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

Kaipin Chen, Qin Kaili, Li Xiaofeng, Caihong Wang, the Second Hospital of Shandong University, Taiyuan, China

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 1 gene (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 IL-6, IL-6+MTX (0.1μg/ml), IL-6+MTX (0.1μg/ml), IL-6+MTX (1μg/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.

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

Acknowledgement: National Natural Science Foundation of China (No. 81471618).

Disclosure of Interests: None declared


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

Beth Dibnah1, Emmanuella Traianos2, Jessica Tam3, Dennis Lendrem4, Wan Fai Ng5* on behalf of United Kingdom Primary Sjögren’s Syndrome Registry.
1Newcastle University, Institute of Cellular Medicine, Newcastle, United Kingdom; 2NIHR Newcastle Biomedical Research Centre, Newcastle, United Kingdom

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 responsive luciferase bioassay. Expression levels of TGFBR1 and TGFBR2 were analysed using quantitative PCR. Fatigue was measured using the fatigue impact scale (FIS). FIS was categorised into three groups; ‘mild’ (0-80), ‘moderate’ (81-120) and ‘severe’ (121-160). Linear and ordinal regressions were performed on the continuous FIS and FIS categories respectively.

Results: Serum TGF-β concentrations in the CFS group did not differ significantly compared with the HC group (p=0.58). TGF-β concentrations showed no correlation with disease duration but there was a trend towards decreased TGF-β with increasing symptom duration. There were no significant differences between the levels of TGFBR1 and TGFBR2 in any of the fatigue groups, or between HCs. Active TGF-β concentrations were significantly elevated in the ‘severe’ FIS group compared to the ‘mild’ FIS group (p=0.04). Active/total TGF-β levels were significantly higher in the ‘severe’ FIS group than the ‘mild’ and ‘moderate’ FIS groups (p=0.02, p=0.03 respectively).

Conclusion: These data suggest no differences in serum concentrations of TGF-β or expression of TGFBR1 and TGFBR2, between the HC and CFS groups. It also suggests no differences in expression levels of TGFBR1/2 between any of the CFS fatigue groups. However, active/total TGF-β levels were increased in more severely fatigued patients based on FIS. This finding could be due to higher levels of circulating TGF-β, or increased amounts of TGF-β activation. Further work is necessary to confirm this finding in a larger cohort of CFS patients, and to explore how this increase in TGF-β relates to fatigue.

REFERENCES

Disclosure of Interests: None declared


P75NTR IN THE MODULATION OF INFLAMMATORY RESPONSE MEDIATED BY SYNOVIAL FIBROBLASTS

Luciaia Faresina1, Gaetana Minnone1, Marzia Solgo2, Luigi Manni2, Gian Marco Moneta3, Ivan Caiello1, Luigi Manzo3, Fabrizio De Benedetti1, Luisa Bracci-Laudiero1,2, 1Bambino Gesù, Division of Rheumatology, Roma, Italy; 2Consiglio Nazionale delle Ricerche, Istituto di Farmacologia Traslazionale, Roma, Italy; 3Università Degli Studi di Pavia – Scientific Center, Department of Medicine, Pavia, Italy

Background: Our previous studies showed high expression levels of p75NTR, the nerve growth factor (NGF) receptor, in mononuclear cells (MCNs) obtained from blood and synovial fluids of patients with juvenile idiopathic arthritis (JIA) and rheumatiod arthritis (RA), p75NTR binds with high affinity proNGF, the immature form of NGF whose concentration, as we recently demonstrated, is extremely high in the synovial fluids of arthritis patients. In ex vivo experiments we demonstrated that recombinant proNGF increases inflammatory cytokine production in patient MCNs, an effect that was abolished using p75NTR inhibitors. We also found that synovial fibroblasts (SFs) represent the main source of proNGF in the inflamed synovia. A previous study 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.

Disclosure of Interests: None declared
