P111

MMP- AND CYTOKINE-CONCENTRATIONS IN SYNOVIAL FLUID OF PATIENTS SUFFERING FROM UNILATERAL OR PRIMARY OSTEOARTHRITIS DO NOT DIFFER FROM THOSE WITH POST-TRAUMATIC CARTILAGE DEGENERATION OR OSTEOCHONDROSIS DESSICANS


1NMI Natural and Medical Sciences Institute at the University of Tübingen, Tübingen, Germany, 2Hospital for Workers Compensation Tübingen, Tübingen, Germany

Purpose: To date, autologous chondrocyte transplantation (ACT) is mainly used for the treatment of post-traumatic cartilage degeneration or of osteochondrosis dissecans (OD). First studies suggest that ACT might also be used for the treatment of osteoarthritides (OA). However, it is still unclear if the environment in the osteoarthritic joint might hamper cartilage regeneration. Therefore, we compared matrix metalloproteases (MMP) and cytokine levels in synovial fluid from patients currently treated with ACT with those of patients with generalized, primary OA or with unilateral OA combined with axis-malalignment.

Methods: Knee synovial fluid (SF) was collected from OD-patients (n=6, 33 ± 10 years), post-traumatic cartilage degeneration-patients (n=9, 39 ± 12 years), unilateral OA-patients (n=12, 53 ± 17 years), and primary OA-patients (n=19, 71 ± 9 years). Samples were collected before the cartilage harvest for ACT, during diagnostic arthroscopy or before total knee replacement, immediately frozen, and stored at -70°C until measurement.

The concentration of cytokines and MMPs were determined using a multiplex assay based on antibody-coated microspheres (xMAP® Technology, Luminex, USA). IL-1β, IL-6, IL-8, IL-10, IL-12 and TNFα were measured with the Beadlyte® Human Multi-cytokine Detection System 2 (Upstate USA, Lake Placid, NY); MMP-1, -2, -3, -7, -8, -9, -10, and -13 were measured using a multiplexed bead-based assay system developed at our institute.

Results: In SF of all four groups, high concentrations of MMP-1, -3 and -9 (av. 119.7, 471.9, 1311.8 ng/ml respectively), and medium levels of MMP-7 (av. 20.6 ng/ml) were present; MMP-10 (av. 0.4 ng/ml) was only present at very low levels, while MMP-2, -8, and -13 could not be detected. No differences could be detected between the different groups, except that MMP-levels in SF from OD-patient appeared to be generally lower than OA and traumatic cartilage degeneration. However, this only reached significance for MMP-3 compared with post-traumatic cartilage degeneration. IL-6 was the cytokine found in highest concentration in the SF of most of the patients (av. 284.8 pg/ml).

In all groups IL-8 was present in medium levels (av. 39.6 pg/ml), IL-12 was not detected, while IL-1β, IL-10, IL-12 and TNFα were present at baseline (av. 3.6-16.8 pg/ml). Again, no differences between the four groups were found.

Conclusions: We conclude that although both inflammatory cytokines and MMPs were present in the SF of all patients, the levels of those did not differ between the OA, the post-trauma and OD groups. Since patients of the last two groups are routinely treated with the ACT with good results, the presence of these cytokines and MMPs does not hamper the success of this method. It might therefore be expected that the metabolic environment in OA patients also would not prevent the usage of ACT in these patients.

Acknowledgements: This work is supported by the BMBF (grant #0313400C)

P112

CTX I AND CTX II URINE CONCENTRATIONS IN 420 PATIENTS WITH VARIOUS MUSCULOSKELETAL CONDITIONS

P. Mathieu1, T. Conrozier1, F. Colson1, M. Richard1, M. Piperno1, E. Vignon1

1Department of Rheumatology, Centre Hospitalier Lyon SUD, Pierre Bénite, France, 2Department of Biochemistry, Hospital Edouard Herriot, Lyon, France

Purpose: Urine levels of C telopeptides of type I (CTX I) and II (CTX II) collagens have been shown to be useful for the monitoring of patients with bone and articular diseases respectively. The aim of the study was to assess differences of CTX I and CTX II urine concentrations according to the musculoskeletal diseases, and to investigate whether the levels of the biomarkers can help for diagnosis.

Fig. 1. Specificity was examined by mixing together the high, medium and low quality control samples (mQC). This mixture was serially diluted at the following dilutions: 1:2, 1:4, 1:8, and 1:10. The results of this serial dilution were then compared to the standard curve that was derived using the standards provided by the manufacturer by comparing the absorbance to the concentration. The samples demonstrated parallelism to the master standard curve.

Fig. 2. Linearity of dilution was analyzed by serially diluting QC samples (high, medium, and low) at the following dilutions: 1:2, 1:4, 1,8, and 1:10. The concentration of each dilution was then determined from the standard curve and the observed concentration was plotted against the reciprocal of the dilution (1/dilution).
Methods: Urine samples were obtained in 354 consecutive patients, referred to the department of rheumatology for one of the following diseases [Osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis (AS), undetermined inflammatory arthritis (UIA), osteoporosis (OP)] and in 66 controls (CTRL). CTX I and II were assayed using a competitive ELISA method. CTX I and II correlations were studied by linear regression. Differences in CTX levels between groups were studied with analysis of variance, taking into account age, sex and BMI.

Results: Cross sectional study. 144 males and 276 females; mean (SD) age 59(17); mean CTX I 228.9 (204) ng/mL; mean CTX II 375.7(408.6) ng/mL.

CTX I was significantly higher in females than in males (p=0.006). There was a correlation between CTX I and CTX II concentrations (R=0.34, p=0.001). CTX I but not CTX II was correlated with age (p=0.001 and p=0.46 respectively).

CTX I was significantly higher in OP than in CTRL (p=0.02). However there was no significant difference of CTX I levels between OP and OA, RA, SA and UIA. CTX I levels were not statistically different between CTRL and the various articular diseases.

CTX II correlations were higher in all the articular diseases than in CTRL (OA/CTRL p=0.05; RA/CTRL p=0.02; AS/CTRL p=0.004, UIA/CTRL p=0.02) but no difference was found between OP and CTRL (p=0.3).

Lastly CTX II levels were not significantly different between OA, RA, SA and UIA. (all p > 0.2).

Conclusions: Both CTX I and II were increased in muscoskeletal diseases. Compared to CTRL, CTX I levels were significantly increased only in patients with OP. By contrast CTX II were significantly increased in OA, inflammatory rheumatism but also OP. Surprisingly CTX II was as high in OP as in inflammatory diseases and in OA. The influence of the treatments on CTX levels remains to be investigated.

P113
A BIOCHEMICAL APPROACH FOR ANALYZING CHONDROITIN SULPHATE IN BLOOD CIRCULATION AND PURITY TESTING IN SUPPLEMENTS AND DRUGS

A.P. Asimakopoulou1, C.J. Malavaki1, J.-P. Pujol2, N.K. Karamanos3

1University of Patras, Patras, Greece, 2University of Caen, Caen, France

Purpose: Structure analysis of serum chondroitin sulfate (CS) is very important as information could be received in respect to structural alterations of serum CS in several pathological conditions along with its pharmacological profile. Due to the low blood concentration of CS and a general lack of adequate analytical methodology that ensures high recovery of CS and sensitive determination, the factors influencing its structure and concentration have not yet been fully identified. Furthermore, the safety and purity of CS preparations from various sources should be addressed since they are widely used as nutritional supplements and drugs. The aim of this study was therefore to develop: 1) a high sensitive assay that can be used for identification and determination of hyaluronic (HA) contamination in CS commercially available preparations and 2) a serum pretreatment procedure and an accurate analytical methodology for the determination of total CS and its disaccharide composition in serum.

Methods: A reversed polarity capillary electrophoresis (CE) approach has been developed for purity analysis of CS preparations from various tissues as well as for monitoring the various sulphated CS disaccharides in blood circulation. Charge density distribution of CS preparations and purity testing for identification of HA was performed by direct CE analysis of aqueous CS solutions. For analysis of serum samples, they were treated with protease and CS chains were completely recovered by precipitation. CS were selective degraded to variously sulphated disaccharides following enzymic digestion of chondro/dermato-lyases.

Results: Direct analysis of aqueous CS solutions from various tissues ensured the complete separation and identification of CS and HA. Selective enzymic digestion of various CS preparations with chondroitinases and analysis under the same CE conditions, ensures the complete separation between the sulphated CS-disaccharides and HA within 15 min. Analysis as low as 50 μg of CS preparations can be used to identify even 0.3% HA contamination. For analysis of serum samples CS chains were completely recovered by precipitation. Following enzymic treatments and CE analysis, the composition of disaccharides in serum was found to be rich in 4-sulphated and non-sulphated CS-derived disaccharides.

Conclusions: The developed methodological approach is sensitive and accurate and can be easily used for the identification of HA contamination in commercially available preparations of CS, the charge homogeneity of CS as well as for the determination of the CS concentration and sulphation profile in blood serum. The approach will be useful for pharmacokinetic studies and disease monitoring.

P114
PATHOLOGICAL GLYCOXIDATION IN ARTHRITIS AND DIABETES BY MEANS OF PENTOSIDINE DETERMINATION

M. Braun1, P. Špaèek1, M. Adam1, J. Škhrha2, K. Pavelka1, L. Benot1
1Institute of Rheumatology, Prague 2, Czech Republic, 21st Faculty of Medicine, Prague 2, Czech Republic

Purpose: Pentosidine (PEN), a major member of advanced glycation end-products, belongs to promising molecules that elevated concentrations in body fluids and tissues could indicate deleterious changes caused by specific post-translational modifications in metabolism of long-lived proteins, lipids, and carbohydrates. Quantification of PEN in body fluids or tissues by means of sensitive HPLC methods helps to monitor the pathological modifications of proteins in a number of diseases. In this study, we determined PEN in patients with diabetes mellitus (DM) and rheumatic diseases. For that, highly sensitive and reproducible HPLC method combined with sensitive fluorescence detection was developed. Then we determined PEN levels in biological samples from laboratory mice (C57/6 black) with spontaneously induced arthritis. To analyze reduced formation of PEN in vivo, we tested antioxidative and antiarthritic effects of vitamin C and Boswellin supplementation of these mice.

Methods: For PEN determination we established sensitive method based on reversed phase gradient HPLC combined with fluorescence detection (λex/em. = 335/385 nm). The conditions are as follows: compact glass column C8 Separ CG18, 150x3 mm; mobile phase consisted of 0.02 M heptafluorobutyric acid, 0.01M ammonium sulphate and linear gradient is given by variable concentration of acetoni trile (12.5 - 25%), column temperature: 40°C; flow rate: 0.5 ml/min; time of HPLC run: 30 minutes. The method was applied to hydrolyzed and purified body fluid samples from patients with osteoarthritis (OA), rheumatoid arthritis (RA), diabetics of type 1 (DM1) and type 2 (DM2), healthy controls as well as to samples from serum and experimental mice tissue extracts.

Results: In blood samples of patients with RA and DM2, the accumulation of PEN was doubled compared with healthy controls. In case of OA patients, mild elevation of PEN was also observed. We found significant correlation of PEN concentrations between studied body fluids. The association of PEN concentrations with the age of studied subjects was observed.