using monoclonal antibodies (BD Pharmingen TM) with flow cytometry (FACS Calibur, BD) in AA animal spleen. The *foxp3* gene expression in murine spleen cells was assessed by multiplex reverse transcription polymerase chain reaction (RT-PCR). In PCR we used the primer pairs to *foxp3* gene and that of housekeeping: β-actin. A number of gene transcripts was compared basing on the relative semi-quantitative estimation of amplification products using Agilent Bioanalyzer 2100 (USA).

Results: A disordered $CD4^+CD25^+$ cell accumulation in spleen of experimental animals with AA was established and possible use of PCS for its correction was demonstrated. The *foxp3* expression level in AA animal spleen cells reduced on day 28, suggesting a contribution of this factor into AA pathogenesis within a long-term period of pathology development. During this period a decrease in joint swelling correlated with an increased content of T-reg cells and *foxp3* expression level in spleen of animals after either nPCS or CD administration.

Conclusions: Therapeutic effect of either native or cryopreserved PCS in AA animals is manifested on molecular level, as evidenced by an increased *foxp3* expression in spleen cells after suspension administration. The effect of introduced cryopreserved placental cells as for this gene activation and, consequently, T-reg, was herewith determined by cryopreservation regimen.

References:

[1] Goltsev A.N., Lutsenko E.D. Ostankov M.V. Effect of different cryopreservation regimens on manifestation of immune modulating activity of placenta at development of adjuvant arthritis. Problems of Cryobiology 2008; 18(4): 456–459.

[2] Haque R., Lei F. Foxp3 and Bcl-xL cooperatively promote regulatory T cell persistence and prevention of arthritis development. Arthritis Research and Therapy 2010; 12: R.66.

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AB0079 TRANSDERMAL DELIVERY OF METHOTREXATE IN RHEUMATOID ARTHRITIS: ARE WE DEEP ENOUGH?

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Background: Methotrexate (MTX), at low doses, is the first choice in the management of rheumatoid arthritis (RA). Despite its effectiveness, the probability of its discontinuation remains high due to adverse effects such as gastrointestinal intolerance, bone marrow toxicity as well as hepatotoxicity with conventional oral and parenteral therapy.[1] Transdermal delivery epitomizes an attractive alternative for drugs with systemic toxicities. The physicochemical characteristics of MTX such as high polarity and ionisation at physiologic pH make the development of its topical route of delivery challenging.[2] A new class of liposomes termed deformable or flexible liposomes have been reported to possess the virtue of stress-dependent adaptability that enables them to squeeze through interstices of stratum corneum and increase the depth of skin penetration.[3]

Objectives: This study is intended to explore the transdermal route for the delivery of MTX in ameliorating its systemic toxicity without compromising the therapeutic effect in RA.

Methods: MTX entrapped in deformable liposomes were prepared and characterised for particle size (PS) and entrapment efficiency (EE). They were incorporated into a hydroxyethyl cellulose gel base and evaluated for *ex vivo* skin permeation. Optimized liposomal gel was applied on the back of rats (3x4 cm area) and evaluated for its acute dermal toxicity and pharmacokinetics. Biodistribution was studied by topical application of ^{125}I labelled MTX incorporated liposomal gel in rats. Furthermore the efficacy of optimized gel was determined in collagen induced arthritis (CIA) in rats.

Results: The optimized deformable liposomes exhibited a small PS of 110±20 nm and EE 35–50% while the liposomal gel showed a transdermal flux of 17.37±1.5 $\mu g/cm^2/hr$ in *ex vivo* skin permeation study. Topical application of liposomal gel depicted no clinical abnormalities or pathological changes at the site of application in rats. Pharmacokinetic data indicated sustained systemic delivery of MTX from its liposomal gel up to 48 hours. The gel resulted in lower accumulation of MTX in liver, kidneys and gut in contrast to intravenous administration of plain ^{125}I labelled MTX solution. In the CIA model, topical MTX gel administration demonstrated significant reduction in hind paw swelling and arthritic score, also validated by histological and radiographic examination of ankle joints and lowering of serum levels of cytokines like TNF-α and IL-6 in comparison to disease control group.

Conclusions: The liposomal gel displayed dermal safety, sustained systemic delivery of MTX and its lower distribution to the organs of toxicity which may enable alleviating systemic side effects. Moreover, liposomal gel of MTX showed appreciable therapeutic efficacy in the CIA model.

References:

[1] Braun J, Rau R. An update on methotrexate. Curr Opin Rheumatol. 2009;21(3):216–23.

[2] Prausnitz MR, Langer R. Transdermal drug delivery. Nat. Biotechnol. 2008;26(11):1261–68.

[3] Benson HA. Transfersomes for Transdermal Drug Delivery. Expert. Opin. Drug Deliv. 2006;3(6):727–37.

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AB0080 MODUALTION OF IMMUNOGLOBULIN G2B BINDING IN COMBINATION OF METHOTREXATE AND ACONITE IN A COLLAGEN-INDUCED ARTHRITIS SETTING

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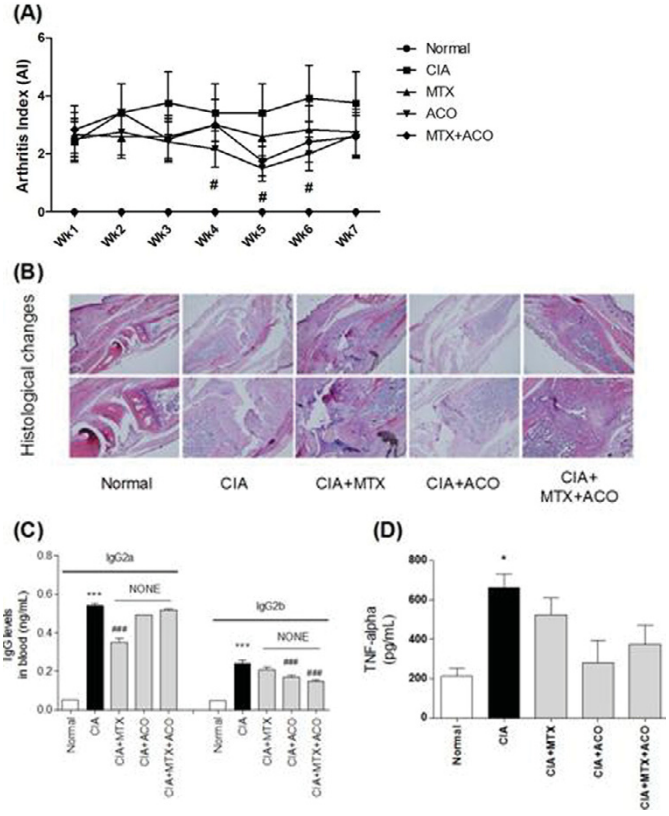
Background: Our previous study showed synergistic responses in TNF-alpha (TNF-α) and interleukin-6 with the combination of methotrexate (MTX) and aconite. Modulation of those cytokines has not been applied to rheumatoid arthritis (RA)-mimicked in vivo models.

Objectives: To translate in vitro effects of MTX, aconite, and MTX/aconite combination towards anti-arthritic responses in vivo, we investigated arthritis index (AI), histopathologic changes, and levels of TNF-α, immunoglobulin G (IgG) 2a and 2b in a collagen-induced arthritis (CIA) setting.

Methods: CIA was induced in five male DBA/OlaHsd mouse per group by intradermal injection of bovine collagen type II and Complete Freund's Adjuvant. In Day21, a bovine collagen type II and Incomplete Freund's Adjuvant were given for booster infection. The mice of arthritis onset were treated daily throughout Day49 with per oral administration of pre-investigated ratios of three to one; MTX (3 mg/kg), aconite (Aconibal®, 1 mg/kg), and MTX/aconite (3 and 1 mg/kg) combination. The AIs were evaluated every week. Histological changes, levels of TNF-α as well as IgG2a and IgG2b in blood using ELISA kit were evaluated at finals. Repeat measure and one-way ANOVA were analysed using SPSS (ver. 18.0 KO for Windows; SPSS Inc., Chicago, IL, USA) to evaluate inter-period and inter-group differences with Tukey's post-hoc tests.

Results: The CIA phenotypes adequately presented through three groups' AI reductions (CIA vs. MTX, aconite, or MTX/aconite; $p<0.001$, for three). There were differences of AI scores in aconite group from MTX one in week 4, 5, and 6 (MTX vs. aconite; $p=0.038$, $p=0.001$, $p=0.042$, respectively). Synergistic responses of AI were not shown any of three groups. The recoveries of synovial tissues were observed in MTX and MTX/aconite groups. The levels of TNF-α were not changed (aconite vs. MTX/aconite; $p=0.200$ and MTX vs. MTX/aconite; $p=0.700$). MTX group showed IgG2a reduction (CIA vs. MTX; $p<0.001$). Interestingly, MTX/aconite combination and aconite group slightly downregulated IgG2b levels as 80.8±5.6% and 90.5±7.4%, respectively (CIA vs. MTX/aconite; $p=0.001$ and CIA vs. aconite; $p=0.010$).



Conclusions: Synergistic in vitro effects of MTX and aconite combination brought

the partial in vivo phenotypic responses: Aconite showed more AI changes than MTX did in 4, 5, and 6 weeks. However, we found the presence of partial FcγRIIB affinity of binding modulation that MTX/aconite could enhance preventing monocyte/macrophage activation via immune complex in RA pathogenesis, as was other benefits of the combination except direct synergies.

References:

[1] Oh DS, Park G, Choi S. Characterising multiple molecule-modulating response of TNF-alpha and interleukin-6 by combination of methotrexate and aconite in interferon gamma-induced toxicity setting. Ann Rheum Dis 2016;75:Suppl2 909–910.

[2] Wijngaarden S, van Roon JA, van de Winkel JG, Bijlsma JW, Lafeber FP. Down-regulation of activating Fcgamma receptors on monocytes of patients with rheumatoid arthritis upon methotrexate treatment. Rheumatology (Oxford) 2005;44:729–734.

[3] Stewart R, Hammond SA, Oberst M, Wilkinson RW. Down-regulation of activating Fcgamma receptors on monocytes of patients with rheumatoid arthritis upon methotrexate treatment. J Immunother Cancer 2014;2:29.

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AB0081 REACTIVE OXYGEN SPECIES INHIBIT CATALYTIC ACTIVITY OF PEPTIDYLARGININE DEIMINASE

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Background: Protein citrullination is catalysed by pepdidylarginine deiminase (PAD) and plays an important pathogenic role in anti-citrullinated protein antibody (ACPA)-positive rheumatoid arthritis (RA), and possibly in other inflammatory diseases. PAD activity is dependent on calcium and reducing conditions.

Objectives: To determine the ability of H_2O_2 and reactive oxygen species (ROS) induced by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to regulate PAD activity.

Methods: Activity of recombinant human (rh) PAD2, rhPAD4 and PADs released from phorbol 12-myristate 13-acetate (PMA)-stimulated leucocytes was measured using an in-house PAD activity assay detecting citrullination of fibrinogen. PAD2 released from cells was measured using a luminex-based assay. The NADPH oxidase inhibitor diphenyleneiodonium (DPI) was used to inhibit ROS production in cells.

Results: At concentrations above 40 μM, H_2O_2 inhibited the catalytic activity of reduced rhPAD2 and rhPAD4. The inhibitory effect increased with increasing H_2O_2 concentration, reaching complete abrogation at 600 μM. PMA-stimulated leucocytes showed markedly higher PAD activity following inhibition of ROS formation with DPI. At a concentration of 10,000 μM, exogenously added H_2O_2 inhibited the catalytic activity of PAD released from PMA-stimulated leukocytes.

Conclusions: The ROS H_2O_2 directly inhibits enzymatic activity of PAD, and generation of ROS by NADPH oxidase down-regulates the activity of PAD released from stimulated leucocytes. This mechanism may play an important role in preventing hypercitrullination of proteins and thereby generation of self-antigens in RA.

Disclosure of Interest: None declared

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AB0082 ALKALINE PHOSPHATASE ELICITS PROPHYLACTIC AND THERAPEUTIC EFFECTS IN ARTHRITIC RATS

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Background: Alkaline phosphatase (AP) functions as a gate-keeper of the innate immune system [1] by detoxifying inflammation triggering moieties (ITMs). As an ectophosphatase, AP thus acts extracellularly by dephosphorylating ITMs that originate and are released from endogenous sources, e.g. by converting ADP and ATP nucleotides into adenosine to establish a key signalling anti-inflammatory effect. Consequently, AP activity prevents the production of pro-inflammatory cytokines by activated leucocytes and their downstream effects. Due to its broad mechanism of action, AP may potentially serve as an attractive therapeutic moiety in chronic inflammatory disorders, including rheumatoid arthritis (RA).

Objectives: To examine the anti-arthritic effects of prophylactic and therapeutic AP interventions in arthritic rats.

Methods: Wistar rats were immunized twice with methylated bovine serum albumin (mBSA), followed by local arthritis induction (intra-articular (i.a.) mBSA injection with 3 repeated injections) in the right knee (arthritic knee) with the contralateral left knee serving as internal control [2]. Interventions were performed using 200 μg human recombinant placental AP, administered subcutaneously, either before i.a. mBSA injections (2x, every 3 days, 2 rats/group; *prophylactic* setting) or after arthritis induction (4x, every 3 days, 4 rats/group; *therapeutic* setting). After *ex vivo* tissue distribution, knees were excised, fixed, decalcified and paraffin-embedded. Knee sections were examined for synovial macrophage infiltration by immunohistochemistry with ED1 (∼CD68) and ED2 (∼CD163) macrophage specific antibodies. Results were compared with untreated arthritic rats and arthritic rats receiving MTX therapy (1 mg/kg, intraperitoneally, 4x, every 3 days, 4 rats/group).

Results: Prophylactic and therapeutic schedules of AP treatment were well tolerated and reduced knee swelling comparable with MTX treatment. Following AP prophylactic intervention, synovial macrophage infiltration in the arthritic knees was reduced 4-fold (ED1) and 6-fold (ED2) when compared with affected knees of untreated arthritic rats, approaching macrophage counts in contralateral (non-arthritic) knees of AP treated rats. Therapeutic AP interventions resulted in 3.5-fold lower synovial infiltration of both ED1 and ED2 macrophages in arthritic knees, comparable with effects of MTX treatment.

Conclusions: AP, both as prophylactic and as therapeutic intervention, demonstrated favourable anti-arthritic efficacy in a rat model of arthritis. These studies warrant further preclinical and clinical evaluation as a putative novel therapeutic entity for arthritis.

References:

[1] Pike AF et al, Biochim Biophys Acta (2013);1832:2044–2056.

[2] Chandrupatla DM et al, Biomed Res Int. (2015), 509295.

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AB0083 SINGLE NUCLEOTIDE POLYMORPHISMS IN LEP – 2548 G>A AND LEPR + 668 A>G IN RHEUMATOID ARTHRITIS MEXICAN MESTIZOS ARE ASSOCIATED WITH AGE AT DIAGNOSIS, DISEASE ACTIVITY AND ANTI-CCP ANTIBODIES

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Background: The role of adipose tissue in RA pathogenesis has been acknowledged since the high frequency of dyslipidemia and insulin resistance in these patients. Leptin, a pleiotropic adipokine, has been associated with inflammation markers and articular damage in RA and the anti-citrullinated protein antibodies. Notwithstanding, these findings have not been constant across different populations. These points towards that single nucleotide polymorphism (SNP) in leptin and its receptor might influence the participation of this adipokine in RA pathogenesis.

Objectives: To determine the association of the SNPs *LEP* -2548 G>A and *LEPR* 668 A>G with adiposity, metabolic and inflammation markers in RA patients.

Methods: We enrolled 116 patients with RA (ACR 1987) matched with 133 control subjects by age, gender, and body mass index (BMI). Subjects were evaluated for fat mass and skinfold thickness. Also, serum glucose, insulin, lipid profile, serum leptin (sLep), soluble leptin receptor, TNFa. In patients with RA we evaluated disease activity and anti-CCP. Genotypes of *LEP* -2548 G>A and *LEPR* 668 A>G were determined by PCR-RFPL using *HhaI* and *MspI* restriction enzymes.

Results: There was no difference in genotypes distribution of LEP -2548 G>A and *LEPR* 668 A>G between RA and control. *LEPR* 668 G allele was associated with higher anti-CCP titers and disease activity score compared to *LEPR* 668A/A homozygotes, 4.2±1.7 vs. 3.46±1.2 *P*=0.012. *LEP* -2548A allele was associated with younger age of RA diagnosis vs. G/G homozygotes, 35.9±11.5 vs. 41.8±13.9 years old (*P* =0.045). OR for diagnosis before 40 years old was 2.7 (CI95% 1.04 – 7.45).

Conclusions: *LEP* -2548 G>A is related with a younger age at diagnosis of RA and *LEPR* 668 G/G was associated with increased anti-CCP titers and disease activity. This suggests that there is an additive effect between chronic inflammation of RA and obesity were leptin may favor humoral immune response against citrullinated proteins and influence the severity of RA.

In preobese and obese patients with RA anti-CCP (+) there is an increased sLep production. *LEP* -2548 G>A is related with a younger age at diagnosis of RA and *LEPR* 668 G/G was associated with increased anti-CCP titers and disease activity. These suggests that there is an additive effect between chronic inflammation of RA and obesity were leptin may favors humoral immune response against citrullinated proteins and influence the severity of RA.

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