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.

P116

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

Y. Kanemoto[1], M. Watanabe[2], Y. Okada[3], S. Sugawara[4], Y. Nomura[1]
[1]Applied Protein Chemistry, Tokyo University of Agriculture and Technology, Fuchu Tokyo, Japan, [2]Koseikai Hospital, Mitaka, Japan, [3]Japanese School of Technology for Social Medicine, Koganei, Japan, [4]Sugawara Orthopaedic Clinic, Matsudo, Japan

Purpose: Synovial fluid as a lubricant of knee joints has its viscosity 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 analysing 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) recieved 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 Techneion) was used to determine the MW of HA. First,the HA fraction was separated by using SEC system and then the MW of HA was calculated by measuring LALLS and refractive index(RI). Triple Detector Array (Viscotek Corporation)which has a LALLS detector, a RI detector and a viscometer was utilized for the measurement. Before analyzing HA samples, the accuracy of the SEC-LALLS system was calibrated with three HA standards (suvenyl, artz and HA(lowMW-type)(Seikagaku Corporation). (MW of suvenyl, artz and low MW HA: 1900-2500, 600-1200 and 100-150(kDa)) Furthermore, hyaluronidase treatment was also done for the confirmation of HA. As a clinical test,visual analogue scale (VAS) of OA patients was also measured.
Results: The SEC-LALLS system was effective in the MW measurement of HA. MW of three kinds of HA standards,suvenyl, artz and HA(lowMW-type)were estimated as 2400, 870 and 160(kDa)respectively. It was succeeded that HA fraction was isolated and identified from synovial fluid samples using this system with high sensitivity. As a proof of HA, it was demonstrated that this peak fraction from HA disappeared after hyaluronidase digestion. The MW of HA (mean$\pm$SD) in synovial fluid of OA patients increased from 4350$\pm$1170 to 5030$\pm$1280(kDa) after glucosamine treatment. VAS for knee pain(mean$\pm$SD) decreased significantly from 4.8$\pm$1.3 to 1.7$\pm$1.0.
Conclusions: We could separate HA from human synovial fluid and determine the MW of HA with high sensitivity by using the SEC-LALLS system. A change in MW of HA was detected after glucosamine treatment, and knee pain was also alleviated. Therefore these changes suggested glucosamine had an effect on the metabolism of HA. Estimating the change in MW of HA by this system may be useful for monitoring a treatment process of symptom modifying OA drugs. Nevertheless, further clinical studies in a lager scale are needed to investigate the improvements in clinical symptoms with alterations in MW of HA in synovial fluid.

Bone Biology

P117

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

N. Kulkarni[1], T. Wei[1], M. Schreiweis[1], K. Brune[1], T. Christopher[1], J. Wolos[1], A. Harvey[1], S. Chandrasekhar[1], M. Carozza[1], T. Engler[1], T. Martin[2], H. Bryant[1], Y. Ma[1], P. Mitchell[1], J. Hale[1], J. Onyia[1]
[1]Lilly Research Labs, Indianapolis, IN, [2]SVIMR, Fitzroy, Australia

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 α_1 (I), α_2 (I), α_1 (V), osteocalcin, osteonectin, bone sialoprotein, biglycan, alphos) and chondrogenic (collagen α_1 (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 10 μg/kg, sc or 603281-31-8 at 3mg/kg/d (GSK-3 inhibitor), po for 60 days. Twenty-four hr after the last treatment, RNA from distal femur metaphysis was analyzed by qPCR to assess the expression levels of these marker genes. 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 in 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 $>$ 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 (1.6 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

or that these markers are not necessarily cartilage specific and could play similar or novel role in bone remodeling. Furthermore, the robust regulation of chondrogenic markers in bone and their good correlation to BMD & BMC suggest that they could be good predictors of disease and treatment outcomes.

P118

PHENOTYPIC CHARACTERIZATION OF OSTEOBLASTS FROM THE SCLEROTIC ZONE OF HUMAN OSTEOARTHRITIC SUBCHONDRAL BONE

C. Sanchez[1], M.A. Deberg[1], A. Bellahcène[1], V. Castronovo[1], P. Msika[2], Y. Henrotin[1]
[1]University of Liège, Liège, Belgium, [2]Expansciences, Epernon, France

Purpose: There is consensus that osteoarthritis (OA) is characterized by subchondral bone thickening, accompanied by an increased osteoid volume and a low mineralization. Until now, phenotypical changes occurring in osteoblasts from the sclerotic subchondral bone remains unexplored. This work was designed to compare gene expression in osteoblasts coming from the sclerotic and non sclerotic zones of human OA subchondral bone.
Methods: Human osteoblasts were isolated from sclerotic or non sclerotic areas of OA subchondral bone. They were cultured for 12 days in monolayer in a differentiation medium composed of 2% Ultroser G as serum substitute, 2 mM proline, 50 microgram/ml ascorbic acid and 10^{-8} M 1,25 dihydroxycalciferol. At the end of this differentiation period, gene expression in sclerotic or non sclerotic osteoblasts was compared. Tissue non specific alkaline phosphatase (TNAP), osteocalcin (OC), transforming growth factor -beta1 (TGF-beta1), osteopontin (OPN), bone sialoprotein (BSP), vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-13, parathormone receptor (PTH-R), transglutaminase (TG)-2, factor XIIIA (FXIIIA), plasma cell membrane glycoprotein 1 (PC-1) and Ank mRNA levels were quantified using real time RT-PCR. Transglutaminase and nucleotide triphosphate pyrophosphohydrolase (NTPPPH) activities were also quantified by enzymatic assays.
Results: MMP-13 (21-fold; $p<0.001$), OPN (2.8-fold; $p<0.001$), TNAP (2-fold, $p<0.001$), OC (2-fold, $p<0.001$), TGF-beta1 (1.4-fold, $p<0.01$) and VEGF (1,5-fold; $p<0.001$) gene expression was significantly higher in sclerotic osteoblasts than in non sclerotic cells. In contrast, PTH-R (-37%, $p<0.001$), PC-1 (-22%, $p<0.01$), Ank (-24%, $p<0.01$) genes were depressed in sclerotic osteoblasts compared with non sclerotic cells. Finally, BSP, TG2 and FXIIIA mRNA levels were similar in sclerotic and non sclerotic osteoblasts. Transglutaminase activity was increased by 53% in sclerotic osteoblasts ($p<0.001$), while NTPPPH activity was decreased by 32% ($p<0.001$).
Conclusions: Osteoblasts from the sclerotic subchondral bone showed an altered phenotype characterized by the overexpression of genes limiting bone mineralization (OPN, PC-1, ...), on one hand, and genes promoting osteoid matrix accumulation (TGF-beta1, OC) on the other hand. These findings suggest that osteoblasts may contribute to subchondral bone sclerosis and as such constitute a potential target for future OA therapies.

P119

WNT SIGNALLING IN OSTEOARTHRITIC BONE

R.M. Aspden, X. Hochet
University of Aberdeen, Aberdeen, United Kingdom

Purpose: Wnts are secreted signalling molecules, traditionally associated with developmental processes. Various members of the Wnt signalling pathway have been implicated in osteogenic differentiation and high and low bone mass phenotypes. In osteoarthritis (OA) there is a massive proliferation of hypomineralized trabecular bone. Our hypothesis is that musculoskeletal cells revert to an earlier, developmental, phenotype and attempt to produce new matrix inappropriately. We have performed a pilot study to analyze the role of Wnt signalling in OA by profiling the expression of Wnt pathway genes in osteoblasts. Bone from patients with osteoporosis (OP), displaying a low bone mass phenotype, were used for comparison.
Methods: Femoral heads were obtained from consenting patients undergoing a total hip replacement for OA (N=5, aged 55-86) or a hemiarthroplasty following a fractured neck of femur for OP (N=5, aged 68-92). Primary osteoblasts were grown from bone chips. Total RNA was isolated from cells using Trizol (Invitrogen) followed by RNeasy purification (Qiagen) and prepared for application as biotinylated cRNA to a Wnt pathway Oligo GE Array (Super Array Biosciences) containing oligo-probes for 128 Wnt-related and housekeeping genes. Intensities were corrected for background and normalised using the inter-quartile median. Mean values of signal intensities (medians if not normally distributed) were found for each disease group, and compared using analysis of variance. The most highly expressed 30 genes (~25%) were examined in each group and the Log_2(OA/OP) (signal log ratio, SLR) calculated and deemed important if greater than 0.5 or less than -0.5.
Results: The Wnt signalling pathway was clearly active in osteoblasts in both dseases. Expression levels for the top 30 genes were significantly greater than those for the lowest expressed gene ($p<0.05$, pairwise comparison, ANOVA on ranks). WISP2, GSK3A, AES and DVL1 were among the most highly expressed genes in both diseases and 27 of the top 30 were common to both groups. High SLR values were found in 7 of these genes, though no differences in signal between the groups reached statistical significance at $p<0.05$. Secreted frizzled related proteins SFRP4 (SLR 0.84) (p=0.056), and SFRP3/FRZB (0.59) were higher in OA than OP. The Na/H transporter regulator SLC9A3R1/EBP50 (-0.85) and transducin-like enhancers of split TLE1 (-0.51) and TLE3 (-0.52) were more highly expressed in OP than OA. Of the Wnt proteins, Wnts 16, 1 and 5a were the most highly expressed but only WNT5A showed any differential expression with an SLR of 0.45 (p=0.057).
Conclusions: Wnt signalling is clearly active in elderly bone. Secreted frizzled-related proteins are extracellular inhibitors of Wnt signalling and, of particular interest, FRZB has been identified as a candidate in genetic linkage studies of OA. However, lower levels of TLEs in OA would indicate higher levels of gene transcription through the canonical signalling pathway. The balance between these processes needs further investigation. Dishevelled-dependent Wnt5a signalling can be transduced through the RHOA pathway, affecting the cytoskeleton and commitment to cell lineage, and via MAP kinases through the JNK pathway affecting gene transcription. Higher levels could be a factor underlying the cellular changes seen in OA.

P120

DIFFERENTIAL EXPRESSION OF GROWTH AND ANGIOGENESIS FACTORS IN PATIENTS WITH ASEPTIC OSTEONECROSIS OF THE FEMORAL HEAD AND PATIENTS WITH HIP OSTEOARTHRITIS

S. Grässel, C. Göttl, M. Vogel, O. Wiech, L. Perlick, J. Grifka, M. Tingart
University of Regensburg, Bad Abbach, Germany

Purpose: Aseptic osteonecrosis of the femoral head (ONFH) is a painful and progressive disorder of the hip, which often leads to collapse and disabling secondary osteoarthritis of the hip joint.