P. intermedia positive cases. There was no statistical significant difference between the three studied organisms regarding serum ACPA level, DAS 28, HAQ score, or X-ray findings of hands.

Conclusions: P.gingivalis is the most prevalent periodontal microbiota in Egyptian RA patients with periodontitis, that associated with significant higher level of gingival ACPA. None of the detected organisms correlated with the degree of RA activity or other disease parameters, apart from significantly higher CRP level with A. actinomycetemcomitans.

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AB0077 INVESTIGATION OF THE INFLUENCE OF TERIFLUNOMIDE PLASMA CONCENTRATIONS ON DISEASEACTIVITY IN LEFLUNOMIDE-TREATED PATIENTS WITH RHEUMATOID ARTHRITIS

R. Tekaya, A. Ben Tekaya, H. Sahli, O. Saidane, I. Mahmoud, L. Abdelmoula. *Rheumatology Charles Nicolle, Tunis, Tunisia*

Background: Leflunomide has become of increasing value for the treatment of rheumatoid arthritis (RA), and leflunomide is used via its active metabolite teriflunomide. The European League Against Rheumatism (EULAR) suggested that both LEF and methotrexate were fundamental medicines for the treatment of RA.

Objectives: To investigate the relationship between teriflunomide plasma concentrations and disease activity in patients with RA.

Methods: This was a cross-sectional multicenter study (four rheumatology departments) over a four years period. Patients with RA on a stable and daily leflunomide dose for >2 months were included.

Socio-demographic data and clinical data were recorded. Respectively, disease status and functional disability were assessed by disease activity score (DAS28) and Health Assessment Questionnaire (HAQ). Treatment response was evaluated according to the EULAR criteria (variation of DAS28).

Quantitative determination of teriflunomide (active metabolite of leflunomide) plasma concentrations was carried out by high-performance liquid chromatography with ultraviolet detection.

Results: A total of 24 patients were enrolled; sex ratio =1. The mean age of the sample was 51,71 ($\pm$ 15,3) years; the mean disease duration at study baseline was 135,2 ($\pm$ 80,26) months; 70% of patients were RF positive, and 58% ACPA positive. The mean score on VAS pain was 43 mm ($\pm$ 22,92). Respectively, the mean swollen and tender joint counts (SJC-28, TJC-28) were 4,3 ($\pm$ 6,1) and 2,8 ($\pm$ 3,09). The mean HAQ was 2,01 ($\pm$ 0,78). At baseline, the mean DAS28 score was 4,17 ($\pm$ 1,12); 56% were good and moderate EULAR responders.

The mean leflunomide treatment duration was 34,94 months ($\pm$ 32,47). The mean teriflunomide plasma concentrations was 38,34 μ g/ml ($\pm$ 24,92).

Residual teriflunomide plasma concentrations were significantly associated with SCJ-28 and DAS28 decrease (p=0,0027). However, all these parameters (VAS pain, TJC-28, HAQ, C reactive protein, prescription duration of leflunomide and EULAR response) were not correlated with residual teriflunomide plasma concentrations.

Conclusions: This study concluded that residual teriflunomide plasma concentration is associated with low active disease.

Disclosure of Interest: None declared

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AB0078 ROLE OF EXPERIMENTAL RESEARCH IN STUDY OF RHEUMATOID ARTHRITIS ETIOPATHOGENESIS

A.N. Goltsev, E.D. Lutsenko, M.V. Ostankov, N.A. Bondarovich. *Cryopathophysiology and Immunology Dept, Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine, Kharkov, Ukraine*

Background: To study the pathogenesis, diagnosis and therapy of rheumatoid arthritis (RA) there are used numerous experimental *in vivo* and *in vitro* models. Topical issue of this problem is to study the causes of disorder and rehabilitation of immune tolerance mechanisms in RA. The concept about the role of different cells in central and peripheral tolerance formation in this pathology is often contradictory and not clear. A tolerogenic activity of products of fetoplacental complex has long been studied at the Cryopathophysiology and Immunology Department of the Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine. The presence of a wide range of immunotropic substances in placenta is a premise to use placental cell suspension (PCS) for immunocompetent sphere recovery in autoimmune diseases.

Objectives: The research aim was to determine the possibilities and features of implementation of a tolerogenic activity by native and cryopreserved placental cells in experimental models of RA development: adjuvant arthritis (AA).

Methods: Research was carried out in CBA/H mice. The PCS was obtained via homogenizing the murine placenta to days 18–19 of gestation. The AA was induced by subplantar administration of the complete Freund's adjuvant. The AA development was expressed as the arthritis index. Either native (nPCS) or cryopreserved PCS were administered intravenously to day 7 after pathology induction. The PCS was cryopreserved under protection of either 10% dimethyl sulfoxide solution (suspension CD) or Propandiosakharol (suspension CP). A number of CD4⁺CD25⁺ T reg cells was determined by direct immunofluorescence