AB0078

SYSTEMIC AND LOCAL IL-17A AND MIR-223 LEVELS IN RHEUMATOID ARTHRITIS PATIENTS

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Background: IL-17 is a proinflammatory cytokine, which overproduction promotes the autoimmune reaction in rheumatoid arthritis (RA). Recent studies have shown that IL-17 production in lymphocytes or its function could be regulated by miR-223 by targeting Roquin ubiquitin ligase or its receptors^{1–4}.

Objectives: To examine a possible correlation between the expression levels of miR-223 and IL-17A in peripheral blood (PB) and synovial fluid (SF) of RA patients.

Methods: Expression levels of miR-223 were determined in matched PB and SF samples of RA patients by relative quantitation method 2^{–ΔΔCt}. As reference control for normalisation RNU6B gene was used. Concentrations of IL-17A in matched serum and SF samples were determined by Human IL-17A ELISA kit (Gene probe, Diaclone). The results were compared to healthy control (HCs) as well as within the RA group.

Results: 58.73% of the RA patients showed overexpression of miR-223 in PB when compared to HCs (p=0.008) with AUC=0.673 (95 CI: 0.562/0.784), with 71.4% sensitivity and 46.9% specificity (p=0.006). miR-223 was overexpressed in 79.17% of RA SF (p=1.64 × 10^{–3}) when compared to HCs SF with AUC=0.841 (95 CI: 0.724/0.958) with 87.5% sensitivity and 72.7% specificity (p=4.6 × 10^{–4}). Within the RA group, SF miR-223 was underexpressed in 58.7% of the patients compared to its systemic levels. Levels of IL-17A were higher in RA SF compared to serum (8.645 pg/ml versus 0.315 pg/ml, p=0.012).

Conclusions: The difference between the systemic and local levels of miR-223 and IL17A in RA patients shows that the inflammatory disease process leads to their altered expression with a possible role of both molecules in the disease pathogenesis. The opposite changes in their systemic and local levels confirm the data about the possible role of miR-223 in regulating IL-17 function.

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AB0079

ALFACALCIDOL SUPPLEMENTATION MODULATES CYTOKINE PRODUCTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS CULTURE OF PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS

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Background: Hormone D and its analogues display immunomodulatory activities that provide a beneficial effect in immunoinflammatory diseases. However, whether this hormone has an additive immunosuppressive effect when it is used with corticosteroids has not been investigated, although these agents are commonly used together.

Objectives: Testing the immunomodulatory *in vitro* effects of vitamin D analogue, alfacalcidol, in peripheral blood mononuclear cells (PBMC) cultures of patients with active rheumatoid arthritis (RA).

Methods: Sixteen patients with active RA were enrolled in the study. Patients PBMCs were isolated, stimulated with PMA/Ionomycin and cultivated for 48 hours at 37°C, 5% CO₂ in cell cultures medium with or without supplementation. *In vitro* effects of supplementation with alfacalcidol (concentration

10 nM), calcitriol (concentration 10 nM), methylprednisolone (concentration 400 nM) and cotreatment with alfacalcidol/methylprednisolone on cytokine production were studied. Stimulated production of cytokines IL-6, IL-17, IL-21, TNF-α, IL-4, IL-10, TGF-β and IFN-γ were determined in cell culture supernatant by standard ELISA method.

Results: *In vitro* alfacalcidol supplementation reduces the production of proinflammatory cytokines IL-17 (p=0.001), IL-21 (p=0.001), TNF-α (p=0.002) and IL-6 (p=0.4), and induce more intense anti-inflammatory cytokine production IL-4 (p<0.0001), IFN-γ (p=0.05), TGF-β (p<0.0001) and IL-10 (p=0.09), in PBMC cultures of patients with active RA. There was no significant difference between the effects of alfacalcidol and calcitriol, except decreased TGF-β production (p=0.535) after calcitriol supplementation. Methylprednisolone supplementation leads to a significant reduction of the IL-6 (p=0.002), IL-17 (p=0.0001), TNF-α (p=0.0001) and IL-4 (p=0.017) production, while increased production of IL-21, IL-10, IFN-γ didn't reach statistical significance. Cotreatment with alfacalcidol and methylprednisolone leads to additional, more significant reduction of IL-17 (p=0.0001) and TNF-α (p<0.0001) in PBMCs of patients with active disease.

Conclusions: Alfacalcidol, *in vitro*, showed a significant immunomodulatory effect through the specific inhibition of Th1- cytokine production, while Th2 cell response was enhanced – “Th2 switch”. Our results demonstrate that alfacalcidol has significant additive effects on glucocorticoid-mediated inhibition of Th1 cytokine production when combined with methylprednisolone. These findings demonstrate the potential use of alfacalcidol as an immunosuppressive agent when combined with corticosteroids in Th1, but not Th2, immune response.

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AB0080

DIFFERENTIAL EFFECTS OF TR14 VERSUS DICLOFENAC ON PRO-RESOLVING LIPID MEDIATORS REVEALED BY RNASEQ

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Background: Anti-inflammatory agents are used widely in treating numerous pain and inflammatory conditions. With a focus on the specialised pro-resolving mediators (SPM) pathways in cutaneous wound repair in mice, the therapeutic activities of Tr14 (Traumeel), a multicomponent/multitarget natural product, and diclofenac (NSAID), a non-selective cyclooxygenase (COX) inhibitor were compared. COX inhibitors can block the synthesis of prostaglandins and thromboxanes from arachidonic acid, and can have important effects on the synthesis of resolvins and protectins produced by similar enzymes from eicosapentanoic and docosahexanoic acid substrates. Differential effects were identified via transcriptome analysis (RNAseq).

Objectives: To compare the transcriptomic changes after administration of Tr14 or diclofenac in a mouse cutaneous wound healing model, with particular emphasis on the SPM pathways, which include resolvins and protectins.

Methods: After abrasive wounding, the wounds were treated with topical Tr14 (34 mg/ml) in combination with subcutaneous Tr14 injections (9.5 mg/ml), or with subcutaneous Tr14 injections only, or topical diclofenac at clinically relevant doses (2 mg/ml). Skin samples were analysed for RNA transcript profiling by RNAseq at specific times (12 hour, 24 hour, 36 hour, 72 hour, 96 hour, 120 hour, 192 hour) after injury. Differentially expressed genes (DEGs) were computed at each time point between diclofenac vs control or Tr14 vs control, using EdgeR.

Results: At early time points (12–36 hour), Tr14-treated wounds, and to a lesser extent diclofenac-treated wounds, showed marked induction of 3 lipoxigenase