THU0058 APOPTOSIS, SEX HORMONES AND ADENOSINE DEAMINASE LEVELS IN CULTURED MONOCYTES (THP-1 CELLS) AND SYNOVIAL RHEUMATOID MACROPHAGES TREATED WITH METHOTREXATE

ME Cutolo, B Villaggio, A Sulli, A Bisso, C Pizzorni, M Briata, M Grosso, C Prete, L Felli, B Seriola. *Department Internal Medicine Division Rheumatology, University of Genova, Genova, Italy*

10.1136/annrheumdis-2001.902

Background Both testosterone (T) and methotrexate (MTX) seem to exert negative effects on cell growth that might partially explain some of their antiproliferative/antiinflammatory effects exerted in rheumatoid arthritis (RA).^{1,2}

Objectives Therefore, we evaluated the T and MTX effects on cell apoptosis (apo) in cultured human myeloid monocytic cells (THP-1) differentiating into macrophages (M) and in cultured synovial M obtained from RA patients. Since extracellular adenosine increase seems implicated in the MTX action, we evaluated also the enzyme adenosine deaminase (ADA) levels. ADA is involved in adenosine metabolism.

Methods Synovial RA M as well as the THP-1 cells induced to differentiate into M (IFN gamma 500U), were cultured in presence of T (10–8M) and 17-beta estradiol (E2,10–8M) alone. The cells were also treated with the MTX concentrations present *in vivo* during RA therapy (= 50 microg/ml) again alone, or in association with T plus MTX or E2 plus MTX. After 24 h early and late apo were evaluated by immunofluorescence (annexin V and propidium iodide assay, respectively). ADA was evaluated on supernatants by spectrophotometry.

Results By considering the THP-1 cells, a significant induction of the late apo in T (17%) (p less 0.05), MTX (80%) alone, and T (72%), E2 (74%) plus MTX-treated cells was observed (all p < 0.001). No significant differences were observed during the early apo for all the treatments during the 24 hr. By evaluating the RA synovial M, a significant (p < 0.001) induction of the late apo (50%) was observed in the T plus MTX-treated M when compared with the alone MTX- or E2-treated M. The treatment of RA M with T alone was found to induce an evident cell apo (30%) (although non significant). ADA levels increased only in the supernatants from all the MTX-treated cells vs MTX-untreated controls, mainly in THP-1 cells.

Conclusion The T and MTX-induced apoptotic effects were more evident in cultured THP-1 cells than in cultured RA M. Therefore, immature differentiating monocytes (i.e. infiltrating monocytes) seem more sensible to the pharmacologic effects of MTX than differentiated cells (i.e. resident M). The significant increase of ADA levels found only in MTX-treated cells, might support the already suggested implication of adenosine level increase as part of the pharmacologic effects exerted by MTX.

REFERENCES

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THU0059 INTEGRATIVE APPROACH TO STUDY OF IMMUNE RESPONSE TO CERULOPLASMIN IN RHEUMATIC DISEASES

EA Sushchuk, NV Petrova, IP Gontar, AB Zborovskiy. *Laboratory of Immunology, Scientific Research Institute for Clinical and Experimental Rheumatology of RAMS, Volgograd, Russia*

10.1136/annrheumdis-2001.903

Background Ceruloplasmin (CP) is a multi-copper oxidase with incompletely determined biological functions. It plays a role in acute phase response, has free-radical scavenger properties, antibodies (AB) to CP in some connective tissue diseases have been reported.

Objectives To determine changes in CP activity and levels in serum, and detect AB to CP in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients.

Methods Serum levels of immunoreactive CP (by commercial ELISA test), oxidase activity of the CP, as well as the specific oxidase activity (enzyme activity per unit of mass) were measured in 76 RA patients (49 female and 27 male) and in 56 SLE patients (96% female) at admittance to hospital and two weeks later. Patients were examined by clinical, laboratory, immunological and radiographic methods, diagnosis was established according to American College of Rheumatology criteria for RA (1987) and SLE (1997). Antibodies to CP were detected using custom developed ELISA method. Antibody binding was considered positive, when optical density readings were above 2 standard deviations of all normal human sera, 46 healthy donors served as a control group.

Results Serum levels of CP, oxidase activity and specific oxidase activity were significantly higher in the group of RA and SLE patients than in the control group (p < 0.05). Increase in oxidase activity and CP levels were more prominent in RA patients with moderate and maximum disease activity, compared with minimal activity of disease (p < 0.001) and correlated with SLE-DAI score in SLE. Antibodies to CP were detected in 78% of RA patients with visceral involvement and in 43% of patients without visceral lesions. In SLE patients AB to CP were detected in 46%. Specific oxidase activity of CP was significantly lower in patients with presence of AB to CP compared to patients with no AB to CP in serum (p < 0.05). After 2 weeks of treatment decrease in serum levels of CP and oxidase activity was observed, correlating with decrease in levels of non-specific markers of inflammation (C-reactive protein, ESR). Decrease in specific oxidase activity of CP in patients without AB to CP in serum was statistically significant (p < 0.001), while no or insignificant decrease was observed in the group of patients with AB to CP. After 2 weeks of treatment the proportion of patients, having AB to CP reduced, but the reduction was statistically insignificant. Specific oxidase activity was more sensitive indicator of active inflammation in these diseases than levels of CP in serum or CP oxidase activity in patients without AB to CP, but this sensitivity was diminished in presence of AB to CP.

Conclusion Antibodies to CP were detected in serum of RA and SLE patients, capable of decreasing oxidase activity of CP, thus leading to diminished specific oxidase activity in such patients. Since oxidase activity of CP is essential for its antioxidant properties, these AB to CP can play an important role in free-radical tissue injury in rheumatic diseases. Levels of CP in serum and oxidase activity of CP can be used as markers of inflammation in RA and SLE.

THU0060 SERUM YKL-40 IN RHEUMATOID ARTHRITIS: CLINICAL CORRELATIONS

M Carrabba, M Chevallard, E Torchiana, PC Sarzi Puttini, S Santandrea, L Boccassini, M Antivalle, G Santalena, MG Marrazza. *Rheumatology Unit, Luigi Sacco University Hospital, Milan, Italy*

10.1136/annrheumdis-2001.904