METHOTREXATE DOWNREGULATES P-GLYCOPROTEIN EXPRESSION AND INHIBITS THE ACTIVATION OF JAK2/STAT3 PATHWAY IN RHEUMATOID ARTHRITIS PERIPHERAL BLOOD MONONUCLEAR CELLS

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Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by abnormal synovial hyperplasia, inflammatory cell infiltration, and destruction of cartilage or bone. Early use of disease-modifying anti-rheumatic drugs (DMARDs) can significantly improve the patient’s condition. However, many patients had no response after treatment or gradually decreased after treatment for some time. DMARDs Multidrug resistance (MDR) is an important cause of the above phenomenon. How to reverse MDR in RA patients, and then control the disease is a major problem in RA treatment. The mechanism of MDR is complex. ATP binding cassette transporter super family consists of 48 transporters, ABCB1/P-glycoprotein (P-gp) is one of the major proteins associated with MDR. The expression of P-gp is induced not only by genotoxic mechanisms, but also by inflammatory cytokines such as IL-2, IL-4, IL-6, and TNF-α in some inflammatory diseases.

Objectives: The multiple drug resistance (MDR) to disease modifying anti-rheumatic drugs (DMARDs) is the key factor in Rheumatoid arthritis (RA) treatment field. P-glycoprotein (P-gp) encoded by multidrug resistance gene 1 (MDR1) is involved in the excretion of numerous DMARDs. We investigated the effects of methotrexate (MTX) alone and combined with interleukin 6 (IL-6) on P-gp expression and examined the signaling pathway involved in Peripheral blood mononuclear cells of RA patients.

Methods: The Peripheral blood mononuclear cells were extracted from Fifteen RA patients who were without any DMARDs and biological agents treatment. These cells were treated with 6L, 6L+MTX(0.1ug/ml), 6L+MTX (0.1ug/ml), 6L+MTX (1ug/ml) for 72h. P-gp expression was measured by flow cytometry and real-time polymerase chain reaction (RT-PCR); JAK2 and STAT3 were measured by RT-PCR; JAK2 and STAT3 expression were induced by IL-6.

Results: Methotrexate produce a dose-responsive reduction of both P-gp, JAK2 and STAT3 expression induced by IL-6.

Conclusion: Methotrexate downregulates p-glycoprotein expression and inhibits the activation of JAK2/STAT3 pathway in rheumatoid arthritis peripheral blood mononuclear cells.

REFERENCES

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INVESTIGATING THE ROLE OF TGF-β AND FATIGUE IN CHRONIC FATIGUE SYNDROME

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Background: Chronic fatigue syndrome (CFS) is estimated to affect up to 5% of people in Europe and is more common in women than men. It is characterised by unexplained fatigue, post-exertional malaise and a range of other symptoms. Recent studies indicate potential immune dysfunction in CFS specifically regarding cytokines and the adaptive behavioural response.

Objectives: This study aims to investigate serum transforming growth factor-beta (TGF-β) and the expression of the TGF-β Receptor 1 (TGFBR1) and TGF-β Receptor 2 (TGFBR2) genes, in relation to the fatigue associated with CFS.

Methods: Serum active and total TGF-β concentrations were measured in 117 CFS patients and 40 HCs using a TGF-β expression in arthritis SFs. Interestingly, after stimulation we observed that recombinant proNGF increases inflammatory cytokine production in patient MNCs, an effect that was abolished using p75NTR inhibitors. We also found that synovial fibroblasts (SFs) represent the main source of proNGF in the inflamed synoviae. At present it is not known whether proNGF can influence the activity of SFs that are key effector cells in synovia inflammation producing inflammatory mediators that regulate chondrocytes and osteoblasts activation.

Objectives: To investigate the mechanisms regulating p75NTR expression in synovial fibroblasts of arthritis patients and to evaluate the effects of its inhibition on the inflammatory response.

Methods: SFs from arthritis patients were used to study the activity of proNGF/p75NTR axis. SFs from osteoarthritis patients (OA) and skin fibroblasts from healthy donors (HD) were used as controls. p75NTR, NGF, and cytokine transcripts were evaluated by quantitative PCR (qPCR). Protein expression of p75NTR, NGF, and proNGF were analyzed by Western Blot. ELISAs were used to evaluate NGF, proNGF and cytokine concentration in supernatants and synovial fluids. p75NTR was inhibited using LM11A-31, a synthetic inhibitor that blocks the binding between p75NTR and its specific ligand proNGF. Results: mRNA and protein expression of p75NTR were up-regulated in arthritis SFs compared to OA SFs and skin fibroblasts. In vitro stimulation with recombinant cytokines (IL-1β, TNF, IL-6), TLR-ligands (such as LPS), and JIA synovial fluids, containing a mixture of inflammatory mediators, strongly increased p75NTR mRNA expression in arthritis SFs. Interestingly, after stimulation we observed that also OA SFs and HD fibroblasts up-regulated p75NTR transcript, suggesting that p75NTR levels are modulated by the inflammatory mediators. SFs of arthritis patients spontaneously produced proNGF and its release was further enhanced by all the above-mentioned inflammatory stimuli, albeit with different ability. Thus patient SFs can both produce proNGF and bind it through p75NTR. The inhibition of proNGF binding to p75NTR using LM11A-31 in arthritis SFs, activated with inflammatory cytokines or with synovial fluids, significantly reduced the expression and release of pro-inflammatory cytokine.