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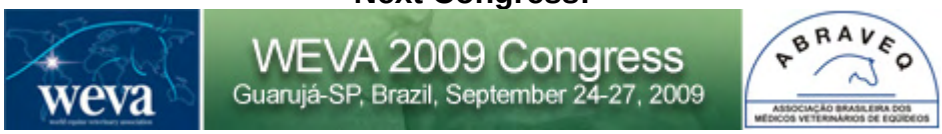

WHAT THE EQUINE PRACTITIONER NEEDS TO KNOW ABOUT THE BIOCHEMICAL MANIPULATION OF EQUINE JOINT DISEASE

C. Wayne McIlwraith

BVSc, PhD, DSc, Drvetmed (hc), FRCVS, Diplomate ACVS
Barbara Cox Anthony University Chair
Professor of Surgery
Director of Orthopedic Research
Colorado State University, Ft. Collins,

A recent survey suggested that 60% of lameness problems are related to OA[1], stressing the importance of advancements of both medical and surgical treatment options. This section reviews medical options currently used for treating joint disease, emphasizing recent and/or future perspectives. The section after this will address these surgical options.

The aim of treatments for acute synovitis, with or without accompanying capsulitis, is to return the joint to normal as quickly as possible. In addition to bringing relief to the patient and allowing it to return to normal work, suppression of synovitis and capsulitis is important in order to prevent the products of inflammation from compromising the articular cartilage and leading to osteoarthritis (processes previously described). In addition to the potential deleterious effects of synovitis on articular cartilage, it is important to provide pain relief and minimize the potential microinstability associated with excessive synovial effusion. It has also been shown experimentally in the rabbit that joint inflammation weakens intra-articular ligaments in addition to affecting the cartilage.

In all traumatic entities in the joint, the goal in addition to returning the joint to normal as quickly as possible- is to prevent the occurrence or reduce the severity of osteoarthritis. In other words, there are two goals; 1) reduce pain (lameness), and 2) minimize progression of joint deterioration. While this section addresses medical treatments, it is important to note that timely removal of osteochondral chip fragments, timely and appropriate reduction of fixation of large intra-articular fractures, accurate diagnosis of ligamentous and meniscal injuries with arthroscopy and the appropriate treatment of osteochondritis dissecans entities are all critical treatments to prevent OA. The remainder of this sections deals with treatments where progress, knowledge, or new treatments have been developed in the past 10 years.

Physical Therapy and Shock Wave Therapy

Swimming and underwater treadmills are popular rehabilitation tools following arthroscopic surgery for joint injury and also, to a lesser degree, rehabilitation of non-surgical injuries. Underwater treadmills have become increasing available and decrease the weight-bearing while potentially providing a massaging effect on the limbs and preventing fibrosis of the joint capsule. Controlled work with some evidence basis for the relative usefulness of these modalities would be an excellent contribution to our knowledge.

The only non-medical or non-surgical physical therapy tool that has been looked at in a controlled fashion in the horse is that of extracorporeal shock wave therapy (ESWT). An equine specific controlled OA study has been done comparing ESWT to Adequan® and a sham treatment group.[2] The study used our established short-term (70 day) OA model, where an osteochondral fragment is created at time 0 and treatments are initiated 14 days later. ESWT was administered on days 14 and 28 using the Versa Tron machine (High Medical Technologies) and a 12 mm probe, and a sham shock wave procedure was performed on the control horses on days 14 and 28.[2] A positive control group involved IM Adequan® treatment every 4 days for 28 days. The shock wave energy was delivered mainly to the middle carpal joint capsular attachments, but some energy was delivered to the area of fragmentation. Significant improvement in clinical lameness, decreased synovial fluid TP (as a marker of synovitis), and less glycosaminoglycan (GAG) levels in the serum (a biomarker of early osteoarthritic change) was observed with ESWT compared to both control and Adequan® treated horses.[2] These results imply promise for this type of therapy in

localized joint disease in horses, but clinical studies with sufficient numbers still need to be reported.

Non-Steroidal Anti-Inflammatory Drugs (NSAID's)

The term NSAID's is used to describe anti-inflammatory agents that inhibit some components of the enzyme system that converts arachidonic acid into prostaglandins and thromboxains and their use in the horse was well reviewed in 1996.[3] All NSAID's inhibit cyclooxygenase activity to some degree[3, 4], but more recently two different isoenzymes for cyclooxygenase (COX) called COX-1 and COX-2 have been reported and this has potential importance in the horse. COX-1 has been associated with the "good" or "housekeeping" functions of the cyclooxygenase pathway.[5] It has constitutively produced and has been shown to be important in the balance of normal physiologic function of the gastrointestinal and renal system, while having a lesser role in the inflammatory COX cascade. COX-2 has mainly been associated with inflammatory events, especially those driven by macrophages and synovial cells it is attributed with only minor roles in normal physiology, thus its "bad" or "inducible" role. There have been developments of drugs that preferentially inhibit COX-2 enzyme. While it appears logical that inhibition should minimize side effects, there has been some suggestion that complete inhibition of COX-2 may not be optimal for the joint or the patient[5] It is felt at this stage that while COX-1 is mainly responsible for the protective functioning of prostaglandins, COX-2 also plays some accessory role, or is, at least, more important than previously thought. The mainstream still feels that the beneficial effects of selective COX-2 inhibition in joint disease are ideal. Anecdotally we have used carprofen (Rimadyl®) at the Orthopaedic Research Center at CSU in horses that have developed high creatinine levels and diarrhea in association with phenylbutazone use. The disappearance of these side effects when the horse is placed on carprofen implies a protective effect with a drug that has more preferential COX-2 inhibiting activity than phenylbutazone.

A new development has been the licensing of a topical NSAID preparation (1% diclofenac sodium cream). Research in humans had previously indicated the topical NSAID's application could be clinically beneficial, while reducing systemic side effects. Anti-inflammatory effects were shown in experimentally induced subcutaneous inflammation.[6] A clinical field trial of the topically applied diclofenac liposomal cream for the relief of joint inflammation showed promising results.[7] The product is now licensed.

A relatively recent paper also raised the issue of whether NSAID's are deleterious to articular cartilage. The topic is not a new one and in 1993 there was a suggestion that inhibition of the E group of prostaglandins could have long-term unfavorable effects on cartilage metabolism.[8] *In vitro* work in the horse had initially shown no evidence of deleterious effects on cartilage metabolism[9], but in a more recent paper based on administering phenylbutazone for 14 days to horses and then testing the serum on articular cartilage explants in vitro concluded there was decreased proteoglycan synthesis to a degree similar to that with rhIL-1β.[10] Until *in vivo* deleterious effects have been demonstrated the author feels that in the absence of any clinical associations between the use of phenylbutazone and articular cartilage degeneration, continued appropriate use of NSAID's is justified.

Intra-articular Corticosteroids

The use of intra-articular corticosteroids for equine joint disease was extensively reviewed in 1996.[11] More recent clarifications of the benefits and deleterious side effects of intra-articular corticosteroids in the horse represent a good example of clinical observation leading to scientific inquiry. Based on the authors observation of an apparent lack of correlation between the prior use of betamethasone esters (Betavet Soluspan®) and articular cartilage degradation during arthroscopic surgery for osteochondral chip removal, experimental studies were initiated of the three most commonly used intra-articular corticosteroid, namely methylprednisolone acetate (Depo-Medrol®[a]), triamcinolone acetonide (Vetalog®[b]), and betamethasone esters (Betavet Soluspan®[c]) were evaluated using the osteochondral fragment model.[12,13, 14] The first product studied was Betavet Soluspan® (later discontinued but then available as Celestone Soluspan®, and this has since been discontinued).

Triamcinolone acetonide (Vetalog®) was shown to have chondroprotective effects- with improved functioning of articular cartilage and no side effects (in particular, no degradative effects on the cartilage). On the other hand, methylprednisolone acetate (Depo-Medrol) had harmful effects to the cartilage and its use needs to be limited as much as possible.

These *in vivo* studies, coupled with some *in vitro* work, have fueled the recommendation that the use of triamcinolone acetonide especially in high motion joints is ideal. There have been some options on "low" dose corticosteroid administration alleviating negative effects of MPA. However, based on in vitro titrations studies, it appears that the lower doses that are commonly used are unlikely to have the same effects and a greater concentration of corticosteroid is needed to inhibit the catabolic compared to the anabolic effects in articular cartilage.[14] On the other hand, clinical improvement is more important to the clinician than in vivo data.

Intra-articular corticosteroids have commonly been combined with hyaluronan and there has been a perception that it might be protective against the effects of corticosteroid. This perception has been based on tradition rather than scientific proof, but has become common thinking amongst equine practitioners.

Hyaluronan (Sodium Hyaluronate)

Hyaluronan is non-sulfated glycosaminoglycan and the biological characteristics and therapeutic use of hyaluronan in an equine osteoarthritis have been reviewed previously.[15,16] Hyaluronan has modest analgesic effects,[17] but more emphasis has been placed on its anti-inflammatory effects that may be physical (steric hindrance) or pharmacological (inhibition of inflammatory cells and mediators).[16] Various in vivo and in vitro studies have shown protection against IL-1, driven prostaglandin synthesis, as well as inhibition of free radicals, but the ability of hyaluronan to inhibit the activity of MMPs is questionable.[18, 19] It has also been pointed out that, because several inflammatory mediators can augment the production of HA by synovial fibroblasts in vitro, elevated synthesis of HA in early osteoarthritis may constitute a protective response by the synovium to joint inflammation.[16] While providing a rationale for exogenous administration, it may explain the elevated levels of HA in response to intra-articular injection of a number of medications.[12, 13]

It has been the authors clinical impression that HA alone is useful for mild to moderate synovitis, but for the treatment of most clinical cases, adjunctive use of a corticosteroid is necessary. It has also been claimed that HA preparations of molecular weight exceeding 1×10^6 daltons may provide superior clinical and chondroprotective events, but this is a controversial claim.[20, 21]

The use of intravenous (IV) HA in the treatment of joint disease is now common. An experimental study documented a significant improvement in clinical lameness, decreased PGE_2 and total protein levels in the synovial fluid, and decreased synovial membrane hyperemia and cellular infiltration.[22]

The prophylactic use of IV HA has been studied in both Quarter Horse and Thoroughbred race horses. One hundred forty horses were entered in the Quarter Horse study and received either IV saline or HA every 2 weeks for the duration of the 9 month study.[23] Trends for HA treated horses to race longer, require an intra-articular injection of corticosteroid earlier, have a better speed index, higher average number of starts, and more money earned was observed when compared to placebo treated horses. A similar study has been conducted in Thoroughbred racehorses using synovial fluid markers and starting with horses without musculoskeletal problems. No significant differences were found, but anecdotal reports from trainers and various equine disciplines have been positive regarding the prophylactic use of IV HA.

Polysulfated Glycosaminoglycan

Polysulfated glycosaminoglycan (PSGAG) belongs to a group of polysulfated polysaccharides and includes, (in addition to PSGAG, pentosan polysulfate, as well as glycosaminoglycan peptide complex (Rumalon®). These drugs have been referred to as chondroprotective, or a more recent definition, slow-acting disease modifying osteoarthritic drugs (SAMOD). Because of this PSGAG has been traditionally used where cartilage damage is considered to be present rather than in the treatment of acute synovitis.[24] Therapy with such drugs

is either meant to prevent, retard, or reverse the morphologic cartilaginous lesions of osteoarthritis with the major criteria for inclusion being prevention of cartilage degeneration. The principal GAG present in PSGAG is chondroitin sulfate and the product is made from an extract of bovine lung and trachea modified by sulfate esterification.

Adequan®[d] was reviewed extensively in 1996.[24] At that time there had been a number of in vitro studies, including one demonstrating that PSGAG that was the only drug tested (others included phenylbutazone, flunixon, betamethasone, and hyaluronan) that inhibited stromelysin.[25] There had been three other in vitro studies on the effect of PSGAG on equine cartilage that were somewhat contradictory. Initially it was reported that PSGAG caused increased collagen and glycosaminoglycan synthesis in both articular cartilage explants and cell cultures from normal and osteoarthritic equine articular cartilage.[26] However, other work had found a dose dependent inhibition to proteoglycan synthesis, little effect on proteoglycan degradation, and no effect on proteoglycan monomer size.[27] Various *in vivo* studies have supported the value of intra-articular (250 mg) of PSGAG in equine joint disease; including a clinical study,[28] a study using a Freund's adjuvant-induced model (a study in dogs)[29] and another equine carpal model using sodium monoiodoacetate.[30] In the latter study, there was significant reduction of articular cartilage fibrillation erosion, less chondrocyte death, and markedly improved Safranin O staining. At the same time, PSGAG had no benefit in healing articular cartilage lesions that were already present.

Studies with intramuscular PSGAG (500 mg every 4 days for 7 treatments) showed relatively insignificant effects with treatment (limited to slightly improved Safranin O staining in sodium monoiodoacetate joints when PSGAG was used).[31] In a more recent unpublished experimental study where IM PSGAG was used as a positive control (administered every 4th day for 28 days starting 14 days post OA induction), there was some improvement in clinical lameness 56 days after initiation of treatment and decreased GAG levels in the serum 14 days post-treatment (GAG is a marker of disease in this OA model).[2] However, there was more impressive improvement in the third test group (shock wave group therapy).[2]

A principal driving force the persistent use of IM PSGAG in preference for intra-articular PSGAG has been the work demonstrating a slightly increased risk of infection (compared to corticosteroids and HA).[32] Apparently receiving less notice is a companion paper reporting all risks could be obviated with concurrent IA administration of 125 mg (0.5 ml) of amikacin sulfate.[33] The author still feels that post-operative IA PSGAG when there is significant exposure of subchondral bone (loss of articular cartilage) is a successful treatment. The main clinical observation is reduction of hemarthrosis, synovial effusion, and improvement in viscosity. There is some insinuation from experimental work that the endogenous repair of cartilaginous lesions can be reduced with PSGAG.[34] This is a potential (but yet to be demonstrated clinically significant) caveat.

Pentosan Polysulfate

The use of this drug in the treatment of joint disease was reviewed in 1996.[35] PPS could also be considered as a disease modifying osteoarthritic drug (DMOAD) and it was pointed out in the review article that PPS unlike NSAID's do not possess analgesic activity.[35] The conclusion was that in order to provide symptomatic relief and efficacy, a drug such as PPS must be capable of correcting the pathobiological imbalances that are present within the OA joint and the authors at that time felt that PPS fulfilled these requirements. However, at this stage the only reports of its use in the horse were anecdotal.

PPS is a heparinoid compound but is unique in that it is derived from beechwood hemicellulose instead of animal sources. Commercial products available include Cartrophen Vet® (licensed in small animals in Australasia, but not in horses) and more recently Pentosan Equine Injection® (pentosan polysulfate sodium 250 mg/ml) which is licensed in Australasia. In studies in sheep, weekly intra-articular injections of PPS for 4 weeks improved joint function and reduced mean radiographic scores and Mankin histologic scores of articular cartilage damage in the femoral condyle.[36]

Recent work from our laboratory has demonstrated favorable results. Using the osteochondral fragment-treadmill model of equine OA in the carpus, there was significant decrease in articular cartilage fibrillation and a strong trend for overall cartilage histologic

appearance (modified Mankin Score). Furthermore, most other parameters showed numerical improvements (including lameness, joint flexion, synovial fluid TP, synovial fluid collagen degradation products, and aggrecan synthesis) although statistical significance less than 0.05 were not obtained. In this study PPS was given at a dose of 3 mg/kg body weight once weekly for 4 weeks.[e] This is the current recommendation for treating horses with mild or early stage OA, particularly with multiple joint involvements (being a systemic drug). On the other hand, based on the previously cited study, there has been some discussion of potentially increasing the dose frequency to 3 mg/kg once every 5 days, for a total of 7 injections.

Oral Joint Supplements

It is important to recognize that none of the oral supplements or oral nutraceuticals is licensed and proof of efficacy is generally lacking. Most products include glucosamine and/or chondroitin sulfate along with other added ingredients. Historically the oral glycosaminoglycan products initially available for the horse included a chondroitin sulfate product from bovine trachea (Flex-Free®) and a complex of glycosaminoglycans and other nutrients from the sea mussel, *Perna canaliculus* (Syno-Flex®). More recently a combination of glucosamine hydrochloride, chondroitin sulfate, manganese, and Vitamin C has been marketed as a nutraceutical (Cosequin®) and a number of other products have simulated Cosequin®. Since that time, other products have attempted to compete on the basis of decreased cost (with no demonstration of comparable efficacy) or other added ingredients. With regard to the commonly used practice of combinations of using glucosamine and/or chondroitin sulfate, glucosamine sulfate is a precursor of the disaccharide subunits of cartilage proteoglycans. While glucosamine salts have been reported as well absorbed after oral absorption in man[37], one study has reported an oral bioavailability of glucosamine hydrochloride in horses to be 2.5%, with a large volume of distribution, which the authors interpreted as poor absorption from the intestinal tract but extensive tissue uptake.[38]

More recent work on the quantification of glucosamine in serum and synovial fluid after nasogastric or intravenous administration of glucosamine hydrochloride to horses questions effective absorption off glucosamine hydrochloride in the horse.[39]

Chondroitin sulfate consists of alternating disaccharide subunits of glucuronic acid and sulfated N-acetylglactosamine molecules and is a principal glycosaminoglycan of aggregating proteoglycan (aggrecan). Chondroitin sulfate is less sulfated, but resembles PSGAG in structure and mechanism of action. Oral absorption of a chondroitin sulfate has been tested in horses. A low molecular weight chondroitin sulfate (0.80 kDa) has been evaluated by quantifying the disaccharide content using a validated method that combined enzymatic digestion of plasma followed by fluorescence HPLC. Low molecular weight chondroitin sulfate was absorbed to a higher extent compared with glucosamine and it was also demonstrated that its absorption may be influenced by the molecular weight of the polymer.[40]

In vitro studies can potentially help determine at what concentrations glucosamine or chondroitin sulfate may inhibit the catabolic response in equine cartilage explants. One study done with cartilage discs incubated with lipopolysaccharide in the varying concentrations of glucosamine, chondroitin sulfate, or both revealed that glucosamine concentrations as low as 1 mg/ml decreased NO production relative to LPS stimulated cartilage, but that chondroitin sulfate at either 0.25 or 0.50 mg/ml did not inhibit NO production. Glucosamine concentrations as low as 0.5 mg/ml decreased PGE_2 production, where as CS did not affect PGE_2. The combination decreased MMP-9 activity, but has no effect on MMP-2 and there was a trend for decreasing MMP-13 protein concentrations.[41]

In vitro dose titration studies of glucosamine hydrochloride (GU) and chondroitin sulfate (CS) alone and in combination have recently been reported based from work in our laboratory. There were no detrimental effects of GU, GS, or GU plus GS on normal cartilage metabolism. Higher doses of GU, CS and GU plus CS appeared to limit total GAG release into the media, where as intermediate doses enhanced GU, CS, and GU plus CS enhanced GAG synthesis and total cartilage content.[42]

The same dosages tested on IL-1 conditioned articular cartilage explants revealed no treatment effects for GU or CS alone, but a protective effect of high dosages of GU plus CS for total GAG release into the media. The study suggested that GU plus CS might be beneficial to

cartilage metabolism by preventing GAG degradation. However the question of effective concentration of GU after oral administration is still an issue[39] and clear *in vivo* demonstration of reduction and degradation would be ideal information.

Other oral joint supplements used include Platinum Performance®[e], which is a combination of rare earth minerals which includes various rare earth minerals and omega-3 fatty acids (making it somewhat unique). Omega 3 fatty acids have been shown to inhibit aggrecanase.[43] This has been used post-operatively but all information is anecdotal. Similarly, oral HA products are new to the market and a recent controlled study in our laboratory did not demonstrate effectiveness in our equine OA model.[e] Recently another experimental study using the CSU equine OA model has demonstrated value for an oral supplement containing soy and avocado**.** This is the first well controlled scientific study demonstrating a positive effect with an oral nutraceutical.

New Biologic Therapies

The knowledge gained from improved understanding of critical mediators in equine traumatic arthritis and OA has lead to the identification of new targets for therapy. Two obvious targets identified include metalloproteinases (MMPs) and IL-1.

Inhibition of metalloproteinases as a therapeutic approach

Metalloproteinase inhibitors include peptide-based inhibitors (including hydroxamic acids), non-peptidal inhibitors (this includes chemically modified tetracycline's such as doxycycyline), and naturally occurring inhibitors (such as N-3 fatty acids, i.e. fish oils). Recent work has demonstrated that N-3 fatty acids, as found in fish oils, will inhibit MMPs and aggrecanase (which as discussed before, is a key enzyme in the degradation of aggrecan).[43]

In vitro in our laboratory with the MMP inhibitor Bay-12-9566 using equine and canine articular cartilage explants in an IL-1 degradation model and using the COL2-3/4C$_{short}$ immunoassay showed that there were significant dose dependent reductions in the catabolic effect of IL-1α on the release of proteoglycans and type II collagen from articular cartilage explants exposed to 10 fold increases in concentrations (1nM:10 μM).[44]

No *in vivo* work has been done in the horse; however, an *in vivo* study in experimental OA in the dog, failed to demonstrate efficacy with an MMP inhibitor and the prospect for these being a valuable biological therapy for horses seems low.

Novel methods of administering therapeutic proteins (including Gene Therapy)

The functional unit of DNA is the gene which can be defined as the set of DNA sequences that are required to produce a single polypeptide (protein). The gene sequence codes for a specific messenger RNA (mRNA) molecule that, in turn, carries the genetic information from the nucleus to the cytoplasm for translation into amino acid sequence (i.e. a protein). While many recognized diseases relate to a lack of or a defect in or an imbalance of a particular protein (S) and since the gene is the basal unit ultimately responsible for protein production, it is also a logical therapeutic target.[58] At the moment most gene therapy protocols (at least the ones we have evaluated) are directed towards increasing levels of selected therapeutic proteins in an attempt to alter specific disease dysfunction. Depending on the natural function of the protein we might be able to enhance or repress certain direct effects on specific cellular processes.

The key component is the efficient transfer and expression of therapeutic genes (and the example used in our laboratory is IL-1ra) by inserting the manipulated gene sequence into a vector. One such example is interleukin-1 receptor antagonist (IL-1ra), which we have used in our laboratory. Previous work when the protein was isolated and administered to laboratory animals with induced OA showed that it inhibited the progression of OA. After the gene sequence of the equine IL-1ra molecule was deduced in our laboratory[46], the value of gene therapy with IL-lra using an adenoviral vector in the treatment of equine OA was then investigated.[45,47]

Proof of principal experiments demonstrating *in vitro* expression of an active equine IL-1ra protein following gene transfer of the equine IL-1ra gene sequence to cultured equine synoviocytes using an adenoviral vector were first performed.[45,48] Following confirmation that the adenoviral vector could infect equine synoviocytes and produce a biologically active IL-1ra protein, an in vivo dose titration study was done. Using the same adenoviral vector carrying the equine IL-1ra gene (AdeqIL-1ra) the optimal vector concentration to provide peak concentrations and duration of IL-1ra protein expression was determined without significant side effects was

determined. Next, using our established experimental model of equine OA this gene therapy treatment was tested and shown to significantly reduce lameness and synovial effusion in the arthritic/fragmented joints. The horses receiving gene therapy also had significantly less pathologic change note on gross examination of the joints compared to placebo treated arthritic/fragmented joints and microscopically there was also significant improvement in the articular cartilage compared to the controls.

Since that time gene therapy, again with IL-1ra but combined with IGF-1, has been tested for its capability of improving cartilage healing and a gene therapy protocol using BMP-2 shown to aid healing in the presence of osteomyelitis in rabbits.[49]

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Footnotes

a) Depo-Medrol®, Pharmacia and Upjohn Co., Kalamazoo, MI 49001.
b) Vetalog®, Bristol Myers Squibb for Fort Dodge, Fort Dodge, IA 50501.
c) Betavet Soluspan®, Schering-Plough Animal Health Corp., Union, NJ 07083.
d) Adequan®, Luitpold Pharmaceuticals Inc, Animal Health Division, Shirley, NY 11967.
e) Platinum Performance®, Platinum Performance Inc., PO Box 990, Buellton, CA 93427.
f) Trumble, TN, PhD dissertation; Colorado State University, Ft. Collins, CO, 2003.