
NEW METHOD TO ASSESS SKELETAL MUSCLE ATROPHY IN COLLAGEN INDUCED ARTHRITIS (CIA) MODEL

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Background: Muscle quality in rheumatoid arthritis (RA) is a new concept that involves morphological features and function. A measurement that associates cell morphology with clinical and physical parameters has not been reported in the literature. CIA is a RA mice model characterized by loss of muscle mass similar to human RA, thereby it is a convenient model to study the disease impact in muscle physiology.

Objectives: Develop an index to measure the morphometry of muscle fibers and correlate the morphological features with the muscle functional performance in CIA.

Methods: 18 DBA/1J mice were induced using complete Freund's adjuvant and a booster after 18 days induction. Along experimental phase, we evaluated muscle strength using grip strength test and clinical disease score after onset of disease. 16 healthy mice were used as control. After 25 days CIA induction, 8 CIA and 8 controls were euthanized for muscle evaluation in mild disease and, at day 50, 8 CIA and 8 control mice were euthanized for evaluation in severe disease. Tibialis anterior were collected for myofiber histological analysis. Using the Image Pro Plus software (Media Cybernetics, China), we segmented muscle fibers and assessed its area and its regularity (the last through an index named Muscle Fiber Irregularity Index, MFII). The index works as a threshold to set to screen the degree of normal, atrophic and hypertrophic based on the morphometry if muscle fibers. Values are compared to area and shape of control (healthy) fibers. Frequency analysis and Pearson Correlations were used and statistical significance was considered as p<0.05.

Results: We found 1.5% atrophic muscle fibers in control animals. Mild CIA showed the same atrophic muscle fibers percentage compared to control. However, severe CIA showed 11.8% of atrophic muscle fibers. Decrease muscle strength in CIA over time were associated with a greater atrophic muscle fiber proportion (r=-0.8, p=0.021) and increased disease score (r=-0.8, p=0.019).

Conclusion: Here we developed a new, objective method applied to screen for muscle quality through the morphometry of muscle fibers. Muscular Morphometric Analysis (MusMA) has potential to be used in combination with clinical parameters in several human pathophysiological analysis. Besides that, we can speculate that although muscle strength is associated with atrophic cell percent-age, loss of strength does not only depend of atrophy, but disease activity also seems to influence muscle strength reduction.

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AB0087 FEATURES OF ANTIPHOSPHOLIPID ANTIBODIES SPECTRUM IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Currently, some data have been accumulated on the participation of antiphospholipid antibodies (aPL) in the development of thrombotic complications in patients with autoimmune rheumatic diseases (ARD), in particular, in patients with systemic lupus erythematosus (SLE). The most studied aPL in this pathology are IgG and IgM antibodies to cardiolipin (aCL), anti-β2-glycoprotein 1 antibodies (aβ2-GP 1), lupus anticoagulant (LA). The participation of IgG and IgM antibodies to prothrombin (aPT) and to annexin V (aAnV), antibodies to oxidized low density lipoproteins (aOxLDL) in hypercoagulation and the development of thrombosis is also discussed. However, the studies focusing on the investigation of aPL in patients with rheumatoid arthritis (RA) are few.

Objectives: To estimate the levels and the frequency of occurrence of aPL in patients with RA in comparison to the SLE patients and the control group.

Methods: The study included 85 female patients with ARD (RA (n=45), mean age 43,0 (33,0; 52,0) years old, disease duration 9,0 (5,0; 13,0) years, disease activity (DAS28= 5,37 (4,69; 5,89) points) and SLE (n=40), mean age 33,5 (27,5; 44,5) years old, disease duration 8,0 (5,0; 14,5) years, disease activity SLE-DAI-2K 7,0 (4,0; 11,5) points). Fifty four healthy women (mean age 38,5 (35,0; 46,0) years old) formed the control group.

The levels of antiphospholipid antibodies (IgG/IgM aCL, aβ2-GP 1, aAnV and aPT, aOxLDL) were determined with ELISA according to the instruction of a manufacturer. LA was determined by one-stage clotting assay using reagents for screening and confirmation (Technoclot LA Screen and Technoclot LA Confirm, Austria).

Results: The frequency of occurrence of elevated levels of all investigated aPL in patients with RA was similar to SLE patients and was revealed in 57,8% of cases for IgG/IgM aCL, 44,4% for IgG/IgM aβ2-GP-1, 26,7% for IgG/IgM aAnV, 8,9% for aPT, 52,6% for LA, 64,4% for aOxLDL. The patients with SLE had an increased levels of IgG/IgM aCL in 60,0% of cases, IgG/IgM aβ2-GP-1 in 57,5%, IgG/IgM aAnV in 15,0%, IgG/IgM aPT in 17,5%, high levels of LA in 68,8%, of IgG aOxLDL – in 80,0% of cases. The control group had a high levels of IgG/IgM aCL in 1,8%, IgG/IgM aβ2-GP-1 in 3,7%, IgG/IgM aAnV in 5,6%, IgG/IgM aPT in 1,8%, high levels of IgG aOxLDL – in 42,6% of cases. None of the controls had an increased level of LA. The frequency of occurrence of elevated levels of aPL and their mean levels in both groups of patients with ARD was higher as compare to the control group (p<0.05).
The mean levels of IgG aI2-GP-1, LA, aRoXXLDL in RA patients and mean levels of LA, aRoXXLDL in RA patients were above standard values but were similar in both groups. Moreover, the mean levels of IgM aCL, IgG aI2-GP-1, IgG1G2M aANV and aPT were comparable in patients with SLE and RA. However, the mean levels of IgM aCL in RA patients was higher than in SLE patients. This may indicate an increased autoimmune activity.

Simultaneous evaluation in four types of aPL levels was observed in 4.4% patients with RA, in 2.5% - with SLE; simultaneous evaluation in three types of aPL was revealed in 11.1% patients with RA, in 17.5% - with SLE; two types of aPL were increased in 35.6% patients with RA and 27.5% - with SLE. Only one type of elevated aPL levels (12.9%) was indentified in the control group.

Conclusion: Thus, patients with RA are characterized by a wide range of aPL. Qualitative and quantitative changes in the levels and types of autoantibodies in patients with RA have been established similar to those in SLE patients.

In patients with autoimmune rheumatic pathology, the presence of simultaneously elevated several types of aPL have been proved.

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**Background:** Many blood vessels are generated in the hyperplastic synovial tissue of patients with rheumatoid arthritis (RA), and lead to chronic tissue inflammation and joint destruction [1]. Janus kinase (JAK) family consisting of JAK1, JAK2, JAK3 and tyrosine kinases 2 (TYK2) are the major regulators of cytokine-mediated signaling pathways. JAKs are important for the proliferation and survival of RA fibroblast-like synoviocytes (RA-FLS), and the angiogenesis of HUVECs activated by VEGF.

**Methods:** The cell line of HUVECs were used for this study. The activity of proliferation and tube formation were analyzed by counting assay and tube formation assay, respectively.

In counting assay, HUVECs (5 × 10⁴ cells/ml) were seeded onto 96-well cell culture plate with 20 ng/ml VEGF including various doses (0.1 μM, 1 μM, 5 μM) of TOF, BAR or PEF. After 48 hours incubation at 37°C in a humidified atmosphere containing 5% CO₂, cell proliferation of each groups was assessed using cell counting kit. In tube formation assay, HUVECs (5 × 10⁴ cells/ml) were treated with 20 ng/ml VEGF including various dose (0.1 μM, 1 μM, 5 μM) of TOF, BAR or PEF for 00 hours, then seeded onto 48-well plate with Matrigel. After 24 hours incubation on Matrigel, the cell activity was photographed using phase contrast microscopy. Tube formation were quantitated by measurement of the length of branch.

Results: HUVECs were activated in proliferation and tube formation by VEGF treatment. And, the proliferation and tube formation of HUVECs activated by VEGF were suppressed by All of TOF, BAR and PEF. In particular, TOF and PEF could suppress them highly.

Conclusion: This study showed the inhibitory effect of JAK inhibitors on proliferation and tube formation of HUVECs activated by VEGF. In particular, the angiogenesis of HUVECs activated by VEGF was highly suppressed by TOF and PEF. VEGF is reported to regulate the angiogenesis through multi JAK-STAT signaling pathways [6]. The inhibitory effects on angiogenesis of TOF, BAR and PEF might depend on the differences in their affinity for JAKs. VEGF has been shown to have a central involvement in the angiogenic process in RA [7]. JAK inhibitors might suppress the angiogenesis in RA synovial tissues by inhibiting VEGF signaling.

References:

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