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.

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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 ¹²⁵I 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 µg/cm²/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 ¹²⁵I 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.

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AB0080 MODULATION 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- α (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-arthritis 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