Purpose: To analyze the gait pattern in a mouse model of knee OA using the CatWalk systemTM, and to assess effects of intra-articular injection of hyaluronan (IAI-HA) on gait pattern.
Methods: C57BL/6 mice (9 weeks old, male) were purchased and destabilization of the medial meniscus (DMM) was performed on the left knee joint as OA knee model. In this model, OA progress slowly and it resembles slowly-progressive human OA more closely than other surgical models and allows for suitable evaluation of interventions. The CatWalk systemTM was used for gait analysis. In this system, mice were placed on glass plate runway and allowed to walk freely. The whole run was recorded by a video camera placed below the runway and gait parameters were analyzed according to foot prints by the computer automatically. To quantify longitudinal gait changes in DMM model, prior to the surgery, mice (n=5) walked on the runway 10 times for acquiring the baseline data. Then gait analysis was performed at the time point of 4, 8, 12 weeks after the induction of DMM surgery. To assess the effects of IAI-HA to the DMM model, 6 mice underwent DMM surgery, 3 underwent sham surgery where only skin incision and patella luxation was performed . Mice with DMM surgery were randomized to either treatment: (1) HA(800-kDa, ARTZ Dispo),(2) saline,3 mice for each treatment group. IAI of HA and saline were given once a week for 5 weeks both started 3 weeks after surgery. For IAI, mice were anesthetized , incision was made to identify the patella tendon and 20μL of HA or saline injection was done through the patella tendon. Gait analysis was performed for each group of mice at the time point of 3 weeks (just before IAI), 8, 12, and 16 weeks after the surgery.
Results: For each gait parameter, ratio of the affected limb to the contralateral limb was evaluated. After the DMM surgery, no significant change was seen in any gait parameter throughout the period of 8 weeks, but significant lower stand phase, single stance phase, duty cycle (percentage of stand phase during the step cycle), swing speed and significant longer duration of swing phase were observed at the time point of 12 weeks. In HA treated experiments, IAI-HA group tended to show better parameters comparing to saline group but they did not reach statistical significance even at 16 weeks.
Conclusion: Gait disturbance was detected at the time of 12 weeks after the DMM surgery. This result corresponded to the previous report that DMM progress OA slower than other surgical models. It was suggested that CatWalk systemTM could be of use to objectively quantify gait disturbance in DMM model. But we could not clarify the effects of IAI-HA on this model by gait analysis using the CatWalk systemTM.

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APPROACHES TO TRANSCRIPTOME ANALYSIS TO STUDY JOINT REGENERATION IN THE RED-SPOTTED NEWT

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Purpose: The adult red-spotted newt *Notophthalmus viridescens* is able to regenerate damaged knee joints after local injury involving surgically induced defects, collagenase-induced joint instability, and monoiodoacetate-induced cartilage degeneration. The mechanism behind this capacity that is not present in mammals is currently not understood.
Methods: Transcriptome analysis with a custom made microarray from a normalized cDNA library was carried out after surgical and collagenase-induced knee damage in newts. Differentially expressed genes in both instances were validated by qPCR localisation studies. To improve the power of this approach, we have established a new whole newt transcriptome library using normalized cDNA from multiple regenerating and normal tissues including heart, extremities, and eyes with the 454 titanium sequencing technique. We are currently performing quantitative transcriptome analysis after knee damage with mRNA sequencing on the illumina platform.
Results: In the initial microarray analysis, a number of gene groups was found deregulated in the course of knee damage repair, most strikingly several matricellular proteins like tenascin. However, several cartilage specific genes like collagen 2 were lacking. The new library is calculated to cover the complete transcriptome with an overlap of 8 fold, annotations are currently being completed.
Conclusions: Conventional array techniques are powerful tools to study differential gene expression. However, the technique is time consuming and has a reduced power compared to the novel techniques that we are currently applying.

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THE PROGRESSION OF MONOIODOACETATE-INDUCED ARTHRITIS INVOLVES SEQUENTIAL EXPRESSION/SUPPRESSION OF MATRIX ASSOCIATED GENES

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Purpose: Osteoarthritis (OA) is an inflammatory disease with progressive loss of cartilage and bone leading to debilitating joint pain and loss of function. Inflammation is the major cause of cartilage and bone loss. In this report, we examined the gene expression and the signaling networks associated with various stages of cartilage destruction in a rat model of monoiodoacetate-induced arthritis (MIA).
Methods: MIA was induced in the right knee joints of Sprague Dawley female rats (n=60) via intra-articular injection of monoiodoacetate (2 mg/50 μl saline). Saline (50 μl) injected knees served as sham controls. The MIA was temporally monitored macroscopically, microscopically and by μCT (micro-computed tomography) at days 5, 9 and 21 post-MIA induction, and compared to sham controls. Gene Chip analysis (Affimatrix) was utilized to analyze the transcriptome-wide changes in gene expression. The functional networks were generated by Ingenuity Pathways Analysis (IPA), and macroscopic and immunohistochemical findings were correlated with the expression of genes/gene products. Signaling pathways involved in the progression of OA were dissected to focus on salient pathways that drive the cartilage damage during the progression of MIA.
Results: The studies demonstrated that the progression of MIA was progressively damaging to cartilage and underlying bone. In this model of MIA, post-monoiodoacetate injection, Grade 1 damage was observed by day 5, which progressively increased to Grade 2 by day 9, and Grade 3 to 3.5 by day 21. The progression of MIA was accompanied by changes in gene expression, belonging to matrix synthesis/degradation. The maximally upregulated genes in the Grade 1 cartilage damage were genes involved in matrix degradation, those associated with Grade 2 damage were involved in matrix synthesis and degradation, and those associated with Grade 3 to 3.5 damage were involved in matrix synthesis. More importantly, many of these genes were those that have been identified as susceptible genes in human osteoarthritis (OA), such as Asporin,Matrixmetalloproteinase-12 (MMP-12), MMP-19, ADAMTS4, ADAMTS5, GDF5, FRZB and DIO2.
Conclusions: These findings suggest that sequential regulation of distinct gene clusters involving inhibition of matrix synthesis and induction of matrix degradation may control the progression of cartilage destruction in MIA. In this process, Asporin may act as central node regulating the processes matrix synthesis, whereas inflammatory cytokines regulate matrix degradation.

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APPA PROVIDES DISEASE MODIFICATION IN PRECLINICAL OSTEOARTHRITIS

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Purpose: APPA, a proprietary combination of apocynin and paeonol, was evaluated for inhibition of cartilage destruction in a well-accepted rat model of osteoarthritis.
Methods: APPA is a synthetic combination of 2 molecules, 4-hydroxy-3-methoxy-acteophenone (apocynin) and 2-hydroxy-4-methoxy-acetophenone (paeonol). Male Lewis rats were anesthetized and aseptic procedures utilized to induce a medial meniscal 'tear', under an IACUC-approved protocol. APPA was orally administered at 80 mg/kg BID (n=15/group) and animals were euthanized at 3 weeks post surgery. Joints were harvested, fixed in formalin, decalcified, halved in the frontal plane, paraffin

embedded, sectioned at three 200um intervals and stained with Toluidine Blue. Joints were scored according to the OARSI criteria, by a Veterinary pathologist blinded to treatment group, with higher scores reflecting worse pathology.

Results: APPA treatment reduced the total Joint score by 21% ($p=0.01$, Mann Whitney U test), when compared to vehicle. Tibial and femoral cartilage degeneration scores were also significantly reduced ($p=0.01$ and $p=0.03$, respectively, Mann Whitney U test). Rats showed no adverse effects at the 80 mg/kg dose and gained weight through the study. A modest (3%) decrease in weight was observed with APPA and also with a non-efficacious drug control, compared to vehicle controls, indicating that a 3% weight difference (351 grams compared to 339 grams) was not responsible for the observed efficacy.

Conclusions: APPA was well tolerated, and had no adverse effects when dosed at 80 mg/kg BID. Significant decreases in measures of cartilage degradation were observed for a number of well-described histologic parameters. These differences were statistically significant with modest group sizes and relatively short follow-up time points. These results, along with decreased lameness in dogs with clinical OA, indicate that APPA should be further investigated for both pain relief and disease modification.

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EXPOSURE TO STATIC MAGNETIC FIELD FOR PAIN OF ADJUVANT ARTHRITIS RATS

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Purpose: In recent years reports, the body of a living animal or person, exposure to static magnetic fields (SMF) has been to be clinically effective. However, the SMF mechanism of pain relief, it is not clear.

In the present study, examine the effectiveness of the application of a SMF upon pain relief, we performed a study on rats with Adjuvant arthritis(AA).

Methods: Eighteen rats(Sprague-Dawley, Female, 5 week, 150 g) were divided into 3 groups. G I and G II, we injected 0.5ml of incomplete Freund's Adjuvant into the left hind foot in order to induce AA. Injected animals were then maintained for a further 8 weeks in order to develop a chronic pain model. Immediately following this 8 week induction period, G I was exposed to SMF treatment for a further 4 weeks (up to week 12 following the onset of AA). G II wasn't exposed to SMF treatment. G III (control) was maintained without any treatment for a total period of 12 weeks. Following SMF stimulation(mean flux density at the center of a cage: 20-80mT, and magnetic surface: 200mT), we measured blood flow volume in the paw and then reactive speed response to thermal stimulation.

Results: Tail and paw blood flow was significantly lower in G I and G II than G III when analysed prior to the exposure of G I to SMF at the 8 week timepoint. After 4-weeks exposure of G I to SMF (between the 14 week and 18 week timepoints), it was found that the blood flow in the tail and hind foot of G I was significantly higher than that of G II. There was no significant difference in blood flow (tail or hind paw) when compared between G I and G III at the 18 week timepoint. The reactive speed response was significantly slower in G I and G II than in G III when tested before the exposure of G I to SMF at the 8 week timepoint. Following exposure of G I to SMF for 4 weeks, we found that the reactive speed response of G I was significantly faster than that of G II. There was no significant difference in reactive speed response when compared between G I and G III at the 18 week timepoint.

Discussion: In the present study, we found that after exposure to SMF, the results of the planter test of AA rats changed to levels observed in normal rats. We also observed a significant decrease in overall reactive speed response (pain-related) in the SMF-treated AA rat, along with an increase in blood flow. Consequently, we postulate that the SMF-induced increase in blood flow observed in the AA rats described in the present study was most likely due to the removal of pain rather than to the induction of stress. Further basic research, using a specific pathophysiological animal model are necessary in order to fully elucidate the precise manner in which SMF relieves pain in a variety of painful conditions, including ischemic pain and inflammatory pain. As complementary and alternative medicine continues to expand, there is increasing interest in the potential therapeutic use of SMF for therapeutic uses.

Furthermore, a clinical study has shown that SMF treatment successfully relieved pain in patients suffering from neck shoulder pain and muscle fatigue as a direct result of ischemic conditions in the microcirculation. We consider it important to combine the application of SMF with Western medicine or exercise therapy.

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PHYSIOLOGIC EFFECTS OF LONG-TERM IMMOBILIZATION OF THE EQUINE DISTAL LIMB

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Purpose: To describe the clinical, biomechanical and physiological effects of distal limb immobilization and remobilization in the equine metacarpophalangeal joint.

Methods: Eight healthy horses were used in the study. One forelimb of each horse was immobilized in a fiberglass cast for 8 weeks, followed by 12 weeks of a standardized training program of increasing exercise. Serum and synovial fluid were collected during the study for biomarker analyses. The metacarpal bone and proximal phalanges were examined using radiography, computed tomography, nuclear scintigraphy, magnetic resonance imaging, and histomorphometry.

Results: All horses were lame in the immobilized limb after cast removal. Lameness in the immobilized limb improved slightly over time however, low-grade lameness was also observed in the contralateral limb at the end of the exercise period. Range of motion of the immobilized metacarpophalangeal joint was significantly decreased and joint capsule thickening and joint effusion were both significantly increased during the exercise period, compared with baseline values. Significant increases in bone sclerosis and lysis were observed radiographically in the immobilized limb during the exercise period, as well as increased osteophyte, enthesiophyte and fragment formation, as compared with baseline. Computed tomography revealed a significant time-by-cast interaction on bone density in the third metacarpal bone and proximal sesamoid bones. Magnetic resonance imaging revealed a significant increase in synovial proliferation, articular cartilage degeneration, osteophyte and enthesiophyte formation, and thickening within the soft tissues of immobilized metacarpophalangeal joints. Significant increases in the uptake of radionucleotide within the bones of both the immobilized metacarpophalangeal joint and contralateral limb were present at the end of the study, compared to baseline, on nuclear scintigraphic evaluation. Gross evaluation of the metacarpophalangeal joint revealed significant increase in lesions in the immobilized limb relative to the non-immobilized limb including wear line formation, articular cartilage erosion, osteochondral fragmentation and palmar arthrosis. Serum biomarkers including CTX-1, BALP and GAG concentrations varied over the duration of the study period and displayed significant concentration increases and decreases. Synovial fluid biomarkers, including PGE_2, WBC and total protein concentration varied significantly at various points in this study as well.

Conclusions: Eight weeks of single-limb immobilization is sufficient to induce significant changes to bone mineral density, articular cartilage and surrounding soft tissue structures in the immobilized limb. Twelve weeks of exercise is insufficient for recovery to pre-immobilization bone and soft tissue conditions and active bone remodeling appears to be ongoing for an extended duration after removal of the cast. Additionally, there is evidence which is suggestive of changes in the bone of the contralateral limb which may result in ongoing lameness and an overall delay in return to function.

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PREVALENCE OF NATURALLY OCCURRING CARTILAGE DEFECTS IN THE OVINE STIFLE (KNEE)

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APPA PROVIDES DISEASE MODIFICATION AND PAIN AMELIORATION IN PRECLINICAL OSTEOARTHRITIS

Sonya Glasson*; Alison Bendele#; Nick Larkins*
*AKL Technologies Ltd; #BolderBiopath Inc.

Background

- Current symptomatic drugs for OA often provide limited efficacy and have significant safety concerns.
- Safe drugs that can provide pain relief as well as modify the progression of pathology are desired.

Aim

An oral small molecule APPA, was evaluated for inhibition of cartilage destruction and for pain relief.

Methods

- APPA is a synthetic combination of 2 molecules, originally obtained from plants: 4-hydroxy-3-methoxy-acteophenone (apocynin) and 2-hydroxy-4-methoxy-acetophenone (paeonol).
- Male Lewis rats were anesthetized and aseptic procedures utilized to induce a medial meniscal 'tear', in a widely published rat model of osteoarthritis[1] under an IACUC-approved protocol.
- APPA was orally administered at 72 mg/kg BID (n=15/group) and animals were euthanized at 3 weeks post surgery.
- Joints were histologically evaluated by a Veterinary pathologist (AB) blinded to treatment groups and following OARSI guidelines[2].
- In a subsequent study, the same model was performed with a synthetic version of APPA (APPA-S) compared to the natural (APPA-N) version.
- Pain was evaluated in the second study by incapacitance testing[3] at 20 days post surgery, and the shift in weight (expressed as grams) from the OA to the normal limb compared across groups.

Results

- Rats showed no adverse effects at the 72 mg/kg BID and gained weight through the study.
- The pathology scores were significantly reduced ($p<0.01$, ANOVA with Dunnett's Multiple Comparison Test) for tibial cartilage degeneration (22% decrease); Medial Femur degeneration (54% decrease); and Total Joint Score (21% decrease, results not shown).
- The pathology scores were significantly reduced ($p<0.01$, Kruskal-Wallis with Dunn's Multiple Comparison Test) for significant Tibial Cartilage degeneration width (30% decrease).
- At 3 weeks post instability surgery, rats in the vehicle group carried 32 grams more weight on the normal limb than the OA limb.
- The shift in weight from the OA limb was significantly less with both APPA-N (33% decrease) and APPA-S (47% decrease), $p < 0.01$, ANOVA with Dunnett's Multiple Comparison Test.

Conclusions

- APPA was well tolerated, and had no adverse effects when dosed at 72 mg/kg BID.
- Significant decreases in measures of cartilage degradation were observed for multiple histologic parameters.
- Weight bearing shifts were significantly normalized, indicating pain relief with both APPA-S and APPA-N treatment.
- These results, along with decreased lameness in dogs with clinical OA (see poster # 572), indicate that APPA should be further investigated for both pain relief and disease modification.

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APPA PROVIDES DISEASE MODIFICATION AND PAIN AMELIORATION IN PRECLINICAL OSTEOARTHRITIS

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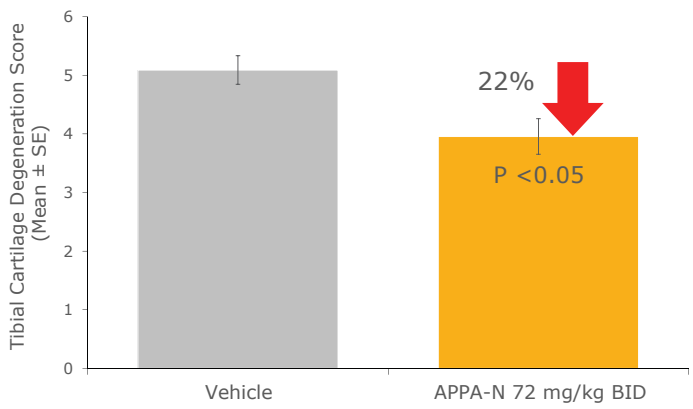
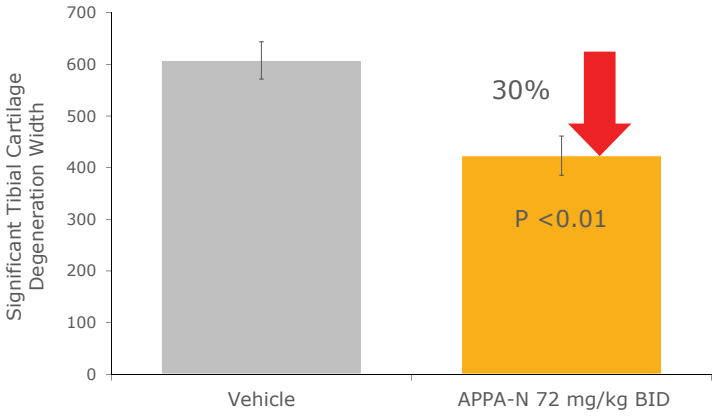
Analysis

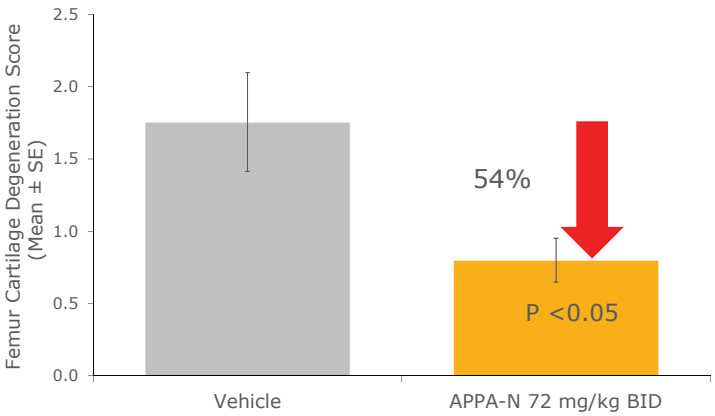
◇ APPA Decreases OA Pathology Tibia Degeneration Score



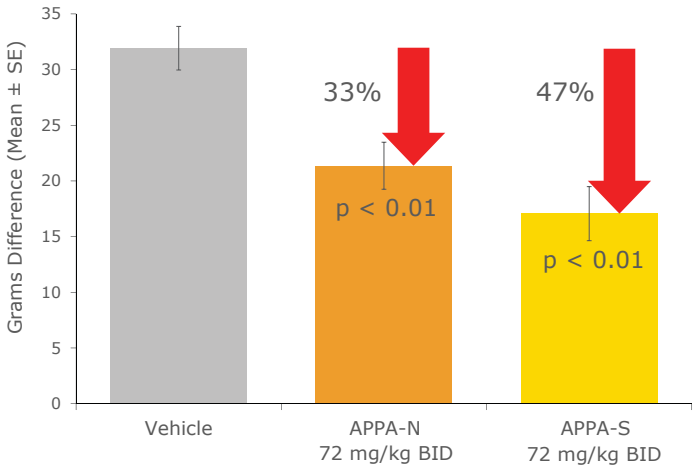
◇ APPA Decreases OA Pathology Tibial Width Measure



◇ APPA Decreases OA Pathology Femur Degeneration Score



◇ APPA Decreases Shifts in Weight Bearing



Both APPA-N and APPA-S were able to decrease weight bearing abnormalities.

This model shows good translation of efficacy to the clinic as oral rofecoxib (COX-2 inhibitor) at 10 mg/kg and gabapentin (a GABA analogue utilized for neuropathic and chronic pain) at 100 mg/kg, were also efficacious at 21 days in this model (45 and 66% decreases respectively)[3].

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