respectively;- Presence of traces of other plant unsaponifiables (like Colza unsaponifiables for example).

Conclusions: These nutraceutical products are therefore completely unrelated to the composition of the original pharmaceutical ASU. The quality of ASU is supported by a global know-how concerning the qualitative management of the plant material from its cultivation, extraction, hemisynthesis to its final concentration. The original ASU alone may therefore claim a relationship between the control of biodiversity, characterization of specific structures and its pharmacological and clinical activity in osteoarthritis.

129 CARTILAGE BIOMARKERS IN URINE - OBSERVATIONS AFTER MENISCECTOMY

E. Lindhorst1, R.X. Raiss2, J. DeGroot3, A. Theisen2, T. Aigner4, L. Wachsmuth2
1University of Marburg, Eppstein, Germany; 2University of Frankfurt/Main, Frankfurt/Main, Germany; 3TNO Quality of Life, Leiden, The Netherlands; 4University of Leipzig, Leipzig, Germany

Purpose: Cartilage biomarkers promise to advance our possibilities to monitor articular cartilage damage and diseases by non-invasive means. Complete medial meniscectomy has been shown to lead to osteoarthritis (OA), in the human as in animal models. In this study, complete medial meniscectomy was used to investigate the potential of the collagenous biomarkers hydroxylsylpyridinoline (HP) and lysylpyridinoline (LP) when measured in urine.

Methods: New Zealand White rabbits (n = 32) had a complete medial meniscectomy of their right knees using a medial parapatellar approach. Open surgery allowed a complete visualisation of the joint structures at all timepoints of the procedure ensuring a safe procedure. Postoperatively, the animals were housed in cages. A group of 32 rabbits were used as unoperated controls. 8 rabbits of each group were sacrificed at 2, 4, 8 and 12 weeks. Macroscopic mapping was performed on the right and left knee joints using the scheme of the International Cartilage Repair Society. Specific areas and sums of areas of all joint sites were analysed for lesions. For microscopic evaluation, sections of the central tibial plateaus were stained with H&E and Safranin O. Histologic analysis was performed with a dedicated grading system accounting for pathologic alterations of proteoglycan content, matrix structure, cellularity, tidemark duplication, and osteophyte formation, basically a modified Mankin scheme.

Urines samples were collected during the 24 hrs before surgery and again during 24 hrs before each sacrifice time point. Measurements of hydroxylsylpyridinoline (HP) and lysylpyridinoline (LP) were performed with HPLC. Data were normalised to the urinary creatinine. Ratios of the markers were calculated. Statistical calculations were performed with SPSS using e.g. ANOVA, Wilcoxon and Mann Whitney U tests.

Results: No signs of infection were noted during the postoperative course of the animals. Macroscopic lesions advanced with time after meniscectomy. First degenerative changes were already present at 2 weeks after surgery, with an increase in number and size of lesions with time. Histologically, OA-like lesions were present at all 4 examined timepoints.

The overall macroscopic, but not microscopic score correlated inversely with the HP/creatinine ratio. The LP/creatinine ratio was inversely correlated with either one. Both HP/creatinine and LP/creatinine correlated inversely with the overall histologic score. At the reported timepoints, both, HP/creatinine and LP/creatinine ratios had generally lower mean levels than the control animals. No simple timecourse which might indicate a worsening osteoarthritic disease could be observed with either ratio. This was also true for HP/LP.

Conclusions: Biomarkers have proven valuable in many biological systems and clinical situations e.g. in gastroenterology, cardiology and oncology. Cartilage biomarkers are still under development coming close to clinical utility. In this study, measurements of HP and LP in the urine of rabbits after complete medial meniscectomy did not show any simple significant postoperative changes. This was true for the respective ratios. Thus we were not able to monitor the development of OA-like lesions of the articular knee joint in a simple manner with these markers. Such somehow negative findings are part of a research development which is still stimulating and will further our detailed knowledge of cartilage and its diseases.

130 URINE PRE-CLINICAL CTX II (CARTILAPS®) ASSAY VALIDATION USING EQUINE URINE

T.N. Trumble1, M.P. Brown1, K.A. Merritt1, R.C. Billinghurst2
1University of Florida, Gainesville, FL; 2St. Lawrence College, Kingston, ON, Canada

Purpose: The CTX II assay has been developed to examine the type II collagen telopeptide that is released from articular cartilage into the systemic circulation. In human osteoarthritis (OA) patients, the Urine Cartilaps® ELISA has been reported to be useful in the prediction of the progression of OA. Since type II collagen is well conserved across species, this assay was modified by the manufacturers into the pre-clinical assay to allow the a broader measuring range of CTX-II concentrations in non-human urine. Horses are one of the few species in which adequate volumes of paired synovial fluid, serum, and urine samples can be collected and compared to allow correlation of this marker in these different fluids. This may lead to translational benefit to human OA patients. The purpose of this report was to validate the use of the Urine Pre-Clinical CTX II assay for use with equine urine for future use with equine models of OA.

Methods: Urine Pre-Clinical Cartilaps® ELISAs (Nordic Bioscience Diagnostics) 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 (99 ng/ml). To create QC samples, fresh urine was collected from 6 normal horses via catheterization. These pooled samples were used for future processing and analysis. Pooled samples were spiked with a known amount of standard to create samples with high, medium and low levels of CTX II. The QC samples were used to determine the precision, specificity, sensitivity, accuracy, linearity of dilution, and stability of this assay with equine urine. To ensure that the assay could detect different biological activity, 3 previously stored urine samples from normal and OA horses were analyzed.

Results: Reproducibility of the standard curve was evaluated (n=6 plates) by computing mean optical density (OD) and percent coefficient of variation (% CV) at each standard concentration. The overall mean inter-assay CV of the standard OD values was 6.5% (range 1.6-24.4%). Samples exhibited acceptable intra-assay and inter-assay precision over 3 plates with an overall mean CV of 4.3% (range; 1.2-12.3%) and 7.2% (range; 4.7-9.7%), respectively. Parallelism and linearity of dilution of equine serum sample dilutions (1:2, 1:4, 1:8, and 1:10) were demonstrated (Figure 1). Lowest detection limit of the assay was determined to be 1.38 ng/ml. Percent recovery was 93% for high, 90% for medium, and 95% for low QC samples. There was no loss in the stability of the CTX II epitope out to 4 freeze/thaw cycles. Differences in biological activity were identified when examining 3 samples from normal (3.23 ± 0.38 ng/ml) and OA (2.25 ± 0.37 ng/ml) horses (P<0.03) (Figure 2).
Figure 1. The linearity was analyzed by serially diluting the mixed QC samples (MQC) at the following dilutions: 1:2, 1:4, 1:8, and 1:10. The observed concentration was plotted against the reciprocal of the dilution to demonstrate linearity of dilutions.

Figure 2. Differences in biological activity were identified when examining 3 samples from normal (3.23 ± 0.38 ng/ml) and OA (2.25 ± 0.37 ng/ml) horses. Significant differences between groups are represented as * P < 0.05 using an unpaired t-test.

Conclusions: Conclusions: The Urine Pre-Clinical Cartilaps® ELISA is a reproducible and valid assay for use with equine urine.

Acknowledgements: Study supported by the University of Florida Pari-mutuel Wagering Trust Fund.

131

A COMPARISON OF METHODS FOR MEASURING CARTILAGE OLIGOMERIC PROTEIN (COMP) IN HUMAN SUBJECTS WITH KNEE OA

T. Stabler1, F. Fang2, J. Jordan2, V. Vilim3, V.B. Kraus1
1Duke University Medical Center, Durham, NC; 2University of North Carolina Medical Center, Chapel Hill, NC; 3Institute of Rheumatology, Prague, Czech Republic

Purpose: The purpose of this study was to compare two commercially available kits and an inhouse method for measuring Cartilage Oligomeric Protein (COMP) in human serum and assessing their utility as biomarkers of knee osteoarthritis (kOA).

Methods: Blood was collected from 80 women participating in the Johnston County Osteoarthritis Project. Serum was separated immediately and stored at -80°C until analysis. Care was taken to insure all samples had undergone the same limited number of freeze-thaws at the time of analysis. COMP concentrations were determined using 3 sandwich ELISA methods: an inhouse method utilizing MAbs 16F12 and 17C10; and separate commercially available kits from Biovendor (Modrice, Czech Rep.) and Anamar (Gothenburg, Sweden). The Biovendor kit utilized the same MAbs as the inhouse method. The inhouse method uses COMP purified from human cartilage as it’s standard while Biovendor uses a recombinant human COMP (Gln21-Ala757). Anamar uses human serum as a standard and results are expressed as U/L instead of ng/ml. OA status was determined radiographically for each knee from posterior-anterior X-rays using the Synaflex positioning device, and a Kellgren-Lawrence (KL) grade of ≥ 2 in at least one knee was considered to be positive for kOA. Linear regression was used to compare the results from the three methods. COMP concentrations were in transformed and one-way ANOVA was used to assess the associations between COMP concentrations and knee OA laterality.

Results: Demographic and clinical characteristics are shown in Table 1. Fifty percent had knee OA, and this was unilateral (1 knee) in 58% of those with kOA and bilateral (both knees) in 42% of those with kOA. Correlations between the methods (Fig. 1) were as follows: Biovendor to inhouse R²=0.897; Biovendor to Anamar R²=0.249; Anamar to inhouse R²=0.210. All three methods showed increasing ln COMP concentrations with the number of OA affected knees (Fig. 2, see page S82), with the following associations: inhouse p=0.0134; Biovendor p=0.0171; Anamar p=0.289.

Table 1. Demographics and clinical characteristics of the sample, n=80 females

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, yr (SD)</td>
<td>64 (9.7)</td>
</tr>
<tr>
<td>Mean BMI, kg/m² (SD)</td>
<td>30.9 (6.8)</td>
</tr>
<tr>
<td>African American %</td>
<td>50</td>
</tr>
<tr>
<td>Knee OA %</td>
<td>50</td>
</tr>
<tr>
<td>Inhouse mean COMP ng/ml</td>
<td>1608 (404-2929)</td>
</tr>
<tr>
<td>Biovendor mean COMP ng/ml</td>
<td>872 (256-1582)</td>
</tr>
<tr>
<td>Anamar mean COMP U/L (range)</td>
<td>11.8 (5.7-18.6)</td>
</tr>
</tbody>
</table>

Conclusions: Correlation between the inhouse method and the Biovendor kit was excellent, as expected since they use the same MAb set. Correlation of the Biovendor or inhouse methods with the Anamar kit was not as robust. The large differences between mean values and ranges for the inhouse and Biovendor assays are probably due to differences in the standards used. Only the inhouse and Biovendor methods showed a significant association with kOA. All results were unadjusted for BMI, ethnicity, or age, which may all be important confounders when using COMP as a biomarker of OA. On the basis of these results, the Biovendor COMP seems the best suited of the commercial kits as a biomarker for kOA.