ALPHA 2 EQ™

Equine Alpha-2 Macroglobulin Safety Study and Clinical Observations

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Osteoarthritis (OA) and inflammatory joint disease are both mediated by numerous biomechanical and biochemical processes. Inflammatory cytokines have been demonstrated to increase production of metalloproteases that degrade cartilage, and the catabolic cartilage products further stimulate production of additional pro-inflammatory cytokines. Recently, increased attention has been given to inhibiting metalloproteases involved in cartilage catabolism, with the intention of modulating cartilage breakdown and preventing the cascade of inflammatory mediators involved in disease progression.

Alpha-2 Macroglobulin (A2M) has emerged as a unique potential treatment of cartilage-based pathology and inflammatory arthritides. The process of concentrating A2M for the treatment of a variety of joint and disc related issues in human medicine is well documented. A proprietary kit has been developed and FDA approved. Autologous concentrated A2M from plasma is currently in use to successfully treat various painful arthritides, including mild to moderate OA, PTOA, enthesopathies, and spinal discogenic back pain in the human market. A2M can not only inhibit the associated inflammatory cascade but also disrupt the catabolic process of cartilage degeneration.

This paper is to document the use of the Astaria Global proprietary kit (Alpha2EQ) system to concentrate equine A2M and utilize it in the treatment of a variety of orthopedic issues. The human kit is easily adopted to the equine patient and the process for preparing autologous A2M is identical.

Two important biochemical networks that contribute to OA pathology include pro-inflammatory cytokines and matrix metalloproteinases (MMPs).[7] A2M is a major plasma glycoprotein best known for its ability to inhibit a broad spectrum of serine, threonine and metalloproteases by a unique bait and trap method (Fig. 1).

A2M uses a 39–amino acid bait region that, when cleaved by a protease, induces a large irreversible conformational change that physically traps the protease within a steric cage. As part of the entrapment, the protease forms a covalent bond with A2M, exposing a receptor recognition site, triggering the endocytosis and eventual clearance of the A2M-protease complex. A2M has also been demonstrated to bind pro-inflammatory cytokines, such as TNF-a and IL-1b, and reduces the cytokine-induced up-regulation of collagenases in chondrocytes (Fig. 2).

Fig. 1. Pictorial representation of the mechanism by which A2M traps proteases. Each dimer traps a single protease. After the second trapped protease, the molecule then undergoes active transport for its elimination.

a_2-Macroglobulin 911

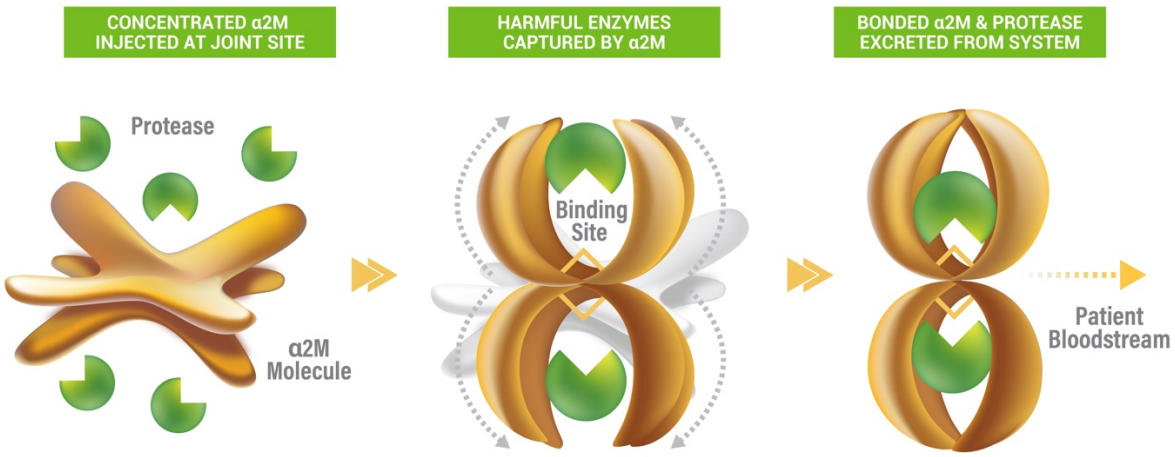
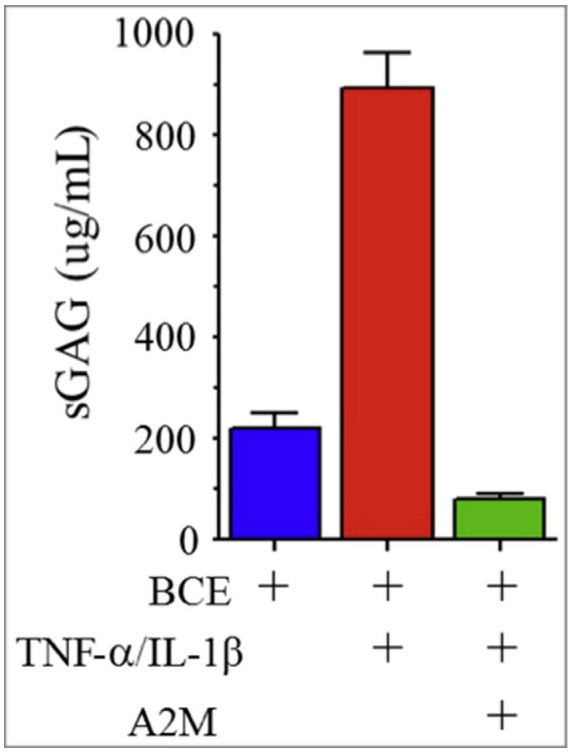


Fig. 2. A2M is chondroprotective against inflammatory cytokines. Treatment of bovine cartilage explants (blue column) with pro-inflammatory cytokines TNF-a and IL-1b (red column) induces chondrocytes within the cartilage to produce or activate proteases resulting in increased production of sulfated glycosaminoglycan (sGAG). Treatment with purified human A2M (green column) potently inhibited cartilage catabolism. BCE, bovine cartilage explant.



Study

For the purpose of establishing safety of the process in the horse, a pilot project utilizing 4 non-lame recipient mares was performed. Each mare was aseptically prepped for a blood draw. Blood was then processed using the kit provided. The medial femorotibial joint of each mare was injected with 15cc of the end product. Patients were evaluated for signs of inflammatory reaction and lameness at 2 hours, 12 hours, 24 hours and daily for the next 3 days. At no time was there any evidence of any reaction associated with the injection.

Based on the pilot project results, a clinical safety study was undertaken. A total of 50 clinical patients were chosen to receive joint injections for a variety of orthopedic conditions that would have traditionally been treated with existing modalities. The clinical study was performed as a multi-clinic study. Five equine hospitals[1] participated in the study and each contributed patients for evaluation of safety as well as clinical response. No controls were utilized, and the results are reported only as reaction to injection and positive or negative clinical response. A positive response was considered if a patient improved more than a full grade of lameness following treatment. In several instances, a second injection was required to achieve a positive clinical response. Patients that required a second injection were all treated with frozen product, which is indicated in the Clinical Response column. All patients were evaluated at 24 hours, 1 week and 2 weeks to determine adverse reactions and clinical response.

Signalment	Site Injected	Adverse Reaction	Clinical Response
QH-Roping	Carpus -Stifle	None	Positive-Received second injection at 6 months
QH- Trail	Coffin Joints	None	Positive
QH-Cow Horse	Coffin Joints-Stifle	None	Positive- Received second injection at 6 months
QH-Cutting	Sifles-MFT	None	Positive on 1
QH-Roping	Coffin Joint	None	Positive
QH-Barrel Racing	Carpus - TMT's	None	Positive
Wmblood-Dressage	Origin LF suspensory	None	Positive
QH-Cow Horse	Coffin Joints-Bursas	None	Positive-Received second injection at 1 month
QH-Roping	LH Coffin joint-Bursa	None	Positive

Signalment	Site Injected	Adverse Reaction	Clinical Response
QH-Roping	Coffin Joint	None	Positive
QH-Barrel Racing	Front Tendon Sheaths	None	Negative
QH-Cow Horse	Carpus	None	Positive
QH-Roping	Stifle-MFT	None	Required second injection
QH-Barrel Racing	Sacroiliac L	None	Positive
QH-Ranch	Coffin Joints-Bursas	None	Positive
QH-Reining	Coffin Joints	None	Positive
Thoroughbred	Fetlocks	None	Positive
QH-Barrel Racing	Coffin Joints	None	Positive
Wmblood-Jumping	Coffin joint-bursa	None	Positive- Received second injection at 6 months
Arabian Show	Coffin Joints	None	Pending
QH-Roping	Coffin joint-bursa	None	Required second injection
QH-Roping	Carpus	None	Positive
QH-Barrel Racing	RH suspensory	None	Positive
QH-Barrel Racing	Carpus	None	Positive
QH-Roping	Coffin joint-bursa	None	Required second injection
QH-Barrel Racing	Coffin Joints	None	Required second injection
QH-Roping	Coffin Joints-Stifles	None	Positive
QH-Roping	Navicular Bursa R&L	None	Positive- Received second injection at 8 months

Signalment	Site Injected	Adverse Reaction	Clinical Response
Arabian Show	Coffin Joints-LF Fetlock	None	Positive
Warmblood	Cervical facets C5-C7	None	Positive
Arabian Hunter	R&L Front Fetlocks-Coffin joints	None	Positive
Warmblood	LF Coffin Joint	None	Positive
QH-Roping	Navicular Bursa R&L	None	Positive
Danish WB-dressage	Coffin Joints	None	Positive
Percheron X-pleasure	RF coffin joint and bursa	None	Positive
Belgian WB-eventing	Coffin Joints	None	Positive
ISH-Foxhunting	Coffin Joints-TMT's	None	Positive
Thoroughbred-eventing	Coffin Joints	None	Positive
Hanoverian X-Dressage	Coffin Joints-TMT's	None	Positive
Thoroughbred-Eventing	Coffin Joints-Bursas - LF digital sheath	None	Positive
Thoroughbred-Eventing	Coffin Joints	None	Positive
ISH-Eventing	RF coffin joint	None	Positive
Warmblood-Jumper	RF coffin joint and bursa	None	Positive
QH-Cutting	TMT's	None	Positive
Warmblood-Jumper	Coffin joint-bursa RF	None	Positive
Warmblood-Hunter	L Inter-carpal joint	None	Pending

Conclusions:

This study indicates that the application of Astaria Global proprietary kit (Alpha2EQ) system for concentration of A2M is safe and seemingly efficacious in the equine patient. No horse experienced any adverse reaction or joint flare post injection. In addition, the several patients that received frozen product injections did not experience any joint flares. The clinical response to frozen product injected was no different than that from freshly processed product.

Research is currently underway at Boise State University. Research to demonstrate the concentration levels of A2M, and the cytokine profiles present in the equine product has been completed and is under evaluation. Studies to determine the molecular size and configuration of the A2M molecule in the horse and other biologically active proteins that may be present is underway.

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