SUMMIT EQUINE INITIAL STUDY

Pilot study: A novel chondroitin sulfate isomer for reducing inflammation in equine joints

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Disclosure: This study was performed prior to acquisition of Summit by Equine Joint Performance. Titleist Technologies Inc is owned and managed by the owners and operators of Equine Performance Veterinary Practice. Drs. Farmer and Costello Chavers were not compensated for work on this project.

Introduction:

Osteoarthritis and synovitis are common conditions in athletic horses that shorten athletic careers and decrease performance and comfort. There are several modalities used in equine sports medicine to alleviate and treat joint pain and inflammation, including steroids, autologous conditioned serums, platelet rich plasma preparations, hyaluronic acids, shockwave, laser, and various nutraceuticals [1,2]. Noninvasive therapies that reduce joint inflammation help prevent further cartilage degradation and may help prolong the athletic life of a horse. Inherent components of articular cartilage are common targets for therapy as they have been shown to be symptom and disease modifying agents[2].

The primary constituents of hyaline cartilage are chondroitin sulfates. Chondroitin Sulfate A (CSA), also known as chondroitin-4-sulfate, is an acid mucopolysaccharide. Acid mucopolysaccharides compose vertebrate connective tissue. CSA is composed of an unbranched chain of repeating units of

disaccharides. The disaccharide unit of the chondroitin sulfates contains glucuronic acid connected by β-1,3 linkages and *N*-acetylgalactosamine. The two major chondroitin sulfates are A and C, and are differentiated by the position of the sulfate groups. Chondroitin sulfate A is sulfated at position 4,

and chondroitin sulfate C is sulfated at position 6 (Figure 1). Thus the names chondroitin 4-sulfate and chondroitin 6-sulfate are used for these two polysaccharides[3]. Chondroitin sulfate chains that are predominantly 4- or 6-sulfated are attached to a protein core, and together help form aggrecan which is the major proteoglycan in articular cartilage [4]. Aging mammalian articular cartilage exhibits changes in aggrecan structure and the multimolecular aggregate that it forms with hyaluronan and link protein. In mammals, active chondroitin sulfate is the 4 moiety with only 1 sulfate group at position 4.

Summit CSA is identical to the natural CSA and is produced directly from pure bovine trachea. No other molecules are added to the structure. The purpose of this pilot study was to evaluate the effects on lameness and inflammatory biomarkers of pure Chondroitin Sulfate A [1] on a small group of horses with varying degrees of documented osteoarthritis and joint pain.

Materials and Methods:

Eight horses with diagnosed osteoarthritis in a joint and varying degrees of lameness were selected for the pilot study (Table 1). There were four gelding and four mares, with mean age of 16 years (range 8-21 years). There were five warmbloods, two Thoroughbreds, and one Appendix Quarter Horse. Treatment with other anti-inflammatories, joint therapy products, or intra-articular therapies were not permitted for 60 days prior to the study or during the duration of the study. Prior to treatment, sterile synovial fluid samples were collected from the affected joint along with systemic blood samples. Horses were injected with 100mg of Summit intramuscularly in the left or right cervical musculature on day 0. Additional synovial fluid and blood samples were then collected at 24 hours, 72 hours, and 7 days following the first injection.

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All fluid and blood samples were handled sterilely, and testing performed immediately. The color, clarity and subjective viscosity were evaluated and recorded for each sample of synovial fluid obtained. Blood samples were tested for C-Reactive Protein (CRP) concentrations [2]. Synovial fluid samples were tested for CRP concentrations and semi-quantitative SAA levels[3] [5–7]. SAA levels were graded 1-4, 1 indicating severe inflammation and 4 indicating no detectable SAA. Statistical significance was assessed using two-tail Student's paired t-test and two sample unequal variances due to small sample size in Excel version 1807 for Windows. Significance was set at $p \leq 0.05$.

Results:

No adverse effects were observed during the study, and none were noted by the owners following its conclusion. Day 7 blood and synovial fluid samples from horse 7 were not obtained. Day 7 synovial fluid sample from horse 1 was not obtained. Mean (± standard deviation) systemic CRP was 38 ± 24 ng/mL before administration. One week following administration it was not significantly different with average of 35 ± 16 ng/mL ($p=0.8$) based on two sample T-test for assumed unequal variances, but was significant ($p=0.01$) with horse 7 removed in the paired t-test. The mean (± standard deviation) intraarticular CRP was 10 ± 8 ng/mL prior to treatment. On day 7, the mean (± standard deviation) intraarticular CRP was significantly different at 3 ± 2 ng/mL ($p=0.05$) based on the two sample T-test for assumed unequal variances, and trended towards significance in the paired t-test ($p=0.066$). Mean (± standard deviation) intraarticular SAA on day 0 was 3 ± 1, which was significantly different from day seven 4 ± 0 in both the paired t-test ($p=0.01$) and the two sample T-test for assumed unequal variances ($p=0.006$). Six of eight horses were noted to have blood tinged and thin synovial fluid on day 0. Of the six horses with synovial

[2] CRP ELISA, ABCAM, San Francisco, CA, USA
[3] EquiChek-SAA, Equine Partners America LLC, Atlanta, GA, USA

fluid retrieved on day 7, five of them were noted to have increased viscosity of synovial fluid and no blood tinting was seen. No subjective notes on horse six were recorded. Due to the small sample size, individual CRP results for all horses are presented in Figure 2.

Discussion:

The results of this study show supportive data for Summit to reduce inflammation in joints. Additionally, there was no indication of increased inflammation systemically or intraarticularly. A small degree of increased inflammation is to be expected with arthrocentesis, as has been demonstrated subsequent to any puncture of the synovium but this was not observed in this small study, and is typically observed with increased synovial fluid protein and cell counts[2].

To the authors' knowledge there are no injectable formulation of CSA currently licensed in the United States. The most similar products are the polysulfated glycosaminoglycans (PSGAG). There is summarized supportive research for PSGAG's ability to modulate symptoms of arthritis, but ability to modulate the disease process is debated [1,2]. PSGAGs differ from CSA in that they add sulfate groups to the basic glycosaminoglycan structure which changes this compound from the chondroitin-4-sulfate found in normal cartilage (Figure 3). This modification likely alters its use and incorporation by cells. CSA is known to have reparative and anti-inflammatory effects in osteoarthritis, but most formulations are oral and not absorbed well [8]. A parenteral formulation would avoid this problem. Direct comparisons of PSGAGs and parenteral CSA in horses are needed.

Synovial fluid was not able to be obtained in all horses at all points as effusion decreased throughout the study. This had a significant impact on the data due to the small sample size. Results were promising none the less. Due to environmental conditions, lameness grade was not able to be tracked throughout

the study, which could have increased the power of the study. Anecdotally, all owners reported improved lameness and comfort within one month of completing the study.

This brief study demonstrates promising data for the utility of Summit to reduce joint inflammation in horses. A larger study demonstrating ability to mitigate lameness or slow progression of arthritis including a larger array of biomarkers is needed to confirm this initial data.

Figures:

Horse	Joint	Lameness (AAEP Grading Scale)
1	LF Fetlock	4
2	RH Fetlock	3
3	LF Fetlock	2
4	RH Fetlock	3
5	LF Fetlock	4
6	LH Fetlock	2
7	R Tibiotarsal	0
8	LH Fetlock	2

Table 1: Horse identification, affected joint, radiographic changes, and lameness grade of horses receiving Summit intramuscularly.

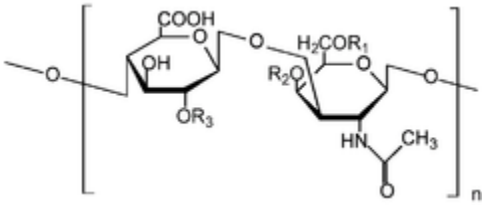

Figure 1: Chemical structure of one unit in a chondroitin sulfate chain. Chondroitin-4-sulfate: R1 = H; R2 = SO3H; R3 = H. Chondroitin-6-sulfate: R1 = SO3H; R2, R3 = H. Note: R2 is position 4.

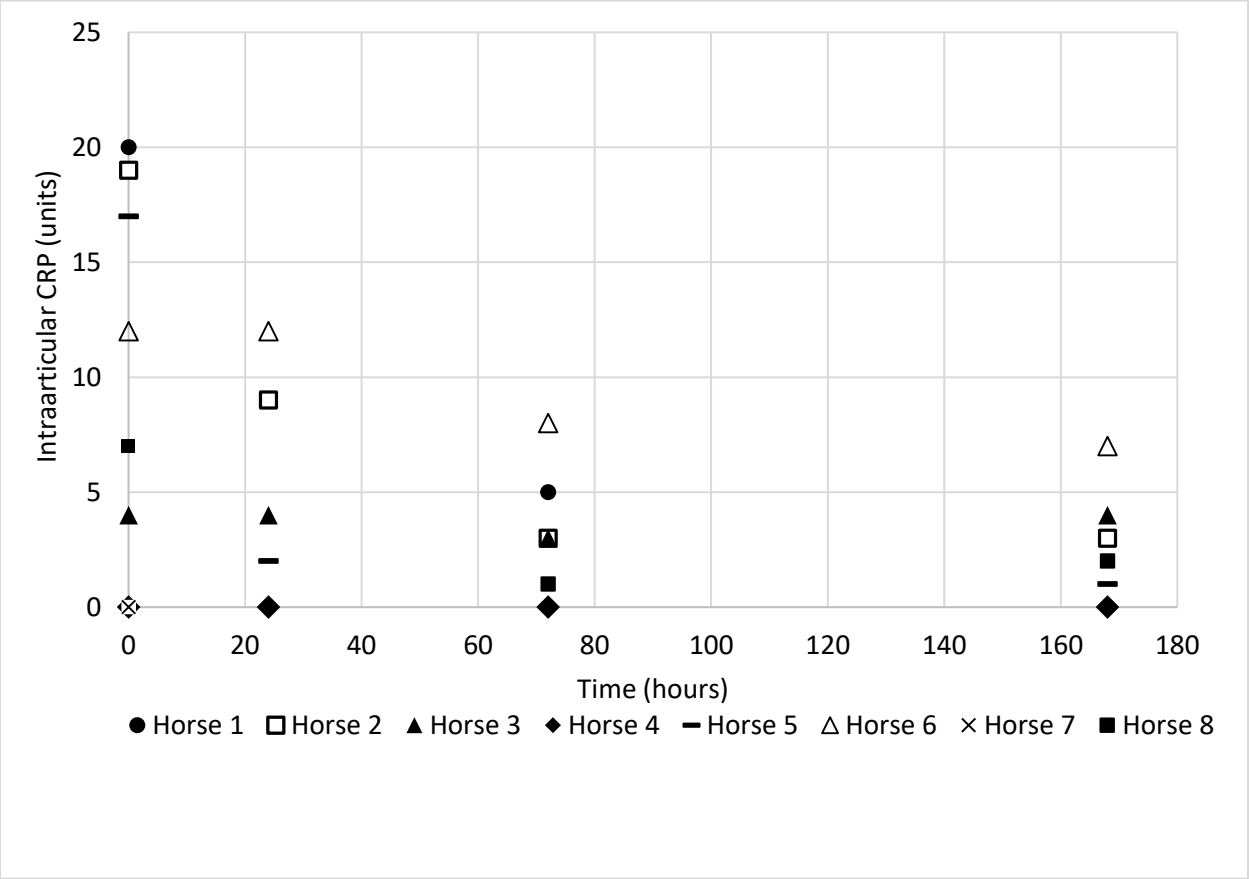

Figure 2: Intraarticular CRP concentrations per horse at 0, 24, 72 hours and 7 days following administration of Summit intramuscularly.

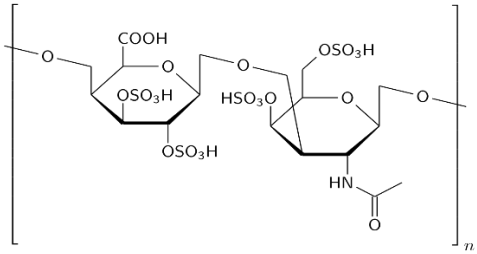

Figure 3: Example chemical structure of one unit of PSGAG with 4 sulfate groups.

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