SAT0117 PROGLUMETACIN AND THE PREVENTION OF ECTOPIC OSSIFICATION FOLLOWING TOTAL HIP REPLACEMENT

¹KK Förster, ²A Thiede, ²J Haasters. ¹Department of Clinical Research, Opfermann Arzneimittel GmbH, Wiehl, Germany; ²Orthopaedic Department, Ostseeklinik (Baltic Sea Clinic), Damp, Germany

10.1136/annrheumdis-2001.513

Background Ectopic ossifications (EO) following total hip replacement are possible and known post-operative complications, developing in about 60% of patients during the first 4–12 weeks after implantation. There is no treatment but surgery, therefore preventive measures are considered to be indispensable. Proven and sufficient measures to prevent EO are radiation treatment or treatment with non-steroidal anti-inflammatory drugs (NSAIDs). These measures are able to reduce EO to occur in about 10% of prophylactically treated patients.

Objectives The objective of this retrospective study was to evaluate our practice of treating patients postoperatively with the NSAID proglumetacin – a molecular combination of indomethacin with the gastroprotective substance proglumid – and to compare the success rate with that of competitive preventive treatments.

Methods During the years from 1995 to 1997 there were 560 patients in our orthopaedic department who underwent total hip replacement, in most of them because of osteoarthritis of the hip. Other reasons for surgery were femur head necrosis, rheumatoid arthritis, femoral neck fractures or loosening of an already existing hip endoprosthesis. Immediately after surgery, 2 weeks and again 2 months later, and, if possible, also 6 months later, control X-rays were performed. Signs of ossification were objectified using the Brooker classification.

Results 545 of these patients (573 women, 188 men; mean age: 66.8 ± 9.7 years; body weight: 75.6 ± 13.2 kg; body length: 167.3 ± 8.5 cm) were postoperatively treated for 3 weeks with proglumetacin. X-ray films of all patients after surgery showed no ossification, just as after 2 weeks the films of 506, after 2 months of 473, and after 6 months of 263 (of 329 re-examined) patients. Clinically not relevant ossifications (class I or II according to Brooker) were seen after 2 weeks in 37 patients (6.8%), after 2 months in 57 patients (10.5%) and after 6 months in 65 (11.9%) patients. Of all patients treated for at least one week no one (0.0%) showed a clinically relevant ossification (class III or IV). Two patients showing class III ossification were prophylactically treated only for 6 and 4 days, respectively. Concerning EO occurrence, in our patients there was no correlation with gender, kind of prosthesis or anaesthesia, duration of hip disease, or risk factors like already performed total hip replacement and diabetes mellitus, respectively.

Concerning safety only 43 patients (7.9%) complained of minor or increasing gastric adverse drug reactions, requiring additional gastroprotective drug therapy or even stop of treatment.

Conclusion In conclusion, the non-steroidal anti-inflammatory drug proglumetacin is very safe and at least as effective as other preventive measures and very safe. In our view, proglumetacin therefore represents a favourable and economic alternative treatment.

REFERENCES

- 1 Brooker AF, et al. Ectopic ossification following hip replacement: incidence and a method of classification. *J Bone Joint Surg Am.* 1973;**55**:1629–32
- 2 Eulert J, Knelles D, Barthel T. Heterotopie Ossifikationen. *Orthopäde* 1997;**26**:399–406

- 3 Förster KK, Haasters J, Schmid K, Thiede A. Prevention of heterotopic ossifications after total hip replacement with a non-steroidal anti-inflammatory drug (abstract). *Eur J Clin Pharmacol.* 1997:A11
- 4 De Pouvourville G. Evaluating the real cost of NSAID therapy; "shadow costs" relating to the treatment of gastrointestinal side effects. *Br J Med Econ.* 1992;**5**:45–50

Cell receptor-ligand interaction signalling and activation

AB0008 CERULOPLASMIN, TRANSFERRIN AND INTRACELLULAR ADHESION MOLECULE-1 IN PATIENTS WITH RHEUMATOID ARTHRITIS

¹S Cogalgil, ¹K Yildirim, ²S Tayli, ²F Akcay. ¹Physical Therapy and Rehabilitation; ²Biochemistry, Arastirma Hospital, Erzurum, Turkey

10.1136/annrheumdis-2001.514

Background

Objectives We studied serum levels of soluble intercellular adhesion molecule-1 (sICAM-1), ceruloplasmin (CP) and transferrin (Tf) and investigated the correlation of these parameters with the disease activity.

Methods Serum sICAM-1 levels were determined with sandwich enzyme-linked immunosorbent assay (ELISA) in sera from 42 patients with RA and in 30 healthy controls. Erythrocyte sedimentation rate (ESR) was determined according to the Westergren method and C-reactive protein (CRP), CP and Tf by nephelometric method. Disease activity was assessed by disease activity criterias.

Results Although decreased serum Tf level, serum levels of sICAM-1 and CP were significantly higher in patients with RA than in healthy controls. It was found that sICAM-1 had a negative correlation with Tf ($r = -0.47$, $p < 0.01$) and a positive correlation with CP ($r = 0.49$, $p < 0.001$). There was a weak but statistically significant positive correlation between sICAM levels with Ritchie articular index (RAI) score and CRP ($r = 0.32$, $p < 0.05$; $r = 0.44$, $p < 0.01$, respectively), whereas no significant correlation was observed between sICAM-1 levels with ESR, age and disease duration. There was no correlation between values of CRP, RAI and ESR with serum CP and Tf.

Conclusion These data show that the decreases in serum CP and Tf levels and increases in sICAM-1, ESR and CRP levels are present in RA, and that the decrease in serum CP and Tf levels in RA might be due to increased sICAM-1, and increased levels of sICAM-1 and correlations with other parameters may be a significant and novel marker for evaluating the disease status and the activity of RA.

OP0001 REDOX-SENSITIVE CHANGES IN CONFORMATION AND CELLULAR LOCALIZATION OF LAT AND DOWNSTREAM TCR SIGNALLING LEAD TO HYPORESPONSIVENESS OF SYNOVIAL FLUID T CELLS IN RHEUMATOID ARTHRITIS

SI Gringhuis, PH Remans, EA Papendrecht-van der Voort, A Leow, EN Levarht, FC Breedveld, CL Verweij. Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

10.1136/annrheumdis-2001.515

Background In rheumatoid arthritis (RA), the synovial fluid (SF) T lymphocytes present in the inflamed joints, display

hyporesponsiveness upon engagement of the TCR/CD3 complex despite phenotypic evidence of former activation. We have previously shown that the central and crucial adaptor protein LAT (linker for activation of T cells), which plays a central and crucial role in the T cell receptor (TCR)-mediated signalling pathways, exhibits deficient phosphorylation due to displacement of the integral membrane protein from the plasma membrane in SF T lymphocytes. SF T lymphocytes exhibit several features of chronic oxidative stress, e.g. severely decreased intracellular levels of glutathione (GSH), and our previous studies have indicated that the subcellular localization of LAT is sensitive to changes in the intracellular GSH levels. The cysteine-to-serine substitutions of several cysteine residues (C26/29 or C117) within LAT creates LAT mutants that are resistant to reduced intracellular GSH levels and remain membrane-anchored in GSH-depleted cells.

Objectives

Methods

Results In this study, we have used the redox-insensitive LAT mutants to study the effect of redox balance alterations, like in SF T lymphocytes, on TCR signalling pathways downstream from LAT and on CD28 signalling pathways. In co-transfection experiments, we show that the presence of the redox-insensitive LAT mutants allows for the partial restoration of the TCR-mediated signalling pathways, but not the signalling pathways induced through the CD28 receptor. The data are indicative that the Raf1-ERK and the calcium-calcineurin pathways leading to transcriptional activation of AP-1 and NFAT, respectively, are very sensitive to reduced intracellular GSH levels, while the activation of the p38/Mpk2 pathway leading to AP-1-mediated transcription is mostly unaffected by chronic oxidative stress. A very proximal event in the CD28-mediated signalling pathways seems to be extremely sensitive to GSH depletion since costimulation did not affect the transcriptional activity of either AP-1 or NF- κ B.

Conclusion We conclude that the signalling pathways in SF T lymphocytes from RA patients are affected at several levels by chronic oxidative stress, all contributing to the observed hypo-responsiveness of these cells.

OP0002 DEOXYSPERGUALIN AND SPERMINE INDUCE APOPTOSIS IN QUIESCENT AND ACTIVATED HUMAN LYMPHOCYTES

M Schiller, C Gabler, N Blank, S Winkler, M Grünke, JR Kalden, HM Lorenz. *Department of Medicine III, Institute of Clinical Immunology and Rheumatology, Erlangen, Germany*

10.1136/annrheumdis-2001.516

Background Deoxyspergualin (DSG) is a novel immunosuppressive drug currently undergoing clinical trials for treatment of transplant rejection or autoimmune diseases. The polyamine analogue DSG is a synthetic derivative of spergualin containing a spermidine moiety. Polyamines are known to induce apoptosis in several mesenchymal cell lines in presence of fetal calf serum (FCS).

Objectives The aims of the present study were

- to test whether DSG alone or in combination with other immunomodulating drugs induces apoptosis or necrosis in immunocompetent cells (fresh isolated cells, PHA generated lymphoblasts) and whether this effect depends on the presence of FCS;
- to analyse which cellular mechanisms are involved in the execution of the cell death program induced by DSG.

Methods Freshly isolated quiescent or activated human PBMCs, PHA generated lymphoblasts and several tumour cell lines (Jurkat, SKW-3, U937, RS4.11., MV4.11.) were incubated with different concentrations of DSG in presence of FCS or human AB serum (hABS). The rate of apoptotic cells was analysed after Propidium-Iodide/AnnexinV (PI/AxV) and PI/Triton staining by flow cytometric analysis.

Mitochondrial-membrane-potential ($\Delta\psi$ M) was measured by flow cytometric analysis after staining with Dihexyloxocarbocyanine (DiOC6).

Results By means of PI/Triton and PI/AxV staining we found an induction of apoptosis by DSG in presence of FCS in quiescent PBMCs, PHA generated lymphoblasts and the tumour cell lines Jurkat, SKW-3, U937. In presence of hABS no induction of apoptosis was found. Necrosis was excluded in any of the experiments.

DSG was also capable to induce apoptosis in the tumour cell lines RS4.11., MV4.11. which are refractory to conventional chemotherapeutic agents operating through the induction of apoptosis. In lymphoblasts coactivation with IL-4, IL-7 or IL-15 did not prevent DSG-induced apoptosis, whereas IL-2 had various effects.

A decrease of $\Delta\psi$ M was seen after treatment of cells with DSG in presence of FCS. DNA degradation measured by PI Triton staining was abolished by adding the caspase inhibitor zVAD FMK.

Combination of DSG with Cycloheximid (CHX), a protein synthesis inhibitor which is described to interact with the regulation of cellular polyamine synthesis, lead to a remarkable increase in the induction of apoptosis by DSG, while CHX alone showed no effects.

Similar effects as described above for DSG were achieved if spermine, a physiological polyamine, was added to the culture.

Conclusion These results show, that DSG in presence of FCS is able to induce apoptosis in several cell systems. Interestingly induction of apoptosis was seen in resting cells and two cell lines, that are resistant to conventional chemotherapeutic agents. Our findings point out that induction of apoptosis by DSG in presence of FCS requires activation of caspases and is associated with a decrease in $\Delta\psi$ M. The structural conjunction of DSG with polyamines and the additive effects in combination with CHX suggest, that alterations of the cellular polyamine contents might be involved in the induction of apoptosis by DSG.

OP0003 STAT1 AND STAT3 ACTIVATION DURING THE PRIMARY AND CHRONIC PHASES OF EXPERIMENTAL ARTHRITIS IN WILDTYPE AND IL-6 DEFICIENT MICE

AS De Hooge, FA Van de Loo, MI Koenders, WD Smallegoor, OJ Arntz, WB Van den Berg. *Rheumatology Research Laboratory, Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands*

10.1136/annrheumdis-2001.517

Background Experimental arthritis in Interleukin-6 deficient (IL-6^{-/-}) mice has shown the importance of this cytokine for chronicity of the synovitis. We^{1,2} and others have found previously that in IL-6 deficient mice the primary inflammation does not develop into a chronic synovitis. The JAK/STAT pathway is important for signaltransduction by IL-6 in the cell. Upon activation of the IL-6 receptor complex, the Signal Transducer and Activator of Transcription molecules STAT1 and STAT3 become phosphorylated and migrate to the nucleus to activate gene transcription.