and cartilage explant models have demonstrated a clear stimulatory effect of Collagen Hydrolysate on chondrocyte metabolism and cartilage growth. The objective of this study was to investigate the efficacy of orally administered Collagen Hydrolysate on the development and progression of osteoarthritis (OA) in an appropriate animal model.
Methods: The inbred mouse strain STR/ort spontaneously develops osteoarthritic lesions of the knee joint by 35 weeks of age resembling human osteoarthritis. The efficacy of Collagen Hydrolysate was tested in a randomly assigned placebo-controlled animal study. In 6 month old male STR/ort mice 0.15 mg Collagen Hydrolysate or BSA/g body weight was orally administered once a day over a treatment period of 3 months. Thin sections of the knees were analyzed for osteoarthritic changes by haematoxylin-eosin staining. OA joint damage was assessed by a well-defined semi-quantitative histopathological score. Additionally, the progression of osteoarthritis in male mice at different ages was determined and the correlation between grade of OA and body weight was investigated. A total number of 48 male STR/ort mice were analyzed in this study.
Results: According to the literature the progression of the determined grade of OA in the non-treated STR/ort mice correlated with the aging of the animals. While female mice developed only mild forms of OA, 85% of the non-treated males showed extensive OA-like lesions at the end of the study. The oral administration of Collagen Collagen Hydrolysate over 3 months led to a pronounced decrease in cartilage tissue degeneration in the knee joints. The incidence of severe joint destruction was clearly reduced after Collagen Hydrolysate treatment and the determined grade of OA decreased statistically significantly in comparison to the untreated control animals.
Interestingly, a more detailed analysis of the data suggested a correlation between the determined grade of OA and the body weight of the STR/ort mice.
Conclusions: The results indicate that orally administered Collagen Hydrolysate was able to slow or even halt cartilage destruction in STR/ort mice. The data suggest that Collagen Hydrolysate may prevent the progression of joint degeneration in OA and could possibly be a potential disease-modifying agent for the treatment of degenerative joint diseases.

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AMBIVALENT PROPERTIES OF HYALURONATES IN EXPERIMENTAL INDUCED OSTEOARTHRITIS RAT KNEE

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Purpose: This experimental study was undertaken to determine whether viscosupplementation with intra-articular (i.a.) low- or high-molecular-weight HA injections influences both chondral and synovial lesions in rats with surgically-induced OA knee.
Methods: Male Wistar rats underwent anterior cruciate ligament transection (ACLT). Rats were divided into 5 groups: naive group (n=10), sham group (n=10), ACLT and saline i.a. injection group (n=10), ACLT + Synvisc® (high molecular weight, HMW) i.a. injection group (n=10), ACLT + Hyalgan® (low molecular weight, LMW) i.a. injection group (n=10). Intra-articular injections of sterile saline or HAs were performed on D7, D14, and D21 after ACLT. Animals were killed on D28 for histological assessment. Grading of chondral lesions was performed according to Mankin's score. Rooney's score was used to assess concomitant synovitis. Concomitant immunostainings of activated Caspase 3 and Hsp70 was also performed.
Results: Articular damages (D28) were significantly reduced in HAs-treated knee joints *vs* control joints: 17.55 for HMW and 19.2 for LMW vs 31.3 for ACLT control rats ($p<0.05$). In the cartilage of ACLT+HAs treated groups, articular surface presented minor fibrillations. A significant increase of histological score of synovial membrane was noted in both ACLT+HAs groups (HMW, $p<0.05$; LMW, $p<0.05$) *versus* matched ACLT+saline group. Both HAs-treated groups exerted an inflamed synovial membrane. A basal expression of caspase 3 (6,7%) was observed in the sham group whereas it was significantly increased in ACLT control rats (23.9%). In contrast, apoptotic events significantly decreased in both HAs groups. Additionally, basal expression of Hsp 70 was quite similar in sham and ACLT groups. In contrast, Hsp70 increased significantly in both HA groups.
Conclusions: Although previous works showed that hylan could be responsible for synovial granulomatous inflammation in the clinics, our pilot preliminary study conducted in the ACLT-induced rat OA knee suggests that HAs may exert ambivalent properties on articular structures, simultaneously exerting chondroprotective properties and promoting synovitis

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ORAL HYALURONIC ACID ADMINISTRATION IMPROVES OSTEOCHONDROSIS CLINICAL SYMPTOMS AND SLIGHTLY INCREASES INTRA-ARTICULAR CONCENTRATION OF HYALURONIC ACID IN A HORSE MODEL: A PILOT SURVEY

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Purpose: The intra-articular content of hyaluronic acid (HA) generally declines in inflammatory arthritis (Carro & Blaya, 2002; Takahasi et al, 2004). Intra-articular administration of HA has been used historically, but less information is available about the effectiveness of the oral route. The objective of this study was to determine the effect of the oral administration of an HA concentrate (Hyal-Joint™) on synovial fluid quality and on the clinical condition of horses with osteochondrosis (OCD).
Methods: The horse was used as an animal model because allows the obtaining of high amounts of synovial fluid at different time points. Twelve horses with a radiographic diagnose of OCD were randomly divided in two groups and assigned to receive orally 250mg of Hyal-Joint (HJ) or placebo during 60 days in a blinded randomized controlled clinical pilot trial. At the end of the treatment (d60) and 30 days after finalization (d90) a sample of synovial fluid was extracted from each animal to analyse HA concentration. The degree of synovial effusion measured with ultrasonographic evaluation and the degree of lameness according to AAEP scale were also evaluated.
Results: On day 0 no differences on intra-articular HA concentration were detected among groups (353 ± 45 vs. 301 ± 137 μg/L for HJ and placebo groups respectively). However during the experimental period intra-articular HA concentration increased numerically in the HJ group but decreased in the placebo group, resulting in differences among groups on day 60 (384 ± 42 vs. 209 ± 104 μg/L; P=0.07) and on day 90 (424 ± 89 vs. 286 ± 119 μg/L; P=0.05) which tended to reach statistical significance. Increases of the intra-articular HA concentration in HJ treated horses were associated on d90 with numerical improvements on the synovial effusion scale (1.25 vs. 2.00 points for treated and control groups respectively) and on the degree of lameness (0 vs. 1.5 degrees for treated and control groups respectively), although differences among groups failed to reach statistical significance due to the reduced number of animals.

Conclusions: The overall results suggests that oral HJ administration could increase HA concentration, which as well could be related to improvements of the clinical condition of the affected joint. However further research is necessary to confirm these findings.

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THE GROOVE MODEL OF OSTEOARTHRITIS APPLIED TO THE OVINE FETLOCK JOINT

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Purpose: Until now there have been no appropriate models for metacarpophalangeal osteoarthritis (OA), even though OA in this joint is a significant medical and economic problem in horses. A good model would be useful to evaluate progression and treatment of OA, particularly in this joint. Therefore, we translated the canine Groove model of knee OA to the ovine metacarpophalangeal (fetlock) joint.
Methods: Cartilage surfaces of the metacarpal side of one fetlock joint were surgically damaged (grooved), followed by intermittent forced loading of the experimental joint. After 15 and 37 weeks, joints were analyzed for macroscopic, histologic, and biochemical features (proteoglycan turnover) of OA, and subchondral bone parameters were evaluated.
Results: Technically, the model was difficult to use because cartilage surfaces were very thin. Nonetheless, all macroscopic, histological, and biochemical cartilage parameters demonstrated features of OA. Macroscopic and histological cartilage damage was present at 15 and 37 weeks (delta change of 1.7 vs.3.3 and 1.8 vs.3.1 for 15 and 37 weeks, macroscopy vs. histology, respectively). Proteoglycan synthesis rate was enhanced (+8% and +15%) while the retention of these newly formed proteoglycans was diminished (+30% and +16%) at 15 weeks and 37 weeks post-surgery (all $p<0.05$). Also the release of proteoglycans was diminished for both time points (+55% and 38%; $p<0.05$). Decreased proteoglycan content suggested slow progression of cartilage degeneration over time (+2% (ns) and -12% ($p<0.02$) for 15 and 37 weeks respectively), while synovial inflammation as measured by macroscopy and histology diminished (delta change of 2.0 vs.1.7 and 0.6 vs.0.5 for 15 and 37 weeks, respectively). Impaired subchondral bone quality, reflected by a decreased trabecular thickness (-6%; $p<0.05$) and cortical bone thickness (-14%; $p<0.04$), and osteophyte formation were found. Although osteophyte formation was progressive, subchondral bone changes diminished over time.
Conclusions: The canine Groove model of OA appears to a limited extent transferable to the ovine fetlock joint. Despite development of features of experimental OA, use of the groove model in the ovine fetlock joint has technical limitations. Using larger animals, such as horses, may significantly improve the technical procedures and with that may provide a more reliable model of metacarpophalangeal osteoarthritis that is based primarily on intrinsic cartilage damage, appropriate to evaluate the progression and treatment of cartilage driven OA changes in particular this joint.

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MODELING INTRAARTICULAR FRACTURE IN ANIMAL JOINTS: A PILOT STUDY WITH AN ORGAN-LEVEL MODEL

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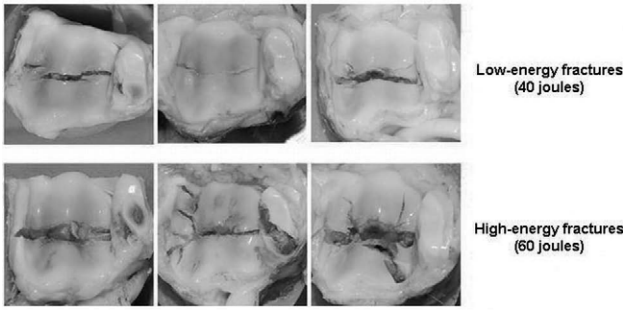
Purpose: Intraarticular fractures (IAFs) are a leading cause of post-traumatic osteoarthritis (PTOA). Commonly, the predominant biomechanical mechanism causing this type of fracture is an axial compressive load of very short duration (milliseconds). Besides this supra-physiologic transarticular impaction load fracturing juxta-articular bone, it also mechanically damages articular cartilage. Cell- and tissue-level damage in fractured cartilage, including death/dysfunction of chondrocytes and disruption of collagen fibers, presumably triggers a pathologic cascade leading to PTOA. An animal model of IAF that accurately replicate pathophysiology of fracture-associated cartilage damage is essential for understanding these pathomechanical details, as well as for developing new treatment strategies to forestall PTOA. In this study, a fracture insult technique for such an animal model was piloted in an organ-level model.
Methods: Three pairs of porcine hock specimens were harvested from juvenile animals immediately after euthanasia, and they were subjected to a quasi-physiologic fracture insult, specifically a transarticular compressive impaction using a drop-tower device. For controlling of fracture morphology by concentrating compressive load onto the anterior tibia, the tibial shaft was tilted 15 degrees posteriorly with respect to the compressive force axis ("offset" impaction technique). One of each pair was subjected to a high-energy impaction (energy delivery = 60 joules levels), and the other was to a moderate-energy impaction (40 joules). Fracture fragments were sampled immediately and incubated over night. TUNEL reaction analysis was preformed on cartilage histological sections for assessing chondrocyte viability in fractured cartilage.
Results: A distal tibial fracture occurred in every specimen, with the fracture morphology very consistent across specimens (Figure). In every pair, fracture displacement was greater with higher energy delivery. Apoptotic chondrocytes were found in every specimen, and the great majority occurring near fracture edges. There was a trend that fractional apoptosis was higher in the transitional to deep zone than in the superficial zone.



Conclusions: With use of the off-set impaction technique, experimental fractures were created with reasonable reproducibility in fracture morphology and severity. The distribution of apoptotic chondrocytes, in terms of relationship to fracture edges, was consistent with human clinical cases (Kim et al., 2002), supporting the validity of the insult technique utilized. A larger-scale experiment using this organ-level model with skeletally mature specimens would allow investigating details of IAF-associated cartilage pathology, as well as testing clinically relevant hypotheses, such as the therapeutic effect of biologic intervention. The concept of the off-set impaction technique is potentially extrapolable to creating a survival animal model of IAF.