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-immuno synovitis by via dual inhibition of NF-κB and EGFR-TKs for possible benefit in rheumatoid arthritis.
Methods: The 1,3,5-triazine-thiazole hybrid derivatives were synthesized via cascade of nucleophillic and cyclo-condensation reaction. These inhibitors was screened for NF-kB 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-κB and EGFR-TK to explicate the inhibitory action.
Results: The designed hybrid analogues showed excellent inhibitory activity against both NF-κB and EGFR-TK. Particularly, against NF-κB, methyl (5c) containing molecule showed most significant activity (respectively with relative NF-κB activity: 1.82±1.87). Docking study suggests that, 5c was deeply buried in the DNA binding domain of NF-κB interacting with Tyr57, Val58, Cys59, His141, and Val142. In EGFR-TK inhibitory assay, the synthesized molecules showed IC_{50} ranging from 4.23–39.32μ 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 inhibitior of NF-κB and EGFR-TKs for the treatment of inflammatory disorders such as rheumatoid arthritis in more efficient way.
References:
[1] Collison J. Features of synovium in RA remission revealed. Nat. Rev. Rheumatol. 12, 316 (2016).
[2] Singh B, Bhat HR, Kumawat MK, Singh UP. Structure-guided discovery of 1,3,5-triazine-pyrazole conjugates as antibacterial and antibiofilm agent against pathogens causing human diseases with favorable metabolic fate. Bioorg Med Chem Lett. 24, 3321 (2014).
[3] Singh UP, Pathak M, Dubey V, Bhat HR, Gahtori P, Singh RK. Design, synthesis, antibacterial activity, and molecular docking studies of novel hybrid 1,3-thiazine-1,3,5-triazine derivatives as potential bacterial translation inhibitor. Chem Biol Drug Des. 80, 572 (2012).
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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

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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.
References:
[1] Brusca SB, Abramson SB, Scher JU. Microbiome and mucosal inflammation as extra-articular triggers for rheumatoid arthritis and autoimmunity. Curr Opin Rheumatol 2014;26:101–7.
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AB0077 INVESTIGATION OF THE INFLUENCE OF TERIFLUNOMIDE PLASMA CONCENTRATIONS ON DISEASEACTIVITY IN LEFLUNOMIDE-TREATED PATIENTS WITHRHEUMATOIDARTHRITIS

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Background: Leflunomide has become of increasing value for the treatment of rheumatoidarthritis (RA), and leflunomideisused via its active metabolite teriflunomide. The European League Against Rheumatism (EULAR) suggestedthatboth LEF and methotrexatewerefundamentalmedicines for the treatment of RA.
Objectives: To investigate the relationshipbetweenteriflunomideplasma concentrations and diseaseactivity in patients with RA.
Methods: This was a cross-sectional multicenter study (four rheumatology departments) over a four years period.Patients withRA on a stable and dailyleflunomide dose for >2 monthswereincluded.
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 concentrationswas 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 (±15,3) years; the mean disease duration at study baseline was 135,2 (±80,26) months; 70% of patients were RF positive, and 58% ACPA positive.The mean score on VAS pain was 43 mm (±22,92). Respectively, the mean swollen and tender joint counts (SJC-28, TJC-28) were4,3 (±6,1) and 2,8 (±3,09). The mean HAQ was 2,01 (±0,78). At baseline, the mean DAS28 score was 4,17 (±1,12); 56% weregood and moderate EULAR responders.
The mean leflunomide treatment duration was 34,94 months (±32,47). The meanteriflunomide plasma concentrations was 38,34 ug/ml (±24,92).
Residualteriflunomide plasma concentrations was significantly associated 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 residualteriflunomide plasma concentrations.
Conclusions: This studyconcludedthatresidualteriflunomide plasma concentration isassociatedwithlow active disease.
Disclosure of Interest: None declared
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AB0078 ROLE OF EXPERIMENTAL RESEARCH IN STUDY OF RHEUMATOID ARTHRITIS ETIOPATHOGENESIS

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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

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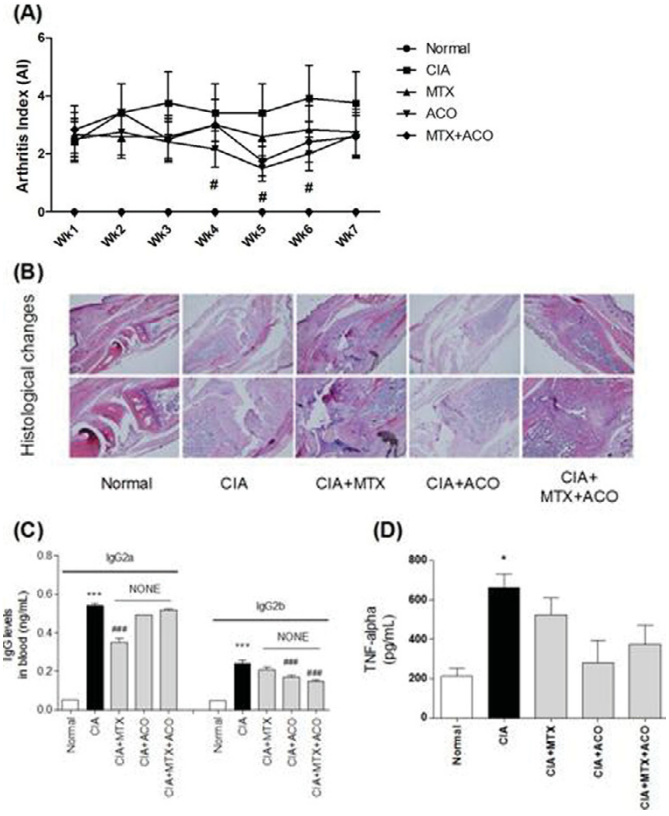
Background: Our previous study showed synergistic responses in TNF-alpha (TNF-α) and interleukin-6 with the combination of methotrexate (MTX) and aconite. Modulation of those cytokines has not been applied to rheumatoid arthritis (RA)-mimicked in vivo models.

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