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### Feeding and Veterinary Management OF THE SPORT HORSE



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## **Management of Joint Disease in the Sport Horse**

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### INTRODUCTION

The joint is an organ, and there are a number of ways in which traumatic damage occurs, ultimately resulting in degradation of articular cartilage. It was recognized in 1966 that articular cartilage change that accompanied osteochondral fragmentation could also be associated with concurrent traumatic damage to the attachment of the joint capsule and ligaments (Raker et al., 1966). However, there was little association made between primary disease in the synovial membrane and fibrous joint capsule and the development of osteoarthritic change in the articular cartilage until an experimental study demonstrated that cartilage degradation could occur in the horse in the absence of instability or traumatic disruption of tissue and that loss of glycosaminoglycan (GAG) staining was associated with early morphologic breakdown at the surface of the cartilage (McIlwraith and Van Sickle, 1984). Surveys have confirmed that approximately 60% of lameness problems are related to osteoarthritis (National Animal Health Monitoring Systems, 2000; Caron and Genovese, 2003). Rapid resolution of synovitis and capsulitis is a critical part of the medical treatment of joint disease because of the principal role of synovitis in causing cartilage matrix breakdown. The goal of treatment of traumatic entities of the joint is twofold: (1) returning the joint to normal as quickly as possible, and (2) preventing the occurrence or reduction of the severity of osteoarthritis. In other words, treatment is intended to (1) reduce pain (lameness), and (2) minimize progression of joint deterioration. Minimizing progression is mainly addressed by medical treatment, but it is also important to remember that timely removal of osteochondral chip fragments, timely and appropriate reduction or fixation of large intra-articular fractures, accurate diagnosis of ligamentous and meniscal injuries with arthroscopy, and the appropriate treatment of osteochondritis dissecans (OCD) entities are also critical treatments to prevent osteoarthritis. This paper will address both medical and surgical treatments.

### **Principles of Therapy**

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 typical work, suppression of synovitis and capsulitis is important to prevent the products of inflammation from compromising the articular cartilage and leading to osteoarthritis. Pain relief, as well as minimizing the potential microinstability associated with excessive synovial effusion, is critical. As information increases regarding targets for therapeutic intervention, the range of treatment options has increased. The term chondroprotection has since been replaced by disease-modifying osteoarthritic drugs (DMOADs). Medications providing pain relief but undefined therapeutic action at the level of cartilage matrix are termed symptom-modifying osteoarthritic drugs (SMOADs).

### Newer Definition of Best Treatments for Osteoarthritis

Various targets have been identified in recent years and confirmed to be of importance in the horse. Mediators of relevance are illustrated in Figure 1.

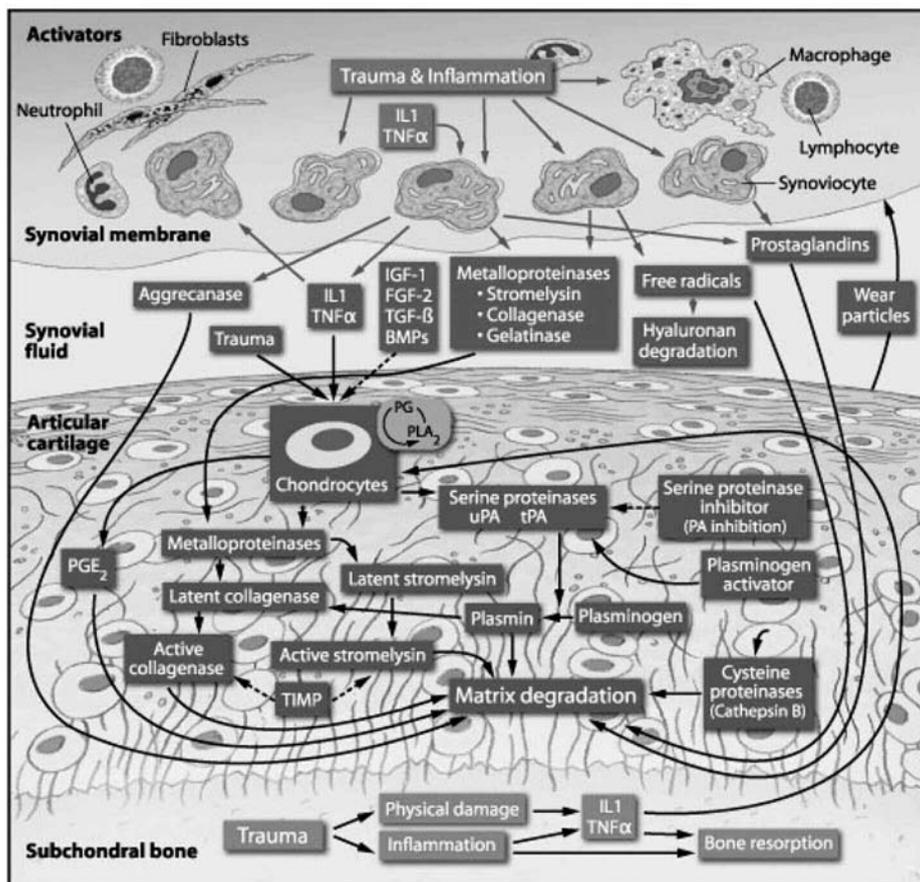


Figure 1. Mediators of relevance.

The principal factor of significance at the top of the inflammatory cascade in equine osteoarthritis is interleukin-1 (IL-1). When considering therapies, attention to inhibition of this molecule is critical.

## Advances in Therapies

This is an update of knowledge on some conventional therapies as well as an introduction to newer biological therapies.

### *Physical therapy and rehabilitation*

The advantages of rehabilitation protocols other than stall confinement or turnout have become recognized in recent years. Experimental data are lacking but currently a study is ongoing at the Colorado State University (CSU) Orthopaedic Research Center examining the value of underwater treadmilling in an experimental model of osteoarthritis.

### *Shock wave therapy*

Shock wave therapy has been used as a treatment for a number of conditions. Most recently, the value of shock wave therapy has been demonstrated with experimental osteoarthritis in the horse. The major usefulness is in decreasing the inflammatory response from synovial membrane and joint capsule as well as symptomatic decrease in lameness.

### *Nonsteroidal anti-inflammatory drugs (NSAIDs)*

The term NSAIDs is used to describe anti-inflammatory agents that inhibit some components of the enzyme system that converts arachidonic acid into prostaglandins and thromboxane. Their use in the horse was well-reviewed in 1996. PGE<sub>2</sub> is the product associated with synovial inflammation and cartilage matrix depletion and has been demonstrated in the synovial fluid of horses with osteoarthritis (Vane, 1971; May and Lees, 1996). Phenylbutazone has been the most commonly used NSAID in the horse at a dose of 2.2 mg/kg once or twice a day, but variable results have been seen in clinical cases (Keegan et al., 2008) as well as a recent study with an equine osteoarthritis model (Frisbie et al., 2009e). Alleviation of clinical lameness was greater after administration of the combination of phenylbutazone with flunixin meglumine, but concerns with secondary side effects (including acute necrotizing colitis) were raised (Frisbie et al., 2009e).

All NSAIDs inhibit cyclooxygenase activity to some degree (Vane, 1971; May and Lees, 1996). 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 (Frisbie, 2004). It has constitutively produced and has been shown to be important in the balance of normal physiologic function of the gastrointestinal and renal systems, 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 the 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 (Kunkel and Chensue, 1985; Dingle, 1993; Frisbie, 2004). 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 view still feels that the beneficial effects of selective COX-2 inhibition in joint disease are ideal. Anecdotally, we have used carprofen (Rimadyl<sup>®</sup>) at the CSU Orthopaedic Research Center 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. Firocoxib, a member of the class of drugs that selectively inhibits the COX-2 isoenzyme, has become approved for use in horses to control pain and inflammation associated with osteoarthritis in general, and its pharmacokinetics during prolonged use have been determined (Legendre et al., 2008).

There is evidence that while prostaglandin inhibition is effective in the production of symptomatic release it may have deleterious effects on the cartilage metabolism in the long term (Dingle, 1993). In vitro work in the horse had initially shown no evidence of deleterious effects on cartilage metabolism (Jolly et al., 1995), 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-1 $\beta$  (Beluche et al., 2001). Until 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 NSAIDs is justified.

A relatively new development has been the licensing of a topical NSAID preparation (1% diclofenac sodium cream, Surpass<sup>®</sup>). Research in humans had previously indicated the topical application of the NSAID could be clinically beneficial while reducing systemic side effects. Anti-inflammatory effects have been shown in experimentally-induced subcutaneous inflammation (Caldwell et al., 2004). A clinical field trial of the topically applied diclofenac liposomal cream for the relief of joint inflammation showed promising results (Bertone et al., 2002). More recently its value has been demonstrated in the experimental chip fragment-exercise model of osteoarthritis developed at CSU and used to assess a number of commonly used medications (Frisbie et al., 2009e). The product is now licensed.

### **Intra-articular corticosteroids**

The use of intra-articular corticosteroids for equine joint disease was extensively reviewed in 1996 (Trotter, 1996a), and the benefits and deleterious side effects of intra-articular corticosteroids in the horse have been more recently clarified. Based on the author's observation of an apparent lack of correlation between the prior use of betamethasone esters (Betavet Soluspan<sup>®</sup>) and articular cartilage degradation during arthroscopic surgery for osteochondral chip removal, experimental studies were initiated of the three most commonly used intra-articular corticosteroids, namely methylprednisolone acetate (Depo-Medrol<sup>®</sup>), triamcinolone acetonide (Vetalog<sup>®</sup>), and betamethasone esters (Betavet Soluspan<sup>®</sup>). These were evaluated using the osteochondral fragment model (Foland et al., 1994; Frisbie et al., 1997; Frisbie et al., 1998). The first product studied was Betavet Soluspan<sup>®</sup> (now available as Celestone Soluspan<sup>®</sup>). Osteochondral fragments were created arthroscopically on the distal aspect of both middle carpal joints in 12 horses. One joint was treated with 2.5 ml of Betavet Soluspan<sup>®</sup> at 14 days after surgery and repeated in 35 days (Foland et al., 1994). The opposite joint was injected with saline as

a control. No deleterious side effects to the articular cartilage were demonstrated, and exercise also did not show any harmful effects in the presence of corticosteroid administration. In subsequent studies with intra-articular corticosteroids (as well as other treatments), the model was modified so that the opposite joint was not used as a control (to evaluate systemic effects from intra-articular treatment). Also, the chip fragment model was modified to more effectively produce early osteoarthritic change. Depo-Medrol® and Vetalog® were tested using three groups (Frisbie et al., 1997; Frisbie et al., 1998).

In joints containing an osteochondral fragment and treated with MPA, there was a reduction, although not a significant one, in the degree of lameness; however, there were significantly lower PGE2 concentrations in the synovial fluid and lower scores for intimal hyperplasia and vascularity (no effect on cellular infiltration in the synovial membrane compared to placebo-treated joints). Of more importance, modified Mankin scores (a score of histopathological change in the articular cartilage) were significantly increased in association with MPA, suggesting deleterious effects of intra-articular administration of MPA (Frisbie et al., 1998). This is in contrast to the results with triamcinolone acetonide (TA or Vetalog®). Horses that were treated intra-articularly with 12 mg TA in a joint containing a fragment (TA-TX) were less lame than horses in the CNT and TA-CNT groups. Horses treated with TA in either joint had lower protein and higher HA and GAG concentrations in synovial fluid. Synovial membrane from CNT and TA-CNT had less inflammatory cell infiltration, intimal hyperplasia, and subintimal fibrosis. Analysis of articular cartilage morphologic parameters evaluated using a standardized scoring system were significantly better from TA-CNT and TA-TX groups irrespective of which joint received TA. The results overall supported favorable effects of TA on degree of clinically detectable lameness and on synovial fluid, synovial membrane, and articular cartilage morphological parameters, both with direct intra-articular administration and remote-site administration as compared to placebo injections (Frisbie et al., 1997).

These studies, coupled with some recent *in vitro* work demonstrating protective effects of TA (Bolt et al., 2008), have fueled the recommendation that the use of triamcinolone acetonide especially in high-motion joints is ideal. There have been some opinions on "low-dose" 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 (Dechant et al., 2003). On the other hand, clinical improvement is more important to the clinician than data.

Fear of laminitis has also reduced the use of triamcinolone acetonide by some equine practitioners, despite scientific studies demonstrating its effectiveness as well as its chondroprotective properties. There have been anecdotal associations made and maximum doses established based on a report of no cases of laminitis in 1,200 horses treated when a dose did not exceed 18 mg (Genovese, 1983). A more recent publication provides the first follow-up study with data on the potential for triamcinolone acetonide to produce laminitis, and the conclusion was that there was no association between the occurrence of laminitis and the intra-articular use of triamcinolone acetonide (McCluskey and Kavenagh, 2004). A relatively recent legal case in the United Kingdom in which a horse developed laminitis after receiving 8 mg of TA in each tarsus and 20 mg of dexamethasone in its back (Dutton, 2007) led to the development of a review of the literature and a retrospective study of one clinician's cases (Bailey and

Elliot, 2007; Bathe, 2007). The review of the literature revealed that good evidence linking laminitis to corticosteroid injection was lacking and that a large-scale multicenter trial was needed (Bailey and Elliot, 2007). In a third publication, the clinician reported that laminitis associated with intra-articular injection of corticosteroids had occurred in 3 of 2,000 cases (0.15%). The majority of the time TA was used and the upper total dose ranged from 20–45 mg (Bathe, 2007).

Another traditional cliché has been that while it is better not to use Depo-Medrol® in high-motion joints, its use in low-motion joints (such as the distal tarsal joints) is appropriate. The implication has been made that we do not care about the state of the articular cartilage in these joints and may be able to promote ankylosis. There is no evidence yet that we can promote ankylosis in this fashion. The flip side of this argument is that we should preserve articular cartilage whenever we can. A recent retrospective study of the effect of intra-articular treatment on osteoarthritis of the distal tarsal joints with MPA or TA (with or without hyaluronan) led to a positive outcome in only 38% of horses (suggesting to the authors that surgical treatment may be more appropriate for long-term prognosis) and also revealed that there was no significant difference between MPA and TA, thereby questioning any clinical advantages to the use of MPA (Labens et al., 2007).

Intra-articular corticosteroids have commonly been combined with hyaluronan (HA), and there has been a perception that the HA might be protective against any deleterious effects of corticosteroids (MPA). This perception has been based on tradition rather than scientific proof but has become common thinking among equine practitioners (Caron and Genovese, 2003). On the other hand, the use of a combination of HA and corticosteroids is still logical based on recent evidence of long-term DMOAD activity with HA after intra-articular use (Frisbie et al., 2009a)

### **Hyaluronan (sodium hyaluronate)**

Hyaluronan is a nonsulfated glycosaminoglycan (GAG). The biological characteristics and therapeutic use of hyaluronan in equine osteoarthritis have been reviewed previously (McIlwraith et al., 2001). Hyaluronan has modest analgesic effects (Gotoh et al., 1993), 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) (Howard and McIlwraith, 1996). Various 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 (Clegg et al., 1998; Lynch et al., 1998). 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 (Howard and McIlwraith, 1996). 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 (Frisbie et al., 1997; Frisbie et al., 1998).

It has been the author's 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. However, it needs to be recognized that based on clinical evidence in humans, the immediate clinical effects may be less dramatic but the evidence for long-term disease-modifying activity is accumulating (Goldberg and Buckwalter, 2005). It has also been claimed that HA preparations of molecular weight exceeding  $1 \times 10^6$

daltons may provide superior clinical and chondroprotective events, but this is a controversial claim (Smith and Ghosh, 1987; Aviad and Houpt, 1994; Howard and Mellwraith, 1996).

A randomized, double-blind, and placebo-controlled clinical study in 77 Standardbred trotters with moderate to severe lameness treated horses with HA, polysulfated glycosaminoglycan (PSGAG), or placebo for 3 weeks. The mean initial lameness score was significantly reduced during treatment and at the last examination in all three groups ( $p < 0.01$ ) (Gaustad and Larsen, 1995). Additionally, the prevalence of sound horses increased significantly from 1 to 3 weeks of treatment and at the last examination in all three groups. The study detected a superior effect on the two drugs (250 mg of PSGAG intra-articularly 4 times or 20 mg of HA intra-articularly twice) compared to placebo for reduction of lameness score during the treatment period and the total study period, time until soundness, and the prevalence of sound horses at the last examination. It was concluded that all three treatments were effective in the treatment of clinical traumatic arthritis in horses, but HA and PSGAG gave better results than placebo. In a second paper, the same researchers compared intra-articular saline with rest alone in 38 Standardbreds with traumatic arthritis. The mean lameness was significantly lower when 2.0 ml of 0.9% NaCl solution was injected. This raises the question: Is this effect due to withdrawing fluid and/or placing a needle in the joint (Gaustad et al., 1999)?

Most recently, intra-articular HA has been tested in the CSU equine osteoarthritis model (Frisbie et al., 2009c). Osteoarthritis was induced arthroscopically in one middle carpal joint of 24 horses. Eight horses received HA (20 mg) (Hyvisc<sup>®</sup>) and amikacin (125 mg) intra-articularly on study days 14, 21, and 28. A second group of 8 horses received PSGAG (250 mg) and amikacin (125 mg) intra-articularly on study days 14, 21, and 28. No adverse treatment-related events were detected. Induced osteoarthritis caused a substantial change in lameness, response to flexion, joint effusion, and radiographic findings. Of those findings, synovial effusion was reduced with PSGAG compared with controls and no changes in other clinical signs were seen with PSGAG or HA compared with controls. Histologically, however, there were significantly less articular cartilage fibrillation seen with HA treatment compared with controls (despite no significant reduction in synovial membrane vascularity and subintimal fibrosis). The conclusion was that HA had beneficial disease-modifying effects and was a viable therapeutic option in equine osteoarthritis (Frisbie et al., 2009c).

### **Polysulfated glycosaminoglycan**

Polysulfated glycosaminoglycan (PSGAG) belongs to a group of polysulfated polysaccharides and includes, in addition to PSGAG (Adequan<sup>®</sup>), pentosan polysulfate. These drugs have been referred to as chondroprotective, or a more recent definition, disease-modifying osteoarthritic drugs (DMOADs). Because of this, PSGAG has been traditionally used where cartilage damage is considered to be present rather than in the treatment of acute synovitis (Trotter, 1996b), but recent work questions that mode of action (see below). 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® was reviewed extensively in 1996 (Trotter, 1996b). At that time there had been a number of *in vitro* studies, including one demonstrating that PSGAG was the only drug tested (others included phenylbutazone, flunixin, betamethasone, and hyaluronan) that inhibited stromelysin (May et al., 1988). There have 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 (Glade, 1990). However, other work found a dose-dependent inhibition of proteoglycan synthesis, little effect on proteoglycan degradation, and no effect on proteoglycan monomer size (Caron et al., 1991). Various studies have supported the value of intra-articular (250 mg) PSGAG in equine joint disease, including a clinical study (Tew, 1982), a study using a Freund's adjuvant-induced model (White et al., 1994), and another equine carpal model using sodium monoiodoacetate (Trotter et al., 1989). In the latter study, there was significant reduction of articular cartilage fibrillation erosion, less chondrocyte death, and markedly improved GAG staining. At the same time, PSGAG had no benefit in healing articular cartilage lesions that were already present, and this was also demonstrated in a study in ponies (Todhunter et al., 1993).

The author has traditionally recommended the use of intra-articular PSGAG following arthroscopic surgery when there is significant loss of articular cartilage (most commonly in the carpus). This was based on clinical observation of rapid resolution of synovitis and hemarthrosis that tends to be persistent following arthroscopic surgery when there is secondary loss of articular cartilage. However, a recent study using the CSU equine osteochondral fragment model in which intra-articular PSGAG was compared to intra-articular HA and intra-articular saline revealed that synovial fluid effusion was significantly reduced with intra-articular PSGAG compared to both controls and the intra-articular HA group. In addition, the degree of synovial membrane vascularity and subintimal fibrosis was significantly reduced with PSGAG treatment as compared with controls (Frisbie et al., 2009c). The conclusion would be perhaps that the main value of intra-articular PSGAG is with severe (and acute) synovitis (and this is most commonly seen following arthroscopic surgery when there is considerable debridement of bone).

Intramuscular PSGAG has become a popular treatment, but a study using intramuscular PSGAG (500 mg every 4 days for 7 treatments) showed relatively insignificant effects with treatment (limited to slightly improved GAG staining in sodium monoiodoacetate joints when PSGAG was used) (Yovich et al., 1987). In a more recent experimental study in which intramuscular PSGAG was used as a positive control (administered every fourth day for 28 days starting 14 days post osteoarthritis induction), decreased GAG levels in the serum 14 days post-treatment (a marker of disease in this osteoarthritis model) was the only significant beneficial effect (Frisbie et al., 2009b). However, there was more impressive improvement in the third test group (shock wave therapy group).

Although a survey to assess the perceived efficacy of PSGAG in 1996 reported that PSGAG was considered more effective than HA for the treatment of subacute degenerative joint disease and less effective for idiopathic joint effusion and acute synovitis (Caron et al., 1996), it now appears that there is very weak evidence for clear-cut efficacy with intramuscular administration. It has been reported that articular cartilage concentrations of PSGAG after intramuscular administration are capable of inhibiting some cartilage-degrading enzymes (Burba et al., 1993), but the duration of effective concentration is unclear. Several degradative enzymes known to be present in articular tissue have been shown to be reduced in

vitro and in studies in other animal models (Howell et al., 1986; Burba et al., 1993), but direct evidence of effectiveness in the horse is lacking. In a study of veterinarians cited previously (Caron and Genovese, 2003), indications for intramuscular use varied widely, including acute or chronic osteoarthritis or both types. PSGAG was also used as a preventative measure and information from the manufacturing company reports that 90% of sales are for such "prophylactic" use. There have been no scientific studies on this application, and it is difficult to prove or disprove efficacy for this usage.

### **Pentosan polysulfate**

The use of pentosan polysulfate (PPS) in the treatment of joint disease was reviewed in 1996 (Little and Ghosh, 1996). PPS is also considered a disease-modifying osteoarthritic drug (DMOAD), and it was pointed out in the review article that PPS, unlike NSAIDs, does not possess analgesic activity (Little and Ghosh, 1996). 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 osteoarthritis joint, and the authors at that time felt that PPS fulfilled these requirements. However, at that 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<sup>®</sup> (licensed in small animals in Australasia, but not in horses) and more recently Pentosan Equine Injection<sup>®h</sup> (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 (Ghosh et al., 1993).

Recent work from our laboratory has demonstrated favorable results. Using the carpal osteochondral fragment-exercise model of equine osteoarthritis, there was significant decrease in articular cartilage fibrillation ( $p < 0.5$ ) and a strong trend ( $p = 0.6$ ) for improvement in 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 was not obtained. In this study, PPS was given at a dose of 3 mg/kg body weight once weekly for 4 weeks.<sup>h</sup>

### **Oral joint supplements**

Oral joint supplements are loosely classified as nutraceuticals. The term "nutraceutical" combines the word "nutrient" (nourishing food or food component) with "pharmaceutical" (a medical drug) (Duren, 2005) and describes a broad list of products sold under the premise of being a dietary supplement (i.e., a food) but for the expressed intent of treatment or prevention of disease. The claims, usually made by manufacturers of these supplements, to aid in equine joint health are often very weak. The potential difference between a feed and a nutraceutical is that a nutraceutical is unlikely to have an established nutritive value. Feeds are required to have nutritive value and are accountable, by labeling, for these values. Joint supplements fall in between food and drug and have advantages over either because they are not required to list ingredients or nutrient profiles as required by feeds, and in many cases are intended

to treat or prevent disease without first undergoing proper drug approval (Duren, 2005). Joint supplements are fed to horses for one of two purposes: (1) to heal the lame or make chronically unsound horses sound, or (2) to prevent joint problems from ever occurring. The first instance is flawed because often the source of lameness is never diagnosed when the owner or trainer elects to use these oral supplements. The second premise is hard to disprove but is the basis for high usage of some licensed drugs as well as nutraceuticals. In 2005 nutraceutical sales reached more than \$1 billion for companion animals, and that figure was expected to double in the next 3 years. To equine practitioners this is a disturbing trend for an industry that for the most part is unregulated by the FDA and has weak in vivo scientific basis (Oke and McIlwraith, 2008).

None of the oral supplements or oral nutraceuticals are 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<sup>®</sup>) and a complex of glycosaminoglycans and other nutrients from the sea mussel *Perna canaliculus* (Syno-Flex<sup>®</sup>). More recently a combination of glucosamine hydrochloride, chondroitin sulfate, manganese, and vitamin C has been marketed as a nutraceutical (Cosequin<sup>®</sup>), and a number of other products have simulated Cosequin<sup>®</sup>. 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 common practice of using combinations of 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 (Setnikar et al., 1993), 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 (Adebowale et al., 2003). A second study in dogs concluded that glucosamine is absorbed orally, albeit low (12%), and it is most likely due to extensive first-pass metabolism in the gastrointestinal tract and/or liver prior to systemic availability (Adebowale et al., 2002).

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 of glucosamine hydrochloride in the horse (Lavery et al., 2005). Eight adult female horses with no evidence of joint disease were studied and were randomly assigned to two different groups for a crossover study. Glucosamine hydrochloride (20 mg/kg) was administered by nasogastric intubation or intravenous injection, and blood samples were collected. Glucosamine was assayed by fluorescence-assisted carbohydrate electrophoresis (FACE) with glucosamine achieving a maximum concentration of 288  $\pm$  53  $\mu$ M following intravenous dose and 5.8  $\pm$  1.7  $\mu$ M following nasogastric dose. Synovial fluid reached a peak concentration at 250  $\mu$ M post-intravenous dosing and 0.3–0.7  $\mu$ M post-nasogastric dosing. It was concluded that the levels of glucosamine obtained in synovial fluid following nasogastric administration with clinically recommended doses are lower than those that have been studied in vitro to elucidate glucosamine action on joint cells.

Chondroitin sulfate (CS) consists of alternating disaccharide subunits of glucuronic acid and sulfated N-acetylgalactosamine 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 (Du et al., 2004). 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 (Du et al., 2004).

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 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 PGE2 production, whereas CS did not affect PGE2. The combination decreased MMP-9 activity, but has no effect on MMP-2, and there was a trend for decreasing MMP-13 protein concentrations (Fenton et al., 2000).

In vitro dose titration studies of glucosamine hydrochloride (GU) and chondroitin sulfate (CS) alone and in combination have been done 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, whereas intermediate doses of GU, CS, and GU plus CS enhanced GAG synthesis and total cartilage content (Dechant et al., 2005). The same dosages tested on IL-1-conditioned articular cartilage explants revealed no treatment effects for GU or CS alone, but did show a protective effect of high dosages of GU plus CS for total GAG release into the media. This 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 (Laverty et al., 2005), and clear demonstration of reduction of degradation would be ideal information.

Other oral joint supplements used include Platinum Performance<sup>®</sup>, which is a combination of rare earth minerals and omega-3 fatty acids (making it somewhat unique). This has been used postoperatively, but all information is anecdotal. Similarly, oral HA products are new to the market and a controlled study in our laboratory did not demonstrate effectiveness in our equine osteoarthritis model.<sup>1</sup> However, a clinical study evaluating the use of an oral HA product after arthroscopic surgery for osteochondritis dissecans (OCD) in the tarsocrural joint revealed that there was significant reduction in postoperative synovial fluid effusion in the horses on oral HA (Bergin et al., 2005).

Recently, another experimental study using the CSU equine osteoarthritis model has demonstrated value for an oral supplement containing soy and avocado (ASU). This is the first well-controlled scientific study demonstrating a positive effect with an oral nutraceutical (Kawcak et al., 2007). The study was a blinded, experimentally controlled, randomized block design that used 16 horses in an established model of osteoarthritis. On day 0 of the study, arthroscopic surgery was performed and osteoarthritis was induced unilaterally in the middle carpal joint of all horses. Also on day 0, horses were divided into two treatment groups: placebo-control group and ASU-treatment group. The placebo-control group received molasses orally one time daily, whereas the ASU-treated group received 6 grams of ASU plus a similar volume of molasses orally; both treatments were continued throughout the study period. On day 14, horses began and continued treadmill exercise for the remaining 8 weeks of the study. All horses com-

pleted the study, and no adverse events were recorded. At the termination of the study, horses treated with ASU were observed to have significantly improved total gross examination score (articular cartilage erosion plus synovial membrane hemorrhage score in their osteoarthritis joint compared to placebo-control horses). There was also significant decrease in intimal hyperplasia in the synovial membrane as well as decrease in the cartilage disease score. There was a trend for decreased lameness. However, significant decrease in the cartilage disease points this product towards that of being a disease-modifying osteoarthritic drug (DMOAD), which is good. Although the improvements were modest, they were more significant than those seen with other parenteral (polysulfated glycosaminoglycan and intravenous HA) and oral (HA) products tested using the same model of equine osteoarthritis.

There has been recent discussion on the potential value of omega-3 polyunsaturated fatty acids (PUFAs) and their value in joint disease. It has previously been shown that the administration of PUFAs reduces inflammatory mediators in equine monocytes, corresponding to an increase in the ratio of omega-3 to omega-6 fatty acids in cell membrane phospholipids (Fenton et al., 2000; Du et al., 2004). An *in vitro* study reported on the role of alpha-linolenic acid, an omega-3 polyunsaturated fatty acid, and its anti-inflammatory potential for the reduction of equine synovial inflammation in an established lipopolysaccharide (LPS) model (Dechant et al., 2005). Challenge with LPS significantly increased production of PGE<sub>2</sub> and decreased production of hyaluronic acid. Treatment with alpha-linolenic acid at the highest dose inhibited prostaglandin production.

### **Combination intra-articular therapy**

HA and corticosteroid have been commonly combined. Without corticosteroids, the efficacy of intra-articular HA (based on short-term clinical response) is limited to mild to moderate synovitis but is markedly enhanced with corticosteroids. Some veterinarians have used HA in conjunction with Depo-Medrol<sup>®</sup>, believing that the former would mitigate the effects of the latter, but this is questionable and has been previously discussed. There is an interesting contrast with the human orthopedic surgeon's rationale for combined therapy. Localized, severe, acute inflammatory reactions (SAIRs) have been associated with the use of highly crosslinked hyaluronan products (Pagnano and Westrich, 2005). Although other people have reported no adverse reactions with the use of hylan G-F 20 in human patients with knee osteoarthritis (Raynauld et al., 2005), this has been challenged by other authors who have reported SAIRs and reduction of the risk with concurrent intra-articular corticosteroids (Hamburger, 2005). Other authors have confirmed SAIRs or pseudoseptic reactions with hylan G-F 20 (Synvic<sup>®</sup>) as occurring usually after the second or third injection (Hamburger et al., 2005). These authors suggested an immunogenic mechanism associated with fermented HA product, as they had not seen problems with naturally extracted HA (Hamburger et al., 2005). The combination of triamcinolone acetonide and IGF-I has been evaluated *in vitro* with positive results (Frisbie et al., 2000b).

### **Newer biologically-based therapies**

The knowledge gained from improved understanding of critical mediators in equine traumatic arthritis and osteoarthritis has led to the identification of new targets for therapy. Biological therapy

specifically modulates key mediators. 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), nonpeptidal inhibitors (this includes chemically modified tetracyclines such as doxycycline), and naturally occurring inhibitors (such as omega-3 fatty acids; e.g., fish oils). Recent work with omega-3 fatty acids has been discussed previously.

In vitro studies in our laboratory with the MMP inhibitor Bay-12-9566 using an IL-1 degradation model and equine and canine articular cartilage explants showed that there were significant dose-dependent reductions in the catabolic effect of IL-1 $\alpha$  on the release of proteoglycans and type II collagen from articular cartilage explants exposed to tenfold increases in concentrations (1nM:10 $\mu$ M) (Billinghurst et al., 1999). No in vivo assessment of MMP inhibitors has been done in the horse; however, a study in experimental osteoarthritis in the dog (ACL transection) failed to demonstrate efficacy with an MMP inhibitor<sup>6</sup>, and the prospect for these being a valuable biological therapy for horses seems low.

*Inhibiting interleukin-1 (IL-1).* As mentioned above, the identification of major mediators driving the joint disease process has fueled considerable advances in clinical treatments. The commonly accepted mediator at the top of the cascade for cartilage degradation in osteoarthritis is interleukin-1 (IL-1), whereas tumor necrosis factor (TNF) has achieved considerable attention in rheumatoid arthritis in humans, leading to novel therapies including the use of an anti-TNF monoclonal antibodies adalimumab (Humira<sup>®</sup>) as well as recombinant human TNF receptor etanercept (Enbrel<sup>®</sup>) (Frisbie, 2005). IL-1 activates MMP, aggrecanase, and PGE2 release by acting through IL-1 receptors on the cell membrane.

There are two potential methods of inhibiting IL-1. The first is through the natural antagonist, interleukin-1 receptor antagonist (IL-1ra), which binds to the cell membrane IL-1 receptor to block IL-1 (but does not elicit any biological response itself). The second method of blocking IL-1 is through the use of soluble receptors, in which IL-1 receptors are released from the cell membrane and bind IL-1 (Frisbie, 2005). While this latter method has been used to inhibit TNF, there are no current techniques of using IL-1 receptors therapeutically. In turn, there are two mechanisms for the therapeutic application of IL-1ra or IRAP<sup>®</sup>: (1) recombinant proteins and (2) gene therapy. This was the first therapeutic application of IL-1ra for equine joint disease in which the equine IL-1ra gene sequence was directly transferred to the synoviocytes of the affected joint using an adenoviral vector (Frisbie and McIlwraith, 2000; Frisbie et al., 2002). Complete inhibition of experimental osteoarthritis in the equine carpus confirmed the critical role of IL-1 in the osteoarthritic process. While both symptom- and disease-modifying effects were demonstrated and the magnitude of therapeutic value was greater than any other medication tested, repeated delivery of IL-1ra using gene transfer necessitates a better vector, which is currently being researched. Because of these limitations with gene transfer therapy, attention has been paid to alternative methods of delivering IL-1Ra to the joint typified by IRAP<sup>®</sup>.

The concept of IRAP<sup>®</sup> was developed in Europe with the advent of a product named Orthokine<sup>®</sup> by a German company Orthogen AG. The principal behind the product is that peripheral blood is collected into a syringe containing glass beads soaked in chromium sulfate and undergoes incubation for 24 hours followed by centrifugation. The autologous-modified serum is then used in a series of intra-articular injections. A publication with Orthokine<sup>®</sup> documented marked elevation of IL-1ra without elevated expression of IL-1 or TNF (i.e., preferential upregulation of "good" cytokines without concurrent upregu-

lation of “bad” cytokines) (Meijer et al., 2002). The product was introduced into the United States in late 2004 by Arthrex Biosystems, and unpublished work in its laboratory confirmed upregulation of IL-1ra but not to the same extent as in the Meijer et al. (2002) study. Because it is believed that upregulation of proteins is through stimulation of monocytes by the beads and syringe, it is expected that more than one molecule is upregulated during the cultures period and this is being examined using mass spectroscopy at the CSU Orthopaedic Research Center.

The IRAP® product has been tested in a double-blind, placebo-controlled, experimental study using the equine osteochondral fragment-exercise model, and IRAP® was shown to have both symptom-modifying effects and disease-modifying effects in horses (Frisbie et al., 2007). There were 8 placebo and 8 ACS-treated horses. Either 6 ml of ACS or 6 ml of PBS solution were injected into the osteoarthritis-affected joint on days 14, 21, 28, and 35, respectively. No adverse treatment-related events were detected. Horses that were treated with ACS had significant clinical improvement in lameness, unlike the placebo-treated horses. ACS treatment significantly decreased synovial membrane hyperplasia compared to placebo, and the ACS-treated joints also appeared to have less gross cartilage fibrillation and synovial membrane hemorrhage. The synovial fluid concentration of IL-1ra (assessed using a mouse anti-IL1ra antibody) was increased following treatment with ACS (Frisbie et al., 2007).

*Mesenchymal stem cells.* Mesenchymal stem cells (MSCs) have received considerable attention and have been used intra-articularly in the horse for a number of “indications.” Both clinical experience and research have been limited to adult-derived MSCs, and because of the early reports using bone marrow as the source of MSCs, this source remains the gold standard. (However, other sources of MSCs such as muscle, cartilage, and adipose tissue have been demonstrated to contain multipotent MSCs.) Isolation of MSCs from marrow or digestive tissue extracts is usually achieved by simple adhesion and proliferation of MSCs to tissue culture surfaces. This technique does not provide a homogenous population of MSCs but rather near-homogenous MSCs populations (Pittenger et al., 1999). Work is proceeding on establishing cell-surface antigens that characterize MSCs. Another important distinction is recognizing MSCs that are isolated and culture expanded from nonculture-expanded sources; an example is the technique utilized by Vet Stem®, in which fat is harvested and the tissue digested, producing a nucleated cell population called stromal vascular fraction, which is injected as the treatment and is believed to contain approximately 2-4% MSCs. Much of the other literature regarding adipose-derived stem cells involved culture-expanded cells and cannot necessarily be extrapolated to the Vet-Stem® technique. With regard to intra-articular use of MSCs, a study comparing adipose-derived stromal cells, bone marrow-derived cultured MSCs, and placebo in the equine osteochondral fragment model revealed no significant treatment effects in any group, with the exception of improvement in synovial fluid PGE2 levels with bone marrow-derived MSCs when compared to the placebo group (Frisbie et al, 2009d). The findings of this study were not significant enough to recommend the use of stem cells for the treatment of osteoarthritis represented in this equine model.

However, a study in goats using a medial meniscectomy and cranial cruciate transection model to induce osteoarthritis using intra-articular of autogenous bone marrow-derived MSCs was encouraging (Murphy et al., 2003). There was regeneration of the medial meniscus and decreased progression of osteoarthritis. It is felt that regeneration of the meniscus could be a critical part in the therapeutic value of decreasing osteoarthritis in this model, whereas osteoarthritis development in the equine osteochon-

dral fragment model has a different pathogenic pathway. Based on these findings from the experimental study in the goat, there has been considerable use of intra-articular MSCs to treat soft tissue injury in the femorotibial joints following arthroscopy with good clinical results.

Much of the material in this paper has been reproduced from a chapter titled "Principles and Practices of Joint Disease Treatment," published in a textbook edited by Ross and Dyson called *Lameness in Horses* (in press).

#### FOOTNOTES

- a) Rimadyl®, Pfizer Laboratories.
- b) Surpass®, IDEXX Laboratories, Greensboro, NC 27410.
- c) Betavet Soluspan®, Schering-Plough Animal Health Corp., Union, NJ 07083.
- d) Depo-Medrol®, Pharmacia and Upjohn Co., Kalamazoo, MI 49001.
- e) Vetalog®, Bristol Myers Squibb for Fort Dodge, Fort Dodge, IA 50501.
- f) Hyvisc®, Boeringer.
- g) Adequan®, Luitpold Pharmaceuticals Inc, Animal Health Division, Shirley, NY 11967.
- h) Pentosan Equine® Injection, Nature Vet Pty Ltd., 11 Moores Road, Glenorie, New South Wales, 2157, Australia.
- i) Platinum Performance®, Platinum Performance Inc., PO Box 990, Buellton, CA 93427.
- j) Frisbie, D.D., C.W. McIlwraith, and C.E. Kawcak. 2005. Unpublished data.
- k) Trumble, T.N. 2003. Ph.D. dissertation. Colorado State University, Fort Collins, CO.
- l) Humara®, Abbott Laboratories, Abbott Park, IL
- m) Enbrel® Wyeth Pharmaceuticals, Giralda Farms, Madison, NJ 07940.

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