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 concentrations 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 musculoskeletal 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 rheumatisms 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.

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

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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 hyaluronan (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 with 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 chondroitinasases 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.

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PATHOLOGICAL GLYCOXIDATION IN ARTHRITIS AND DIABETES BY MEANS OF PENTOSIDINE DETERMINATION

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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 modification 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 antirheumatic 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 (λ_{exc}/λ_{em} = 355/385 nm). The conditions are as follows: compact glass column CGC Separon SGX C18, 150x3 mm; mobile phase consisted of 0.02 M heptafluorobutyric acid, 0.01M ammonium sulphate and linear gradient is given by variable concentration of acetoniitrile (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.