Hyaluronic Acid

Hyaluronic acid (HA), or hyaluronan, is a large unbranched nonsulphated glycosaminoglycan composed of repeating units of D-glucuronic acid and N-acetyl glucosamine. It is synthesized by the Type B synoviocytes of the synovial membrane. In the joint, HA serves various important functions, such as viscoelasticity to the joint fluid and boundary lubrication of the intra-articular soft tissues. HA may also influence the composition of synovial fluid through steric hindrance of active plasma components and leukocytes from the joint cavity. Furthermore, hyaluronan appears to modulate the chemotactic response within the synovial membrane by reducing cell migration and decreasing rates of diffusion and flow of solutes. HA is also an important component in articular cartilage matrix and is locally synthesized by chondrocytes. It is the backbone of the proteoglycan aggregate in the extracellular matrix, and the compressive stiffness in articular cartilage depends on the integrity of the matrix proteoglycans.

It is theorized that exogenously administered sodium hyaluronan supplements, or even replaces, the actions of depleted or depolymerised endogenous hyaluronate in the synovial fluid, thereby restoring viscoelasticity, steric hindrance, and lubrication of the articular soft tissues. In vitro studies of the additional effects of exogenous hyaluronate have identified inhibition of macrophage chemotaxis and reduction in lymphocytic ability to proliferate and migrate. Furthermore, sodium hyaluronate has been shown to decrease the formation and release of prostaglandins from macrophages during phagocytosis. There is some support of the combination of corticosteroids and HA being beneficial in a study of 16 human patients with knee osteoarthritis. This was a one-year, single-blind, randomised study in which 24 patients were treated with intra-articular HA once weekly for 3 weeks and then again at 6 months (total of 6 injections) (Ozturk et al. 2006). Sixteen of these patients also had 1 ml of triamcinolone prior to the first and fourth HA injections and were evaluated using the WOMAC index (assess patients with osteoarthritis of the hip/knee using 24 parameters including 5 indices of pain, 2 of stiffness, 7 of social function and 10 of emotional function). This study found that the results were better where the corticosteroid was combined with HA compared to the corticosteroid alone. Furthermore, there is indirect evidence that the use of HA together with a corticosteroid like triamcinolone acetonide, could provide clinical benefit in the horse. In a recent study the use of intra-articular HA, as well as intra-articular Adequan was assessed in the CSU chip fragment model (Frisbie et al. 2009). Intra-articular injection of 20 mg of HA was done at 14, 21 and 28 days. There was significantly less cartilage fibrillation with HA at Day 70 compared to Adequan (Frisbie et al. 2009). The combination of a potent anti-inflammatory corticosteroid line like triamcinolone and the chondroprotective HA is therefore a good option.

There is still some controversy over the effectiveness of high versus low molecular weight HA. Proponents of the visco-supplementation theory believe higher molecular weight products are more effective. Conversely, others minimize the importance of size and suggest the activity of HA is mediated pharmacologically rather than physically. More controlled clinical research is necessary to define the relationship between molecular weight and efficacy.

Intravenous administration of HA is a non-invasive mode of administration and a route for treating multiple joints. Beneficial effects are most likely due to its localization in the synovial membrane since plasma half-life is no greater than 5 min. The synovial membrane is highly vascularised and intravenous administration may allow greater
exposure to synoviocytes than intra-articular administered HA. The only study to evaluate intravenous administered HA used an osteochondral chip model and exercised horses following induction of the osteochondral fragment. Horses treated with IV HA had reduced lameness, better synovial membrane scores, significantly lower total protein concentrations and, most importantly, a reduction in prostaglandins in joints compared to joints of horses receiving saline. The study extended up to 72 days following injection. Interestingly, HA did not have any effects on GAG content, synthetic rate, or morphologic scoring in articular cartilage. This gives credence to the theory that HA has mainly anti-inflammatory effects in the joint.

**Polysulphated Glycosaminoglycans (PSGAG’s)**

Adequan is produced from an animal source and is in a class of drugs that exhibits chondroprotective properties in cartilage. Pentosan polysulphate is produced from a plant source but is within the same class of drugs. These drugs alter OA progression by sustaining or promoting chondrocyte metabolic activity and inhibiting the detrimental effects of cytokines or prostaglandins on cartilage. Additionally, these drugs may have an inhibitory effect on inflammation in other tissues such as the synovium. The International League Against Rheumatism (ILAR) guidelines categorize these drugs as “disease modifying” OA drugs (DMOADs). Therapy with this class of drugs can prevent, retard or reverse morphologic cartilaginous lesions of OA.

PSGAGs are glycosaminoglycans (GAGs), primarily chondroitin sulphates, extracted from cartilage such as the bovine trachea and subjected to sulphate esterification. While there has been convincing evidence of the beneficial effect on cartilage, the exact mechanism of action remains unknown. It has been theorized that PSGAGs form stable complexes with fibronectin and collagen fibres and are deposited in cartilage. It has been shown that PSGAGs inhibit a plethora of degradative enzymes that contribute to the OA process.

Adequan is approved for intra-articular and intramuscular administration, but less evidence exists about the efficacy of intramuscular administration. Furthermore, intra-articular injection has occasionally caused severe joint infections that resulted in devastating complications and it seems to inhibit complement activity in the joint, thereby potentiating a subinfective dose of *Staphylococcus aureus*. This has resulted in wide intramuscular administration despite less clinical data available on this mode of administration. However, anecdotal reports of intramuscular efficacy abound, regardless of the lack of clinical data.

Intramuscular Adequan® is recommended at 500mg every 4 days for a total of 7 injections. Therapeutic levels are achieved and maintained in articular cartilage for up to 96 hours. Some authors suggest that a course of 4 injections should be initially given and if a good response is observed then a further 4 injections are administered. Evidence of the benefit of intra-articular PSGAG is usually seen at the time of the second injection. In cases of experimental carpitis, significant improvements were seen in joint mobility, circumference and synovial fluid protein at the $3^{rd}$ injection and even more so at the $4^{th}$ injection. Antibiotics should be combined with intra-articular injection due to the previously mentioned complement inhibition.

**Nutraceuticals**

There are also products on the market called nutraceuticals, which are oral supplements that claim to help regenerate and replace the damaged cartilage. Examples are Cosequin® and GCS-Max®. These products contain chondroitin sulphate
and/or glucosamine and are available as a dietary supplement for the treatment of osteoarthritis in horses. The practical and financial limitations of intra-articular and intramuscular PSGAG make the use of orally active products highly desirable. Due to limited scientific evidence of these products as anti-arthritis agents, unsubstantiated and incorrect claims appear in the marketing literature. However, anecdotal evidence gained from many thousands of human patients or animals that suffer with joint pain associated with degenerative joint disease, suggest that reduced symptoms of pain and improved joint function following supplementation with oral chondroprotective agents can be achieved. Chondroitin sulphate and glucosamine have both been suggested as being slow-acting, disease-modifying agents in osteoarthritis or as chondroprotective agents.

**Chondroitin sulphate**
Chondroitin sulphate (CSu) is the predominant glycosaminoglycan (GAG) in adult articular cartilage. In the ground substance of cartilage, CSu aggregates with hyaluronic acid, other GAG’s and proteins to form macromolecules. The CSu chains of these aggregates bind with collagen to form the characteristic resilient matrix of articular cartilage. The sequence of the GAG side chains in proteoglycans enables them to have specialised biological functions within tissues as diverse as articular cartilage, tendons, ligaments, skin and bone.

Oral administration of GAG’s has been controversial because large, highly charged molecules are less likely to cross the gastrointestinal mucosal barrier. The literature provides conflicting evidence on the bioavailability of orally administered CSu. Recent studies using radiolabeled, purified, low-molecular weight CSu have shown oral absorption in rats, dogs and humans. In contrast, absence of absorption has been reported in man and rabbits. It appears therefore, that CSu is not absorbed following oral administration but that low molecular weight desulphahted degradation products probably are. Positive clinical responses to oral supplementation with CSu thus may be explained either by biological activity of its low molecular weight degradation products, or from the activity of other substances such as glucosamine.

**Glucosamine sulphate**
Glucosamine sulphate is classified as a symptomatic slow-acting drug and is a precursor of articular cartilage GAG. Glucosamine is a fundamental metabolite required for biosynthetic production of glycolipids, glycoproteins, glycosaminoglycans, hyaluronan and proteoglycans. Chondrocytes normally manufacture glucosamine from glucose but when glucosamine is available it is preferentially taken up by cartilage where it is the preferred substrate for and stimulates the synthesis of GAG.

Glucosamine is a structural component of glycosaminoglycans (GAGs) and chondroitin sulphate being an important GAG constituent of proteoglycans, in particular aggrecan, the largest and most predominant proteoglycan in cartilage. In *vitro* studies have shown a number of effects on the expression or activity of many mediators of osteoarthritis, including matrix metalloproteinases (MMPs), aggrecanases, nitric oxide (NO), and prostaglandin E$_2$.

The hydrochloride salt yields approximately 50% more bioactive glucosamine than the sulphate. However, many of the molecular events in chondrocytes and synoviocytes, which result in production of macromolecules incorporating glucosamine, cannot effectively utilise this exogenous source of glucosamine for the direct synthesis of these macromolecules. The primary role of glucosamine within the joint is a structural component that is incorporated into proteoglycan side chains within the cartilage and hyaluronic acid in synovial fluid. Glucosamine has been proven to stimulate proteoglycan and collagen synthesis. It has also been proposed that glucosamine can
enhance production of hyaluronan by synovial membrane cells, is utilised by mammalian tissue as a primary substrate for the biosynthesis of cartilage macromolecules and may, when concentrated in the cartilage matrix, trigger an increase of matrix synthesis. Studies by Hanson *et al* demonstrated clinical improvement in animals diagnosed to be suffering from navicular syndrome when fed a compound containing a combination of CSu and glucosamine. Several studies in humans have been published demonstrating the clinical benefits of oral CSu/glucosamine.

The oral dose rate of glucosamine is 22 mg/kg, while the oral dose rate of chondroitin sulphate is 8.8 mg/kg. However, effects are dose dependent so higher doses may be more effective.

Studies have demonstrated that GU and CSu may inhibit cartilage matrix degradation through a number of mechanisms, including

- Inhibition of degradative enzymes and other cytokines,
- Prevention of inducible nitric oxide synthase upregulation by IL-1 or other cytokines,
- Inhibition of matrix metalloproteinase protein expression and activity and inhibition of nuclear factor kappa B (NFκB), which is a key inflammatory regulator.
- CSu and GS also provide dietary sulphur.

Sulphur is important for the synthesis of many biological molecules, including proteins and glycosaminoglycans, and a sulphur deficiency can inhibit glycosaminoglycan synthesis. It has been proposed that osteoarthritis may exacerbate sulphur deficiencies by increasing demand as the stressed cartilage enters a hypermetabolic state and upregulates synthetic processes.

**Surgery**

In some cases, for example, where there has been extensive new bone formation or where a chip fracture has occurred as a consequence of DJD, arthroscopic surgery may be helpful to give the joint a better chance for recovery. The choice of appropriate therapy must be based upon an accurate and complete diagnosis and the particular needs of the individual case. If, in spite of all efforts, severe chronic osteoarthritis remains and the horse has a persistently painful joint with little movement, it is sometimes possible to restore soundness by athrodesis of the affected joint. This is occasionally used for the pastern and lower hock joints but only as a last resort after all other forms of treatment have been tried.

**IRAP**

Interleukin Receptor Antagonist Protein, also known as Orthokine in human medicine, is a novel therapy and showing promising results for the treatment of osteoarthritis in horses and humans. IRAP was developed to counteract the inflammatory protein Interleukin – 1 (IL – 1) that is produced in the joint during synovitis and to slow the progression of OA. Blood is collected from the patient in specially prepared syringe, which contains coated glass beads that stimulates monocytes to produce up to thirty times more of the antagonist protein. This protein is then injected into the affected joint and prevents Interleukin – 1 from binding to IL – 1 receptors on tissues within the joint, and therefore blocks the action of and stops the damage caused by IL – 1. This relatively simple procedure is costly although its benefit lies in improving lameness and decrease joint swelling. Horses likely to benefit from IRAP are those with synovitis or mild to moderate OA. IRAP cannot reverse any permanent damage that often exists in joints with OA, but may serve to prevent further inflammation and reduce the progression of the disease.

On the human side specialists have had very good results with IRAP treatment as well. Dr Axel Baltzer of The Centre for Molecular Orthopaedics, Königsallee Clinic
(Gemeinschaftspraxis Königsallee) in Düsseldorf, Germany reports that virtually all of his IRAP patients at Gemeinschaftspraxis Königsallee have been able to discontinue pain medications – with an impressive number delaying or completely avoiding joint replacement or spinal surgery.

**Platelet Rich Plasma**
Platelet-rich plasma (PRP) is a fraction of plasma that contains high levels of multiple growth factors. Like stem cells, the use of platelet rich plasma (PRP) for treatment of joint pain or arthritis is quite new, but unlike stem cells, there is a rapidly growing body of literature to support its use. In animal models, PRP prevents progression of arthritis. In a retrospective cohort study in human beings with knee arthritis, PRP was significantly better than hyaluronan in reduction of pain and improvement of function (Sanchez et al Clin Exp Rheumatol 2008; 26:910-913). Although the molecular mechanisms behind the decreased pain and improved function are not well elucidated, one study suggests the PRP enhances the secretion of hyaluronan by synovial fibroblasts in arthritic patients. Zavadil also showed that application of PRP at the time of total shoulder arthroplasty significantly lowered post-operative pain scores and functional internal rotation index improvement factors (Savadil et al. J Extra Corpor Technol 2007; 39:177-182).

There are no clear guidelines for treatment protocols, but most studies use 3 injections at weekly intervals. The number of platelets or volume of injection is not well documented in most studies. PRP isolated from autologous blood may be useful as a source of anabolic growth factors for stimulating chondrocytes to engineer cartilage tissue. The principle clinical use is post-surgery and also for OA that is no longer responsive to HA and/or corticosteroid combination therapy.

**Other products**
One of the world’s leading clinicians and researchers in equine joints, Wayne McIlwraith, BVSc, PhD, DSc, FRCVS, Dipl. ACVS, University Distinguished Professor, Barbara Cox Anthony Chair in Orthopaedics, and director of Orthopaedic Research at Colorado State University, recently commented on alternative therapies. There is some information that omega-3 fatty acids can help OA, noted McIlwraith. He said in humans, three to four months of supplementation reduced joint pain.

A clinical trial at Colorado State with unsaponified avocado soy (Vetoquinol) concluded that the product significantly reduced the severity of joint damage and significantly increased the synthesis of cartilage glycosaminoglycans (i.e., the “building blocks” of articular cartilage) in joints with OA, compared to horses treated with a placebo. McIlwraith said this was the “first well-controlled equine study demonstrating a positive effect with an oral nutraceutical.” He said controlled studies of the product in humans were also positive.

**Summary**
In conclusion, there are currently many options for treating joint disease in the horse. The practitioner should add rest and physical therapy to treatment regimens of therapeutic drugs. The extent of an injury is important to consider, and rest should be recommended according to the predicted time of inflammation. Inflammation is the greatest “enemy,” since ongoing inflammation and promotion of the cascade of events...
surrounding the release of inflammatory mediators in the joint perpetuates injury to articular tissues. Many therapies mentioned here are combined to have an additive, if not a synergistic, response to joint injury. Often NSAIDs are combined with intra-articular injection of HA and steroids; and horses are placed on parenteral as well as oral GAGs. While no studies have evaluated this “shotgun” approach, each drug has beneficial effects and no adverse effects have been reported to date from using these many different modes of therapy.

Selected References
CPD Questions:

Question 1
Which one of the following substances would normally be absent in cartilage matrix:

a) Calcium phosphate  
b) Chondroitin sulphate  
c) Keratin sulphate  
d) Collagen  
e) Water

Question 2
The repeating unit of hyaluronic acid is

a) Glucuronic acid and N-acetyl D-galactosamine  
b) Galacturonic acid and N-acetyl D-galactosamine  
c) Galacturonic acid and N-acetyl D-glucosamine  
d) Glucuronic acid and N-acetyl D-glucosamine  
e) Glucuronic acid and keratan sulphate

Question 3
The plasma half-life of intravenous Hyaluronic acid is no greater than:

a) 30 Minutes  
b) 5 Minutes  
c) 15 Minutes  
d) 20 Minutes  
e) 60 Minutes

Question 4
The predominant glycosaminoglycan in adult articular cartilage is:

a) Keratan sulphate  
b) Chondroitin sulphate  
c) Heparin sulphate  
d) Galactosamine  
e) Dermatan sulphate

Question 5
The primary role of glucosamine within the joint is:

a) Structural  
b) Lubrication of the cartilage  
c) Anti-inflammatory  
d) Providing building blocks for cartilage  
e) Lubrication of the soft tissues
Question 6

It is thought that chondroitin and glucosamine may inhibit cartilage matrix degradation through a number of mechanisms except:

a) Inhibition of degradative enzymes and other cytokines
b) Prevention of inducible nitric oxide synthase downregulation by IL-1
c) Inhibition of matrix metalloproteinase protein expression and activity
d) Providing dietary sulphur
e) Prevention of inducible nitric oxide synthase downregulation by IL-16

Question 7

IRAP was developed to counteract the inflammatory protein:

a) Interleukin 3
b) Interleukin 5
c) Interleukin 7
d) Interleukin 1
e) Interleukin 13

Question 8

Interleukin receptor antagonist protein is produced by:

a) Neutrophils
b) Monocytes
c) Eosinophils
d) Basophils
e) Synovium

Question 9

The use of platelet rich plasma supplies which factor to tissue:

a) White blood cells
b) Growth factors
c) Sulphur
d) Matrix metalloproteinases
e) Glycosaminoglycans

Question 10

Which one of the following may have some anti-inflammatory effects in a joint?

a) Purified aloe vera
b) Acetyl L-Carnitine
c) Omega 3 fatty acids
d) Rhino horn
e) MSM