

of persons with painful knee effusions. Both groups made significant gains in peak flexion and extension of the knee.

**Conclusions:** The physiotherapy programme tested in this trial reported positive effects on pain and disability in OAK and improve muscle power, but isotonic exercise are useful for improving the function of persons with painful knee. This data suggest that isokinetic training improve joint stability and increase the range of movement.

## Therapy – Pharmacologic

### 528] INTRA-ARTICULAR HYALURONAN TREATMENT REDUCES SYNOVIAL PATHOLOGY AND IMPROVES GAIT IN AN OSTEOARTHRITIS MODEL

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**Purpose:** Synovial fluid hyaluronan (HA) content and molecular weight are decreased in osteoarthritis (OA). According to a Cochrane review intra-articular (IA) HA injection provides symptomatic relief that has slower onset but is more sustained than corticosteroids. The mechanism for the benefit is unknown, but MRI shows a strong association between synovitis, capsular fibrosis and knee pain in OA. We determined the effect of two HA preparations on gait, synovial and cartilage pathology in established OA in a sheep model.

**Methods:** Four months after bilateral meniscectomy, sheep (n=6/group) received IA saline or Hyalgan<sup>®</sup> (Fidia Farmaceutici) weekly for 5 weeks or IA HYADD4<sup>®</sup>-G (an amide derivative; Fidia) every two weeks for 3 injections. Ground reaction forces (GRF) were determined pre- and 6, 12, 16, 22 and 26 weeks post-surgery, when sheep were sacrificed. Tibial cartilage damage and osteophytosis and modified Mankin and OARS histopathology were scored. Expression of MMP-1, -13 and -14, ADAMTS-4 & -5 and TIMP-1, -2 & -3 in cartilage were assessed by RT-PCR. Synovial sections were scored for intimal hyperplasia, inflammatory cell infiltrate, vascularity and sub-intimal fibrosis and intimal cell number and depth of fibrosis ( $\mu\text{m}$ ) were quantified. Pro-fibrotic and inflammatory factors (TGF $\beta$ , CTGF, HSP47, CD44, TNF, iNOS) were immunolocalized in synovium. HA synthesis by isolated synovial fibroblasts was quantified.

**Results:** OA reduced all GRF parameters ( $p < 0.01$ ) and abolished the normal two-peak vector. GRF were partially restored by both HA preparations: Hyalgan<sup>®</sup> increased peak vertical force at 6 weeks, whilst HYADD4<sup>®</sup>-G increased vertical impulse at all times. Both HAs restored a two-peak composite force vector at 6 weeks. OA increased gross cartilage damage and osteophytosis ( $p < 0.001$ ), and modified Mankin and OARS histopathology scores ( $p < 0.005$ ), with no effect of either HA. ADAMTS-5, MMP-1 and MMP-13 mRNA were increased in OA cartilage versus non-operated controls (NOC). Hyalgan<sup>®</sup> did not change cartilage gene expression while HYADD4<sup>®</sup>-G decreased MMP-13 and TIMP-1. Subintimal fibrosis, vascularity, aggregate score, intimal cell numbers and depth of fibrosis were higher in OA synovia versus NOC ( $p < 0.01$ ) but inflammatory cells did not increase. Both HAs decreased vascularity ( $p < 0.03$ ) while intimal hyperplasia was only reduced by HYADD4<sup>®</sup>-G ( $p = 0.03$ ). Fibrosis depth was decreased by Hyalgan<sup>®</sup> ( $p = 0.03$ ). Staining for CTGF, iNOS and CD44 increased in OA synovium but were not altered by either HA. HA synthesis was increased only in cells from HYADD4<sup>®</sup>-G treated joints ( $p = 0.04$ ).

**Conclusions:** GRF following meniscectomy in sheep mimic data for human meniscectomy and OA. In addition to cartilage damage, there is significant synovial pathology in this model, as in human OA. HA treatment of established OA resulted in improved GRF, and our results suggest this clinical benefit was achieved by modifying synovial pathology. The effect of HA on synovium was not associated with changes in staining for the inflammatory or pro-fibrotic molecules examined. As cartilage damage was already established at the time of initiating HA therapy, the change in the ensuing 10 weeks may have been insufficient to detect a therapeutic effect. The reduced expression of MMP-13 by HYADD4<sup>®</sup>-G could limit continued cartilage erosion. The more prolonged clinical benefit with HYADD4<sup>®</sup>-G may be related to its effect on endogenous HA synthesis. HA preparations such as HYADD4<sup>®</sup>-G, with increased joint retention and reduced treatment frequency, may offer advantages in OA treatment.

### 529] EFFICACY OF INTRAARTICULAR HYALURONAN INJECTION FOR KNEE OSTEOARTHRITIS IN HEMODIALYSIS PATIENTS

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**Purpose:** To clarify whether intraarticular hyaluronan injection is effective for knee osteoarthritis in hemodialysis patients.

**Methods:** The subjects were 15 knees of 10 hemodialysis patients, 3 males and 7 females, with symptomatic knee osteoarthritis. Mean patient age was 63.5 years, range 55 to 82 years. The mean duration of hemodialysis was 14.7 years, range 10 months to 31 years. There were 3 knees with Kellgren-Laurence (K-L) grade II, 6 with grade III, and 6 with grade IV. Intraarticular injection of hyaluronan (ARTZ Dispo<sup>®</sup> 25 mg, Kaken Pharmaceutical Co. Ltd., Tokyo, Japan) was performed 5 times per week. We assessed the visual analogue scale (VAS) of knee pain and the Japan Knee Osteoarthritis Measure (JKOM) score for evaluation of knee function. We also analyzed the concentrations of MMP-3, chondroitin 6-sulfate (C6S), chondroitin 4-sulfate (C4S), and hyaluronan in aspirated synovial fluids.

**Results:** Intraarticular hyaluronan administration reduced the degree of pain in 11 of 15 knees (73.3%), most markedly in 9 of 12 knees (75%) with K-L grades III and IV. JKOM scores were improved in 5 of 10 patients (50%). The concentrations of MMP-3, C6S and C4S were decreased in 5 of 6 knees (83.3%).

**Conclusions:** Intraarticular hyaluronan injection reduced the degree of knee pain and improved knee function in hemodialysis patients with knee osteoarthritis by decreasing the concentrations of MMP-3, C6S and C4S in synovial fluid.

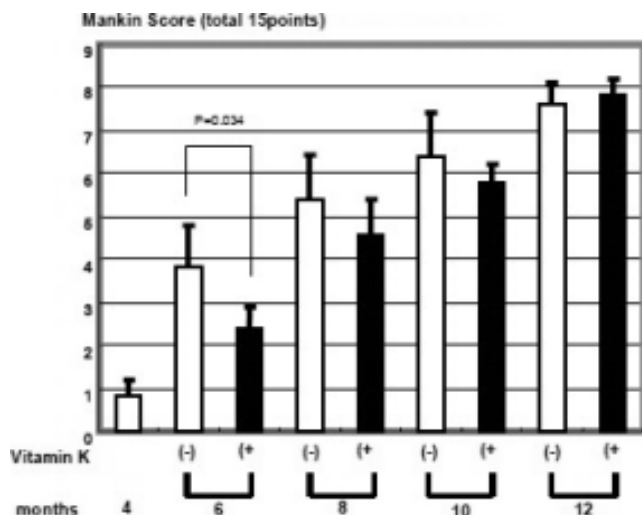
### 530] VITAMIN K2, MENAQUINONES, DELAYS THE PROGRESSION OF KNEE OSTEOARTHRITIS CHANGES IN HARTLEY GUINEA PIG

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**Purpose:** Vitamin K is a family of structurally similar, fat-soluble, 2-methyl-1,4-naphthoquinones, including phyloquinone (K1), menaquinones (K2), and menadiolone (K3). Among these molecules, menaquinones exerts an influence on bone building, especially in osteoporosis. This effect is demonstrated by its ability of posttranscriptional modification of a number of vitamin-K dependent proteins such as osteocalcin or matrix GLA protein (MGP), a bone protein containing gamma-carboxyglutamic acid. Our previous study revealed that MGP played a critical role in regulating endochondral chondrocyte maturation and ossification processes by inhibiting cartilage mineralization *in vitro* and *in vivo* assays. MGP is not only present in the mineralizing zone of the growth cartilage, but also present in articular cartilage. Hence, there might be the possibility that vitamin K influences physiological or pathological course of articular cartilage. This study demonstrates the effect of oral administration of vitamin K2 on the pathological progress of OA using Hartley guinea pigs.

**Methods:** Hartley strain female guinea pigs (n=45) were obtained at 4 months of age from Charles River Laboratories (Wilmington, MA). Animals were divided into 9 groups (n=5) at random. Food and water were available ad libitum. One group was harvested at 4 months of age as control subjects. Eight groups were raised for the subsequent 2, 4, 6, and 8 months with or without food containing vitamin K2 (content 30 mg/g). Animals were euthanized by administration of lethal doses of pentobarbital, and bilateral knee joints were then dissected for following experiments. The dissected joints were fully exposed by disarticulating the patella and severing the cruciate ligaments. After application of India ink, gross morphologic changes of tibial plateau were evaluated. The gross appearance was judged according to the criteria as described by Yoshioka et al. Furthermore, the area of ink-retained portion and total cartilage surface of tibial plateau were calculated in each individual. The ratio of ink-retained area to total cartilage surface was determined.

Coronal plane sections (5  $\mu\text{m}$ ) cut at 200- $\mu\text{m}$  intervals of tibial plateau were stained with hematoxylin and eosin, and Safranin-O. Histological scoring of serial sections of each knee was also performed. Histopathologic alterations in the knee joints were graded according to the Mankin criteria. All sections were graded and median scores were determined for statistical analysis. All data are expressed as the mean  $\pm$  SD. Statistical analysis was performed by using the un-paired t-test.



**Results:** Progressive histopathologic changes characteristic of developing OA were observed concomitantly with aging. This change was initiated by the disruption of the weight-bearing regions of articular cartilage at 6 months of age, and subsequent changes such as cloning of chondrocytes or loss of Safranin-O staining were recognized from 8 months of age onward. The figure shows the Mankin scores obtained from sections stained with Safranin-O. As shown in this figure, the scores were increased with aging in both groups. Statistical significance was recognized between two groups only in the early phase at 6 months ( $p=0.032$ ). This data was consistent with the data of gross appearance. **Conclusions:** The present study demonstrated that menaquinones could delay the progression of osteoarthritis. To our knowledge, this is the first report to discuss the effect of menaquinones on the pathological feature of OA.

### 531 EFFECT OF HYALURONIC ACID IN SYMPTOMATIC HIP OA: A MULTICENTRE, RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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**Purpose:** To evaluate the efficacy and tolerability of one single hyaluronic acid (Adant<sup>®</sup>) intra articular (IA) injection for hip osteoarthritis (OA).

**Methods:** A randomised, double-blind (investigator blinded to the procedure), placebo-controlled trial. Patients (age over 30) with symptomatic hip OA (pain level on VAS >40 mm), Kellgren Lawrence grade II or III, were randomly allocated to receive one fluoroscopically guided IA injection of hyaluronic acid (2.5 ml) or placebo (2.5 ml). Patients were followed up every month for 3 months. The mean outcome measure was the level of pain recorded on a VAS (0–100 mm) at month 3 and compared with baseline. Secondary outcomes included the percentage of responders according to the OMERACT-OARSI criteria, the Western Ontario and McMaster Universities (WOMAC) OA index subscores on pain, stiffness and disability, the patient's and physician's global assessments. Safety was assessed at each visit. Statistical analysis was performed on the inter-group difference in the intent-to-treat population (ITT) and in the per protocol (PP) population. Missing data were replaced by carrying forward the last outcome.

**Results:** Eighty-five patients were included, and were randomized in the hyaluronic acid group (n=42) or in the placebo group (n=43). Baseline characteristics were similar between the two groups. The number of drops out was 5% (n=4). At end point, the decrease in pain was -7.8 (24.95) and -9.12 (27.37) in the hyaluronic acid and placebo groups respectively, in the ITT population ( $p=0.98$ ). Same result was found in PP analysis. The OMERACT-OARSI responder rate was 33.3% in the hyaluronic acid group and 32.5% in the placebo group ( $p=0.94$ ). There was no significant

difference, both in ITT and in PP analysis, in secondary end points as well as in the consumption of rescue medication between placebo and verum. There was no difference in the frequency of adverse events between groups.

**Conclusions:** This study failed to show a superior symptomatic effect of a single IA hyaluronic acid injection (Adant<sup>®</sup>) over placebo in patients with hip OA. Further studies are required to explore the potential efficacy of more than one single intra articular injection in hip OA.

### 532 HYALURONIC ACID INTERACTION WITH BUPIVACAINE IN INTRAARTICULAR ADMINISTRATION

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**Purpose:** Elucidate if a daily common clinical administration of two intraarticular drugs as Hyaluronic Acid (H.A.) used to treat osteoarthritis and Bupivacaine used as local anaesthesia, cause increase degradation of Hyaluronic Acid.

**Methods:** *In vitro* studies with five commercial H.A. have been used in this study, (Synvisc<sup>®</sup>, Coxarthrum<sup>®</sup>, Go-on<sup>®</sup>, Hyalgan<sup>®</sup> and Durolane<sup>®</sup>) with Bupivacaine at three different concentrations (0.25%, 0.50% and 0.75%) with/without adrenaline (1/200,000), with 24 hours incubation at 4°C and 37°C. Chromatography procedures (molecular exclusion HPLC) have been used for determination of molecular weight and degradation percentage of H.A. Anova-Manova and Kendall's correlation have been used to determinate statistical significance.

**Results:** Synvisc<sup>®</sup> and Durolane<sup>®</sup> have shown less degradation and different behavior than Coxarthrum<sup>®</sup>, Hyalgan<sup>®</sup> and Go-on<sup>®</sup> (Kendall's correlation  $p<0.05$ ). Temperature of incubation modified the degradation of H.A. Durolane<sup>®</sup> and Synvisc<sup>®</sup> at 4°C showed higher degradation than at 37°C ( $p<0.05$ ), otherwise happens for Coxarthrum<sup>®</sup>, Go-on<sup>®</sup> and Hyalgan<sup>®</sup> that increased degradation at 37°C ( $p<0.05$ ). Higher concentration of Bupivacaine increased the degradation of H.A. in all cases ( $p<0.05$ ) and the concomitant use of adrenaline increased the degradation in the three concentrations used at the present study for Synvisc<sup>®</sup>, 0.25 and 0.75 for Coxarthrum<sup>®</sup>, only at 0.75 for Durolane<sup>®</sup> and 0.25 for Go-on<sup>®</sup> ( $p<0.05$ ). Adrenaline seems not to increase degradation over Hyalgan.

**Conclusions:** Bupivacaine administration (with/without adrenaline) with H.A. must be valued before concomitant intraarticular administration because Bupivacaine increased H.A. degradation of all the H.A. studied. Durolane<sup>®</sup> and Synvisc<sup>®</sup> have shown less degradation (6 to 20%) and different behavior than Coxarthrum<sup>®</sup>, Hyalgan<sup>®</sup>, Go-on<sup>®</sup> (12 to 20%, 27 to 29% and 28 to 39% respectively). Higher concentration of Bupivacaine and the concomitant use of adrenaline increased the degradation of H.A. in all cases except the concomitant use of adrenaline that seems not to affect Durolane<sup>®</sup> degradation except at highest Bupivacaine concentration.

### 533 CHRONIC ADMINISTRATION OF CHONDROITIN SULFATE DOES NOT AFFECT CYTOCHROME P450 AND NADPH P450 REDUCTASE IN THE RABBIT

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**Purpose:** Chondroitin sulfate (CS) is a SYSADOA eliciting an anti-inflammatory effect. Since patients take CS over long periods, it was of interest to assess whether CS modulates the activity of cytochrome P450 isoforms (P450).

**Methods:** Two models were used, chronic intake of CS in control rabbits and in rabbits with a down-regulated P450 by an inflammatory reaction (IR). Six groups of 5 rabbits were used; three were used to assess the effect of CS on P450, one without CS and two receiving orally around 20 mg/kg/day CS for 20 and 30 days; three groups received turpentine s.c. generating an aseptic IR (AIR) 48 h before their sacrifice, e.g. days -2, 18 and 28, exposed to CS for 0, 20 or 30 days, respectively. CYP3A6, CYP1A2 and NADPH P450 reductase (NADPH) activity, expression and RNAm were assessed in the hepatocytes.

**Results:** Compared with control rabbits, 20 and 30 days CS did not affect the activity of CYP3A6, e.g. 15582±1330, 13480±3052 and 14701±841, and of CYP1A2, e.g. 6532±1203, 11612±2403 and 7494±746, arbitrary