Less frequently utilized joint therapies including Stanozolol and Pentosan.

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Objectives:

- Know the potential benefits of intra-articular use of stanozolol.
- Be able to select cases that may benefit from intra-articular stanozolol.
- Understand the regulations that may affect you use of IA stanozolol.
- Learn about pentosan polysulfate.
- Think about what horses in your practice are good candidates for pentosan.

Stanozolol

Stanozolol is a synthetic anabolic steroid that can reduce inflammatory processes and act on synoviocytes and chondrocytes promoting anabolic processes when administered intra-articular. Stanozolol acts via local autocrine response stimulating production of anabolic growth factors. Stanozolol reduces apoptosis in equine chondrocytes *in-vitro* by a combination of stimulating IGF-1 production and decreasing nitric oxide production.[1] *In- vitro* gene expression of inflammatory mediators MMP-13, MMP-1, IL-6 and COX-2 was decreased in normal and IL-1β exposed equine chondrocytes.[2] In humans with chronic osteoarthritis, decreased joint pain was associated with increased TGF-β1.[3]

A dose response study utilizing doses of 1, 2.5 and 5 mg per joint of intra-articular stanozolol has been completed.[4] During the study they noticed a mild effusion in some joints. The results showed that the lameness was improved at all three of the dosages. However, the fastest positive results were with the 5 mg weekly dosing after 2 treatments. However, the lameness decreased by more than 50 % at all doses after four injections. The 5 mg dose has been used in the available studies to date.

An abstract published in 2012 on the retrospective evaluation of the use of stanozolol in performance horses evaluated 60 horses, 50 being racehorse, many which were refractory to previous treatments.[5] Horses received a mean of 4 treatments of 5 mg at weekly intervals. There was greater than 6-month follow-up available for 77% of the cases. There were no adverse reactions and a beneficial effect in 39% of cases, uncertain in 39%, not beneficial in 22%. In another study that was double-blinded, weekly intra-articular doses of 5 mg of stanozolol were administered to horses with acute and chronic osteoarthritis.[6] Acute osteoarthritis was defined as less than 1 month of symptoms with chronic osteoarthritis being more than 1 month of symptoms. The maximum treatment period was 21 days and 35 days for acute and chronic groups respectively. A positive outcome was at least a 1 grade reduction in AAEP lameness score. The lameness score was 0/5 in 15 of 21 horses in acute group after 2 treatments and in 7 of 19 horses in chronic group after 4 treatments. They also examined the quality of the synovial fluid which was considered be normal only in the acute group, after the third treatment. Similar to what was noted by Rinnovati *et al* all in the dose response study, there was a mild swelling of the treated joints for a few days after treatment which regress rapidly and spontaneously and did not require intervention.

Because stanozolol is not a mainstream therapeutic with FDA approval, much of the information is more opinion from veterinarians who have been utilizing it. Stanozolol is an underutilized intra-articular therapy, likely a result of limits of availability and controlled

substance regulations. The objective of utilizing stanozolol is the normalization of the joint including cartilage, synovium, subchondral bone cartilage and associated ligaments and menisci. Therefore, this is not generally considered a quick fix, and therapy should include an overall rest and rehabilitation schedule. In most cases a series of injections are done at weekly or greater intervals. With these factors in mind, the 2 more common applications are osteochondrosis lesions in young horses and performance horses with lameness localized to the joint, but less likely to have osteoarthritic degeneration to the point that returning the joint to normal function is unlikely.

Subchondral cysts of the medial femoral condyle, when identified in young horses may be one of the more frequent uses of stanozolol. Early screening of western performance horses for subchondral cysts is frequently done because of the relatively high incidence in the population. Resolution of the cysts have been reported following serial administration of stanozolol. Administration of stanozolol into the lining of the subchondral cyst via ultrasound guidance or during arthroscopy are also discussed and this may also be done in concert with a transcondylar screw. Furthermore, in the stifle, the application of stanozolol in horses with meniscal injuries in conjunction with rest and a rehabilitation program may provide a positive outcome.

The studies presented above were done in horses with both acute and chronic osteoarthritis. The response of horses with an acute arthrititis/synovitis of less than a month was positive, however there was no control group.

Racehorses with palmar/plantar osteochondral disease can be difficult to confirm diagnostically and are consistently difficult manage. Anecdotal reports of 5 mg weekly serial intra-articular stanozolol treatments and a controlled exercise program have improved the likelihood of return to performance of these horses.

The 5 mg/ml micronized stanozolol is a suspension. The micronized particle size is used for intra-articular therapy because the large crystals in the intramuscular product may potentially damage the joint. Stanozolol is frequently dispensed in 5 ml vial, 5 mg/ml concentration. With a 5 mg dose, there are multiple doses in a vial. The suspension needs to be established by agitation prior to drawing a dose from a bottle. The micronized stanozolol is not viscus and easily administered with routine intra-synovial injection techniques. There can be some increased joint effusion following treatment, as noted in the two studies above. There will be an occasional flare which can be managed with phenylbutazone and ice. The co-administration of a corticosteroid may inhibit the desired anabolic pathways, so this is not routinely performed. It is utilized with and without the addition of hyaluronic acid and the addition of antimicrobials can be done based on the veterinarian's preference.

4. Limitations

The primary limitations to stanozolol use are availability and controlled substance requirements in addition to the lack of large case-controlled studies. There is not major manufacturer source available in the United States. In Europe stanozolol is available as Sungate®. In the United States 5 mg/ml micronized stanozolol is available from compounding pharmacies. These sources have been changing and I will present at least some compounding pharmacies that will provide the material. The compounding pharmacy guidelines can require extensive information about the patient/client that it is being to be treated. The pharmacies must be licensed to ship to the state you are in, so this can also be a limiting factor. Stanozolol is a prohibited substance in many testing jurisdictions because of the anabolic effects of larger doses

administers intramuscularly. The administration of 5 mg stanozolol intra-articular is detected in plasma a relatively brief time, but contamination, injection technique, combination therapy can all potentially result in violation of foreign substance rules. The disposition of stanozolol in plasma after intra-articular administration of 5 mg stanozolol administered into both the right and left tarsocrural joints of 12 sound horses was determined.[7] The drug was quickly found in systemic circulation and was eliminated rapidly and could not be detected after 36 hours following intra-articular administration. Particle size of the stanozolol, joint health, and additional factors can all result in a different outcome following administration.

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Pentosan Polysulfate

Pentosan polysulfate sodium (PPS) is a semi-synthetic polysulfated xylan used for the relief of various medical conditions including osteoarthritis (OA) in horses.[1,2] In the osteochondral fragment model, intramuscular administration of PPS for the treatment of experimentally induced OA in horses indicated that 4 weekly injections at a dose of 3.0 mg/kg may be a therapeutic option for OA in horses.[2] In a pilot in 39 horses with OA of metacarpophalangeal/metatarsophalangeal joints, PPS reduced the clinical signs of lameness, and increased range of motion and stride length. In a survey on the use of PPS in Australia, the respondents reported they used PPS for prophylaxis and treatment of OA despite the limited evidence available.[3]

PPS may provide a disease modifying effect in the management of OA. *In-vitro* PPS resulted in a concentration related stimulated proteoglycan synthesis in chondrocyte monolayers.[4] *In-vitro* evaluation of PPS shows there is a dose related inhibition of stromelysin.[5] While there are direct inhibition of degradation effects, there are also direct anabolic effects.[6,7] Chondrocyte uptake of PPS can stimulate production of matrix proteins and also have anti-inflammatory and fibrinolytic effects.[7] In a rat model PPS may preserve proteoglycan within

cartilage matrix.[8] Studies have confirmed that intramuscular dosing of PPS in the horse can achieve concentrations of PPS in the synovial fluid that can result in an effect on synoviocyte metabolism, stimulate proteoglycan synthesis, and reduce metalloproteinase activity.[9] In the osteochondral fragment model, PPS was shown to reduce cartilage fibrillation and increase chondroitin sulfate (CS 846) epitope concentrations in the synovial fluid of treated horses compared with saline-treated controls.[2] PPS is approved for use in Australia[a].

The subsequent data is part of a study of field safety and effectiveness of PPS when injected intramuscularly once weekly for 4 weeks in horses with naturally occurring osteoarthritis. The study was a multi-centered study conducted at 12 veterinary practices in the USA. Horses were maintained by their owners on their current diets and housing. Radiographs of the affected joint were obtained pre-treatment for the purpose of inclusion and on SD28 to verify there were no unexpected changes. The horse's weight was measured at enrollment by weight tape using the heart girth circumference and body length to calculate the weight.[10] The investigational product (IVP) was pentosan polysulfate sodium (PPS) formulated as a 25% (250 mg/ml) injectable sterile solution. Once weekly injections of 3.0 mg/kg PPS on SD0, 7 (±2 days), 14 (±2 days) and 21 (±2 days) were administered. A 0.9% saline solution was used as a control product (CP), with a volume calculated to be equal to the IVP volume. The IVP/CP was administered by intramuscular injection in the neck with the gauge of needle of the clinician's preference. The subsequent injections were given on the opposite side of the neck from the previous injection site and at least 3 inches from the previous injection when on the same side. Any concomitant treatment during the study period was documented in the study records.

At enrollment, the investigator identified a single limb with lameness due to OA with radiographic evidence to support the diagnosis. This limb was then followed in each subsequent lameness evaluations and used in the assessment of treatment effect. The endpoint for efficacy was the improvement in lameness score at SD28. SD28 lameness scores were compared to SD0 lameness scores. Animals with an improvement of at least 1 category in the SD28 lameness score as compared to the SD0 lameness score were considered a treatment "Success"; otherwise, the animal will be classified as a "Failure". Should an animal have been withdrawn from the study for perceived inefficacy or failure to improve, or at any time for treatment-related reasons, they would have been classified as a "Failure". The "Success Rate" of the IVP treated group was then compared to the negative control group to determine overall treatment effect. Any horse removed from the study for treatment-related reasons were considered treatment "Failures". Horses that did not complete all study visits due to treatment-related reasons are included in the effectiveness analysis as treatment failures. In addition, horses that completed all or any of the interim visits but did not complete the follow-up (Day 38 ±2) phone call visits were included in the effectiveness analysis and be deemed as "success" or "failure" based on the last evaluation.

For this study, an adverse event (AE) was defined as any unfavorable and unintended observation in a horse that occurred any time following administration of the IVP or CP, whether or not it was considered product related. Any AE which occurred during the study was reported to the Investigator who recorded the AE and any associated concomitant medication administered.

The study was a negatively controlled, randomized, blinded field efficacy study. The investigator doing the physical examinations and lameness examinations and the owner/agent were blinded. The treatment administrator could not be blinded because of differences in color and packaging of the control and treatment materials. Each animal was randomized in presentation order using randomization generated by SAS statistical package also used for analysis. Descriptive statistics (number of observations, mean, standard deviation, minimum, and maximum

(or number of observations, median and frequency counts for binomial data) were generated for all variables at all visits. All hypotheses were tested at a two-sided 0.05 level of significance. Effectiveness was determined by the results obtained on Day 28. Animals having major protocol deviations were not included in the effectiveness evaluation. The lameness scores from Day 28 were compared to the lameness scores at Day 0, and an animal was classified as a 'Success' if there was an improvement of at least 1 category in the Day 28 lameness scores; otherwise the animal was classified as a 'Failure.' Animals withdrawn from the study for perceived inefficacy or failure to improve, or at any time for treatment-related reasons, were classified as 'Failure. Statistical analysis was performed on a "Per Protocol" (PP) population set (comparison of treatment groups that includes only those cases which completed the treatment originally allocated without major deviation). This population takes into consideration all protocol deviations which would be considered to impact the results and conclusions drawn from this case data. Statistical analysis was also performed on an "Intent To Treat" (ITT) population set. This population reports the efficacy for all cases on the study regardless of any protocol deviations. This population set is included as it gives an indication of the true field situation and how the product would perform once it comes to market. The ITT population will be used for safety summary and analyses. Body systems were evaluated using categorical observations (normal/abnormal). Injection site and physical examination data were evaluated with descriptive statistics.

Enrollment included 237 horses of multiple breeds including Appaloosa, Arabian, Belgian, Crossbred, Dutch Warmblood, Friesian, Grade, Hanoverian, Miniature Horse, Morgan, Oldenburg, Other, Paint Horse, Percheron, Quarter Horse, Saddlebred, Standardbred, Tennessee Walking Horse and Thoroughbred from 3 to 32 years of age. There were 82 females, 151 castrated males and 4 intact males ranging from 153 to 904 kg,

A total of 237 horses were randomized to either the CP (n = 117) or IVP (n = 120), the intent to treat (ITT) population. Of these, 113 CP and 106 IVP horses were included in the per protocol (PP) population. Horses excluded from the PP population included cases with major protocol deviations (16) and sites which enrolled less than 2 cases in one of the treatment groups (2). No horses were eliminated for severe adverse events.

Treatment success rate in the PP population was significantly higher in the IVP group (58.92%) as compared to the CP group (36.29%) on Day 28. Similarly, for the ITT population, treatment success rate was significantly higher in the IVP group (59.19%) as compared to the CP group (36.98%) on Day 28. Descriptive statistics of categorical observations of body systems, heart rate, respiration rate and temperature resulted in no difference noted between the treatment groups. A small percentage of PPS treated horses exhibited an injection site reaction approximately 3 hours post treatment [maximum number on any given study day: edema/swelling 11 (9.17%), heat 1 (0.87%), pain 4 (3.33%) and redness 1 (0.87%)].

No serious adverse events were reported during the study period. The majority of non-serious AEs reported were considered to be transient and did not require treatment, with the vast majority of AEs to be typical of the population under treatment.

The objective of this study was to generate pivotal data to evaluate the field safety and effectiveness of PPS when injected intramuscularly once weekly for 4 weeks. The study demonstrated a significantly higher success rate in the PPS treated group (58.92%) compared to the saline group (36.29%) on SD28 (p=0.0419). The administration of PPS was well tolerated with no serious AEs reported. Injection site reactions were observed in relatively few animals, and the maximum duration was 5 days.

PPS has thrombolytic properties via mechanism similar to heparin, acting on the intrinsic coagulation pathway.[12] Heparin utilizes anti-thrombin III to catalyse thrombin-heparin cofactor II and PPS independently catalysis this cofactor without the use of anti-thrombin III. Lipids and thrombi have been reported to be present in the microvasculature of subchondral bone in joints with osteoarthritis, causing osteonecrosis and pain.[13] Even though the anti-coagulant effects of PPS is significantly lower than heparin and is cleared from plasma concentrations relatively quickly, it has been reported to improve blood flow in subchondral bone and reduce joint inflammation in animal model studies.[12,13] In this study at a dose of 3.0mg/kg given systemically, PPS did not cause any adverse events such as, hemorrhage or pain at injection site, thrombocytopenia, etc.

In this study 58.92% of the horses treated with PPS improved at least one grade of lameness. When PPS was evaluated in the osteochondral fragment model, there was not a significant difference in lameness between control horses and horses treated with PPS.[2] There were however indications of disease modifying effects including decreased cartilage fibrillation. While the methodology of these two studies is quite different, the findings of both studies are very relevant. In this study, enrollment criteria required radiographic evidence of OA. While this helps create a more homogenous study group, it does select for more advanced cases where subtle disease modifying effects may not be evident. The improvement of almost 60% of the cases enrolled in this study is notable, because of the more advanced nature of cases being enrolled. Ultimate clinical use may be more effective earlier in the disease process. Where PPS is approved for use, a survey of veterinarians indicated PPS had a higher efficacy as a prophylactic drug than for treatment OA.[3] Similarly respondents perceived PPS had moderate treatment efficacy. This would suggest that the methodology of this study found a significant outcome in a challenging model of treating advanced OA with radiographic evidence of degeneration.

References and Footnotes

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