Conclusions: These results indicate that pentosidine may play an important role in the process of pathophysiology of chronic rheumatic diseases and DM complications. Supported by the Ministry of Health of the Czech Republic (grant No. NR/7895-3 and research project No. 00023728).

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BONE ALKALINE PHOSPHATASE (BAP) ASSAY VALIDATION USING EQUINE SERUM AND SYNOVIAL FLUID

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Purpose: Bone undergoes reformation in response to the demands placed upon it. Examination of both the serum and synovial fluid BAP concentrations in joint disease may be valuable in determining the different local and systemic effects that occur to bone in response to exercise and injury. BAP has been shown to be a good biomarker for early osteoblastic activity when joint injury is present. The investigators have performed previous studies demonstrating the capability of the commercially developed Metra® BAP ELISA to determine differences in biological activity of equine serum and synovial fluid (SF) samples. BAP is well conserved across species, and the Quidel Corporation has demonstrated species cross reactivity with the horse. Thus, because the horse is a good translational model of OA for biomarker evaluation, the objective of this study was to validate the use of equine serum and SF with the Metra® BAP ELISA.

Methods: Metra® BAP ELISAs (Quidel Corporation) were used for this validation study according to manufacturer protocols. Internal quality control (QC) samples were prepared using the highest concentration standard provided by the manufacturer (140 U/L). To create QC samples, fresh serum was collected from 3 normal horses and fresh SF was collected aseptically from 12 middle carpal and 12 radiocarpal joints from 6 normal horses. The samples were respectively pooled together for further processing and analysis. Each group of pooled samples were spiked with a known amount of standard to create samples with high, medium and low levels of BAP. The QC samples were used to determine the precision, specificity, sensitivity, accuracy, linearity of dilution, and stability of this assay with equine serum and SF.

Results: The standard optical density (OD) values exhibited acceptable reproducibility of the standard curve over 10 plates with an overall inter-assay precision mean coefficient of variation (CV) of 8.1% (range; 4.9-12.3%). Serum and SF samples exhibited acceptable intra-assay and inter-assay precision over 3 plates with an overall mean CV of 3.3% (range; 0.1-8.1%) and 7.2% (range; 5.7-9.6%), respectively for the serum, and 2.6% (range; 0.2-7.5%) and 7.1% (range; 4.8-8.8%), respectively for the SF. Parallelism of serum and SF sample dilutions (1:2, 1:4, 1:8, and 1:10) was demonstrated when compared to the standard curve (Fig. 1). Lowest detection limit of the assay was determined to be 1.6 U/L. Percent recovery for serum was 103.1% for high, 111.6% for medium, and 116.1% for low QC samples. Percent recovery for SF was 64.8% for high, 86.1% for medium, and 91.2% for low QC samples. Linearity of serum and SF sample dilutions (1:2, 1:4, 1:8, and 1:10), was demonstrated (Fig. 2). The results also demonstrated that no more than 2 freeze/thaw cycles should be...
performed per sample for the serum, but that there is minimal loss of the BAP analyte out to 5 freeze/thaw cycles for the SF.

Conclusions: The Metra BAP assay is a reproducible and valid assay for use with equine serum and synovial fluid.

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ANALYSIS OF MOLECULAR WEIGHT OF HYALURONAN IN HUMAN SYNOVIAL FLUID AFTER GLUCOSAMINE TREATMENT: A PILOT STUDY

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Purpose: Synovial fluid as a lubricant of knee joints has its viscoelasticity depending on the molecular weight (MW) and concentration of hyaluronan (HA). HA concentration in synovial fluid have been frequently reported, but there are few reports analyzing MW of HA in synovial fluid of patients with osteoarthritis (OA).

The purpose of this study was to measure the MW of HA by using SEC-LALLS (Size-exclusion chromatography (SEC) - a low-angle laser light scattering (LALLS)) system, and to evaluate the change in MW of HA after glucosamine administration.

Methods: Five knee OA patients (3 men, 2 women, mean age: 60.6 years) received 1.5g of glucosamine hydrochloride per day for one month. Synovial fluid samples were collected before and after glucosamine administration. The SEC-LALLS system (Asahi Technoel) was used to determine the MW of HA. The HA fractions were separated by using SEC system and then the MW of HA was calculated by measuring LALLS and refractive index (RI).

Results: The SEC-LALLS system was effective in the MW measurement of HA. MW of three kinds of HA standards, suvenyl, artz and HA (low MW type) (Seikagaku Corporation) were measured for use with equine serum and synovial fluid.

Conclusions: The Metra BAP assay is a reproducible and valid assay for use with equine serum and synovial fluid.

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CHONDROGENIC MARKERS ARE EXPRESSED AND REGULATED IN BONE AND CORRELATE WITH BONE MASS

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Purpose: The expression of chondrogenic markers (collagen type II and X, aggrecan, cartilage oligomeric matrix protein) are largely restricted to cartilage and play a role in cartilage metabolism and function. Recently we have shown that (unpublished) these markers are also expressed in adult rat bone and primary osteoblasts. In this study, we sought to confirm their expression at mRNA and protein level in bone; compare the temporal expression of these markers to established osteogenic markers in ovariectomized (Ovx) rat model of osteopenia; and evaluate the regulation of these markers upon treatment with known bone anabolic agents.

Methods: Six-month old, female Sprague Dawley rats (n=10), were Ovx or sham-operated. The animals were sacrificed at 2, 4, 6, 8, and 12 weeks post-ovx and left femur was analyzed for BMD and BMC using quantitative computerized tomography. The right femur (distal metaphysis without epiphysis) was processed for mRNA analysis of osteogenic (collagen α1 (I), α2 (I), α1 (V), osteocalcin, osteonectin, bone sialoprotein, biglycan, alphos) and chondrogenic (collagen α1 (II), aggrecan, COMP, Cdrap) markers by quantitative real-time PCR (qPCR). Biochemical markers of bone and cartilage turnover (collagen type I and II degradation products in the serum CTX-I and CTX-II) were measured by ELISA. Type II collagen and aggrecan proteins in bones were confirmed by Western analysis and LC/MS. To assess the effects of bone anabolic agents on the regulation of bone and cartilage markers, Ovx rats permitted to lose bone for one month were treated with PTH (1-38) at 100-150(kDa) and then the expression levels of these marker genes were measured. Pearson correlation coefficients were used to measure correlations between gene expression and phenotypic parameters.

Results: qPCR confirmed the expression of the chondrogenic markers in bone and primary osteoblasts. LC/MS and Western analysis validated the presence of type II collagen and aggrecan proteins in both metaphyseal and diaphyseal bone. Ovx for 12 weeks resulted in significantly lower BMD at whole and distal femur (10-15%) relative to sham controls. This was associated with a temporal decrease in the expression levels of chondrogenic markers (2 to 5-fold) with optima observed at 8-12 weeks post-ovx. In contrast, the expression levels of osteogenic markers steadily increased with maxima observed at 6 weeks (1.2 to 4-fold) followed by decline below sham levels. The levels of CTX-I and CTX-II were increased in the serum of Ovx rats and returned to near sham levels by 12 weeks. The magnitude of change in CTX-II levels (4.8-fold) was greater than in CTX-I (1.9-fold). Treatment with bone anabolics, PTH or GSK-3 inhibitor restored bone mass in Ovx rats and was associated with a robust increase in the gene expression of chondrogenic (1.8 to 4.7-fold) compared to osteogenic markers (1.1 to 1.8-fold). Interestingly, overall the chondrogenic markers showed a better correlation (r=0.71) to BMD & BMC compared to osteogenic markers (r=0.5).

Conclusions: These results demonstrate the presence and the regulation of chondrogenic markers in rat long bone. This suggests either the continued presence of cartilage in adult rat bone