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AB0128

ANTIOXIDATIVE PROTECTION AND MONOXIGENASE SYSTEM ACTIVITY OF CYTOCHROME P450 (MOG) OF NEUTROPHILIC LEUKOCYTES (NL) FROM SYNOVIAL FLUID (SF) IN RHEUMATOID ARTHRITIS (RA)

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Background: MOG system is present not only in the liver but also in far less quantities in other organs and tissues, including SF.

Objectives: SF in RA patient contains up to 95% of NL that determines the inflammation also by inhibiting MOG.

Methods: SF of knee joints of 8 patients with RA with active synovitis and 4 donors (D) were examined. NL have been isolated by standard methods. After the application of Triton-X-100 0,1% solution, NL has been centrifuged at 25000 g for 30 minutes. The supernatant has been used in the experiment. The activity of MOG has been estimated according to the level of cytochromes P450. P420 and b5 have been estimated by applying the method of differential spectrophotometry. Active forms of oxygen have been registered by applying electron paramagnetic resonance (EPR). The activity of the antioxidant enzymes - Cu-Zn superoxidismutase (Cu-Zn SOD), Se-glutathione peroxidase (Se-GPO) and catalase (CAT) was estimated by employing classical enzymology methods.

Results: It has been found out that in RA, the level of active oxygen forms increases by 68%. Meanwhile, the enzymes level of antioxidant defense is insufficient. In RA the activity of SOD, GPO and CAT enzymes is inhibited at 52, 66 and 39% respectively. In RA the activity of MOG (P450 and b5) in NL is 4 times lower than normal. The concentration of the inactive form of cytochrome P420, however, doubles. Nevertheless, their ratio P450/P420 (when normal = 5) in case of RA = 5,6 hardly ever changes.

Cytochrome concentration P450, P420 and b5 (nmol/g of protein), level of active oxygen forms (Units/mg of protein) and antioxidant system activity (SOD, GPO - Units/mg of protein; CAT- μ mol/min-mg of protein) in NL in RA

NL	P-450	P-420	b5	Level of active forms	Cu-Zn SOD	Se-GPO	CAT
D	1,25 ± 0,4	0,025 - 0,029 (0,027)	0,43 ± 0,07	15,7 ± 4,1	19,7 - 34,4 (27,1)	1,8 - 2,3 (2,1)	10,8 - 15,3 (13,1)
PA	0,3 ± 0,1	0,054 - 0,063 (0,058)	0,11 ± 0,03	49,4 ± 8,6	10,7 - 15,3 (13,1)	0,6 - 0,8 (0,7)	0,76 - 0,84 (0,8)

Conclusion: A fall in the level of free radical oxidation and antioxidant defense and faster generation of active oxygen forms in RA leads to NL being destroyed and creates favorable conditions for the development of autophagy and apoptosis. The oxidative stress during RA is accompanied by a fall in the activity of MOG and activation of lipid peroxidation and cytokine production in NL as shown earlier. A general fall in the activity of MOG (P-450 and b5) in NL in RA occurs against the background of enhanced conversion of P-450 into the inactive cytochrome P-420. This fact proves that the expression of isoenzymes P450s (CYPs) is reprogrammed whilst the autoimmune pathology is being developed and there is an unbalance of the immune oxygenase homeostasis in RA.

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AB0129

ULTRA-MICROSCOPIC ANALYSIS OF NURSING PHENOMENON IN SYNOVIAL TISSUE OF RHEUMATOID ARTHRITIS

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Background: Applying recent advances in electron microscope technology, cells and cell-cell interactions are observable using virtual slides, three-dimensional (3D) imaging, and serial sections of ultrathin thickness. Inflammation in rheumatoid arthritis (RA) is caused by multiple cell types, including infiltrating inflammatory cells, such as lymphocytes, neutrophils, macrophages, and spindle-shaped fibroblasts. Especially, we are focusing on fibroblast-like synoviocytes (FLSs). These cells have been reported to be positive for multiple markers including CD14, CD68, HLA-DR and vimentin, and we have shown that these cells are dendritic cells in our previous study using electron microscopy. Therefore, FLSs are presumed to function as dendritic cells. and these cells involved in the nursing phenomenon.

Objectives: The objective of this study is to investigate the chronicity mechanism of immunological inflammation in RA by using electron microscopy.

Methods: Synovial tissues collected from RA patients undergoing elbow or knee joint surgery were prepared for this study. Using wide field of view images of the synovial tissue, areas containing the nursing phenomenon were selected by using multiscale electron microscopy. 3D structures of the features were constructed from series of micrographs obtained by using focused ion beam-scanning electron microscopy and those by using transmission electron microscopy with double-axis electron tomography (DA-ET).

Results: From multiscale electron microscopy, many synovial dendritic cells and plasma cells were present in various areas of the lymphnodular infiltration. Serial cross-sectional observation with an FIB-SEM and 3D images revealed that synovial dendritic cells patronized the plasma cells, wrapping around the plasma cells, by the long slender axes. A tomogram of an ultra-thin sliced section created by using DA-ET revealed that synovial dendritic cells formed a membranous fusion with the plasma cells.

Conclusion: The nursing phenomenon involves characteristic histopathological features, which may be a factor in the chronicity of the inflammation mechanism in RA synovial tissue.

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AB0130

THE EFFECTS OF METHOTREXATE ON THE VASCULAR ENDOTHELIUM

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Background: Anti-inflammatory therapy has been recently validated as a treatment option for patients with cardiovascular disease (CVD) [1]. The anti-inflammatory drug methotrexate (MTX) reduces cardiovascular risk in patients with systemic inflammatory rheumatic diseases [2]; possibly by improving endothelial function [3]. However, the molecular basis of the drug's anti-inflammatory and potential anti-atherogenic effects on the vasculature is not well described.

Objectives: Our goal was to characterize the actions of MTX on vascular endothelial cells (EC).

Methods: MTX-treated human umbilical vein (HUVEC) or aortic endothelial cells (HAEC) were analyzed by quantitative real-time PCR (qRT-PCR), immunoblotting and phospho-kinase activity arrays. EC were treated with tumor necrosis factor (TNF)- α to induce pro-inflammatory activation. EC viability and cell cycle were evaluated by flow cytometry using Annexin V and/or propidium iodide staining. For selected experiments, EC were exposed to laminar (LSS; 20dyn/cm²) or oscillatory shear stress (OSS; 0 $\pm$ 5dyn/cm², 2Hz) using a parallel plate model.

Results: Under static conditions, MTX independently increased the activity of multiple kinases in quiescent and TNF α -activated EC. MTX-activated kinases