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Evidence for alpha-2-macroglobulin as an orthobiologic osteoarthritis therapy: a narrative review

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Abstract

Orthobiologics rich in alpha-2-macroglobulin (A2M) are being used with increasing frequency to treat equine and human osteoarthritis (OA). The glycoprotein is concentrated from whole blood, typically prepared by a commercial device, and is administered IA with the goal of ameliorating inflammation and associated pain. In recent years, numerous investigations have elucidated A2M's mechanism of action and its effects on joint cells; however, none have used the horse as a model nor have they investigated the commercially available kit for equine orthobiologic preparation, Alpha2EQ. This narrative review presents the most pertinent work on A2M, which supports its current clinical use as an OA treatment. Alpha-2-macroglobulin has been studied in a variety of preclinical models, in vivo, and in clinical patients. As a naturally occurring and potent protease inhibitor, A2M acts to regulate key components of the OA inflammatory cascade, from cytokines and chemokines, which propagate synovitis, to disintegrins and metalloproteinases that degrade the cartilage extracellular matrix. Three main mechanisms of action contribute to A2M's modulation of joint inflammation and concomitant OA progression across species: the bait-and-trap mechanism, direct binding interactions, and regulation of gene expression. In vivo, A2M treatment results in improved histopathology scores, gait improvement in animals, and improved patient-reported outcomes in people. Though substantial evidence exists for A2M's anti-inflammatory effects and role in OA treatment, further studies will be required to elucidate the mechanisms of the equine orthobiologic.

Keywords: alpha-2-macroglobulin, osteoarthritis, synovial fibroblast, orthobiologic, equine

Osteoarthritis (OA) is a debilitating joint disease affecting people and animals worldwide. It is reported that nearly 600 million people suffer from OA, and this number is projected to increase markedly by 2050.[1] In our canine companions, stifle OA had an estimated prevalence of 36.4% in a clinic population comprised of dogs over 8 years old, and the prevalence of OA in the shoulders of the same dogs reached 57.4%.[2] Osteoarthritis is the reported cause of approximately 60% of lamenesses in horses of all ages.[3,4] In one study[5] examining the prevalence and severity of metacarpophalangeal joint OA, the disease was reported to affect one-third of all 2- and 3-year-old racehorses, and lesions were most severe in horses over 5 years. Not only is OA seen in early-career athletic horses, but it is prevalent across all breeds and disciplines. Of 200 geriatric horses and ponies in the United Kingdom, 83.5% had reduced range of motion of at least 1 joint, likely resulting from OA.[6] More recently, a high rate of OA and lameness associated with fetlock-region pain was described in working dressage horses.[7]

Driven by multiple inflammatory cascades, the pathogenesis of OA can be summarized as a complex crosstalk between cytokines, chemokines, and metalloproteinases produced by joint tissues, leading to the hallmarks of synovitis, cartilage degeneration, osteophyte formation, and subchondral bone sclerosis seen across all species.[8–10] There are currently no approved disease-modifying drugs for the treatment of OA.[11] Historically, NSAIDs and IA corticosteroids have been the mainstays of therapy; however, pain amelioration comes with considerable systemic risks, including kidney or gastrointestinal insult and laminitis[12] in equine patients alongside possible chondrotoxic effects[13,14] with IA administration.

Orthobiologics, including platelet-rich plasma (PRP), autologous conditioned serum (ACS), and autologous protein solution (APS), have more recently become part of the equine veterinarian's biologic armamentarium for treating OA. Such autologous therapies may deliver a safer, efficacious[4] alternative to traditional pharmacologic approaches and are typically prepared stall-side. Autologous protein solution treatment has been shown to

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reduce inflammation via increasing concentrations of the chondroprotective cytokines interleukin (IL)-1Ra and IL-10 in vitro[15] and to improve synovial membrane histology and gross joint appearance in an experimentally induced model of synovitis.[16] Similarly, in vitro, ACS downregulates IL-1β expression in cartilage and matrix metalloproteinase (MMP)-1 expression in synovial membrane explants in coculture.[17] As a result of its ubiquitous nature, all blood-based orthobiologics contain some amount of alpha-2-macroglobulin (A2M).[18,19]

Alpha2EQ (Creative Science [formerly Astaria Global, LLC]) has emerged as a commercially available equine orthobiologic for the treatment of OA. The Alpha2EQ kit uses 2 cycles of centrifugation to first separate plasma from whole blood and then concentrate acellular A2M for autologous IA injection. While Alpha2EQ is being used in clinical practice,[20] no studies to date in the horse have explored the effects of Alpha2EQ or isolated A2M on equine joint cells; however, there is substantial evidence to suggest that A2M from blood would have potent anti-inflammatory action within the osteoarthritic joint. This narrative review presents the body of work on A2M across species in both the clinical and research settings, which supports its current clinical use as a treatment for equine OA and motivates our ongoing investigations into the mechanisms by which this orthobiologic could alter disease progression.

A2M Structure

Alpha-2-macroglobulin is a highly conserved and ubiquitous glycoprotein produced primarily by the liver[21] and found in the blood of all mammals.[21,22] As part of the innate immune system's acute-phase response, it is an inhibitor of all types of proteases.[23,24] Alpha-2-macroglobulin is a large macromolecule comprised of four 180-kD monomer domains, which are essential to its dynamic activity as a protease inhibitor.[25] Alpha-2-macroglobulin in its tetrameric form (720 kD), where these dimers are joined by noncovalent interactions, works through a bait-and-trap mechanism to sterically capture proteases, thereby preventing substrate binding and halting downstream catabolism.[22, 25–27] This mechanism represents the transformation of native A2M to induced A2M[25]; however, there is evidence that structural intermediates, including dimers, may have novel regulatory functions.[28] In fact, inflammation and oxidative modification of A2M changes the affinity of the A2M for its targets.[28–30] Herein, we will discuss the evidence for the 3 main mechanisms through which A2M acts to inhibit protease and cytokine activity in the context of OA: the bait-and-trap mechanism, direct binding interactions, and regulation of gene expression, all of which directly relate to A2M's complex structure.

Primary Evidence for A2M Function

Studies in vitro **(Table 1)** and in vivo **(Table 2)** have elucidated A2M's function. Though in its clinical use as an orthobiologic A2M is patient-derived and concentrated from autologous whole blood, a commercially available recombinant human A2M protein is often utilized in experimental studies. While beneficial for study reproducibility, the use of recombinant protein precludes the examination of the effects of the whole orthobiologic with its full repertoire of effector molecules. Regardless of A2M source, these investigations have established a comprehensive understanding of A2M's molecular mechanisms as they relate to OA pathogenesis.

Bait-and-Trap Mechanism

Characterization of A2M derived from human plasma using cryogenic electron microscopy has revealed the bait-and-trap mechanism of the glycoprotein with exceptional resolution.[25] In its native state, A2M, with its 4 identical monomeric subunits, presents a large cavity within each monomer pair, sufficient for entry of substrate protease. Inside, the active protease cleaves a specific domain termed the bait region, inducing a conformational change and retraction of the monomers to encapsulate the substrate protease in its new cage-like[26,28] structure. A schematic of this bait-and-trap mechanism is illustrated in **Figure 1**. During this transformation to the induced state of A2M, a buried thioester site is exposed, which covalently binds the substrate to further prevent its escape. Only then can A2M's receptor binding domain expose a lysine residue that is critical to its receptor (low-density lipoprotein receptor-related protein 1 [LRP1]), and A2M binds LRP1 for internalization by the target cell and clearance of the A2M/trapped protease complex from the circulation.[25] In plasma, LRP1 receptors on hepatocytes are responsible for this endocytosis; however, immunohistochemistry has demonstrated that LRP1 receptors are expressed in a spectrum of cell types, including fibroblasts, which may explain a second mechanism of A2M/protease clearance from tissues.[42]

A range of proteases are inhibited via the bait-and-trap mechanism of A2M. Digestion assays by 2 groups have demonstrated that A2M is an endogenous inhibitor of the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of proteases, the principle enzymes that break down articular cartilage in OA through the cleavage of extracellular matrix components.[43] Alpha-2-macroglobulin acts to inhibit the aggrecanase activity of ADAMTS-4 and -5 in a concentration-dependent manner[32] and also prevents cartilage oligomeric matrix protein degradation through baiting and trapping of ADAMTS-7 and -12.[33]

Similarly, the MMPs, their relatives, break down collagen and proteoglycans and are therefore major contributors to OA pathogenesis.[44] Binding kinetic studies[45] indicate that A2M regulates the activity of extracellular MMPs, with A2M being a 150-fold better substrate for collagenase (MMP-1) than collagen type I. In monolayer chondrocyte culture (human primary OA as well as IL-1β–stimulated human chondrocyte cell line) and IL-1β–stimulated human cartilage explants, recombinant A2M inhibits MMP-13 as

Table 1—In vitro experimental studies presenting primary evidence for alpha-2-macroglobulin (A2M) function relevant to the treatment of osteoarthritis (OA).

Study design	Subject species	A2M species	A2M source	Cell type	Evidence	Reference
Cartilage explants stimulated with IL-1α	Bovine	Human	Plasma	N/A	Bovine and human cartilage degradation is inhibited by A2M in the presence of plasminogen, but degradation is not inhibited when explants are stimulated with IL-1α (bovine) or IL-1β (human) alone	Oleksyszyn et al[31]
Cartilage explants stimulated with IL-1β	Human	Human	Plasma	N/A		
Digestion assay: ADAMTS-4 and -5 incubated with A2M and cleavage products measured by SDS-PAGE	N/A	Not reported	Unable to interpret[a]	N/A	A2M is a concentration-dependent endogenous inhibitor of ADAMTS-4 and -5, inhibiting aggrecanase activity via proteolysis of A2M's bait region and physical entrapment of ADAMTS-4 and -5	Tortorella et al[32]
Digestion assay: same methods as above	N/A	Human	Recombinant[b]	N/A	A2M is a novel substrate of ADAMTS-7 and -12, acting as a competitive inhibitor of COMP	Luan et al[33]
Monolayer cell culture and cartilage explants stimulated with IL-1β	Human	Human	Recombinant[b]	Chondrocyte	A2M dose-dependently inhibits MMP-13	Wang et al[34]
Monolayer cell culture stimulated with IL-1β	Human	Human	Serum	Chondrocyte	(1) Reduces MMP-13, TNF-α, and IL-6 protein levels and expression of MMP-3 and -13 (2) Increases COLII and ACAN expression	Zhao et al[35]
Monolayer cell culture stimulated with IL-1β	Human	Human	Recombinant[b]	Chondrocyte	(1) A2M binds and neutralizes IL-1β both intra- and extracellularly, blocking NF-κB–induced catabolism (2) A2M decreases MMP-1 and -3 and TNF-α expression and increases COLII and ACAN expression	Sun et al[36]
Monolayer cell culture	Murine (ATDC5 cell line)[b]	Human	Recombinant[b]	Chondrocyte	(1) A2M promotes proliferation and reduces apoptosis (2) A2M increases COLII and ACAN expression (3) A2M promotes PCNA gene expression and protein production	Guo et al[37]

ACAN = Aggrecan. ADAMT = A disintegrin and metalloproteinase with thrombospondin motifs. COLII = Collagen type II. COMP = Cartilage oligomeric matrix protein degradation. IL = Interleukin. MMP = Matrix metalloproteinase. N/A = Not applicable. NF-κB = Nuclear factor κB. PCNA = Proliferating cell nuclear antigen. TNF-α = Tumor necrosis factor–α.
[a]Serva. [b]Sigma Aldrich.

evidenced by significantly lower MMP-13 concentrations with A2M treatment.[34] In the primary OA chondrocytes, multiple doses of A2M were investigated, and this MMP-13 inhibition was shown to be concentration dependent.[34] In synovial fluid, ELISA results have also shown that recombinant A2M treatment decreases the concentration of MMP-13 in rats with anterior cruciate ligament (ACL) transection-induced OA[34] and MMP-1 in pigs with OA induced by modified IA drilling (mIAD)[40] when compared to untreated controls in both studies. Interestingly, a single study[31] has shown that A2M effectively inhibits glycosaminoglycan release from IL-1–stimulated human and bovine cartilage explants, but this effect is only observed in the presence of plasminogen. The authors implicated the possible role of the fibrinolytic cascade in the proteolytic activation of the MMPs, postulating that plasminogen is necessary to have active forms of the MMPs and to therefore see measurable A2M activity in this model. Therefore, substantial evidence, across mechanistic, in vitro, and in vivo studies, demonstrates that A2M protects the cartilage extracellular matrix from degradation by modulating the clearance of a variety of proteases.

Table 2—In vivo experimental studies presenting primary evidence for A2M function relevant to the treatment of OA.

Study design	Subject species	A2M species	A2M source	Induction model	Evidence	Reference
Animal model	Murine (rat, unspecified)	Human	Recombinant[a]	ACL transection	IA A2M: (1) reduces MMP-13 concentration in SF (2) decreases MMP-13 expression (3) Increases Col2A1 and ACAN expression	Wang et al[34]
Animal model	Murine (DBA/1)	Not reported	Not reported	COLII-induced arthritis for RA	(1) Less inflammatory infiltration and synovial hyperplasia; more typical cartilage/bone organization (2) Increases COLII and ACAN and decreases TNF-α and MMP-13 staining on cartilage histology (3) Reduces MMP-3, -9, and -13 and increases COLII and ACAN expression in cartilage	Li et al[38]
Animal model	Murine (Sprague-Dawley rat)	Human	Serum	ACL transection	A2M reduces MMP-13 mRNA and protein levels in cartilage, reduces OARSI scores for cartilage degeneration, and reduces chondrocyte loss	Wang et al[39]
Animal model	Minipig (unspecified)	Porcine	Serum	"Idealized" ACLR	IA A2M reduces IL-1β, IL-6, and TNF-α protein levels in SF, reduces microscopic OARSI scores, and improves gait	Zhao et al[35]
Animal model	Yucatan minipig	Human	Recombinant[a]	Modified IA drilling	IA A2M: (1) Lowers IL-1B, NF-κB, and TNF-α expression in synovium (2) Lowers SF MMP-1 levels (3) Reduces macroscopic and microscopic cartilage degeneration and synovitis	Sun et al[40]
Double-blinded, randomized, controlled clinical trial	Humans with naturally occurring OA	Human	Plasma	N/A	(1) Significant improvement in VAS, WOMAC, and KOOS scores at 12 weeks after injection (2) Significant improvement in VAS, WOMAC, KOOS, and Tegner scores at 6 weeks, whereas PRP-treated patients showed no significant changes at 6 or 12 weeks after injection (3) Between A2M, PRP, and CC groups, there were no significant differences in baseline to 6- or 12-week change in PROs	Thompson et al[41]

ACL = Anterior cruciate ligament. ACLR = ACL Reconstruction. CC = Corticosteroid. Col2A1 = Collagen type II. KOOS = Knee Injury and Osteoarthritis Outcome Score. N/A = Not applicable. OARSI = Osteoarthritis Research Society International. PRO = Patient-reported outcome. PRP = Platelet-rich plasma. RA = Rheumatoid arthritis. SF = Synovial fluid. VAS = Visual analog scale. WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

[a]Sigma Aldrich.

Direct Interactions

Alpha-2-macroglobulin also binds several cytokines and growth factors directly in a mechanism distinct from that which entraps proteases in its cage-like structure.[27] The biochemical interactions between A2M and these substrates are dependent on A2M conformation, and binding affinity is increased for TGF-β1 and tumor necrosis factor–α (TNF-α) when A2M is in its induced (protease-activated) form.[23] In equine plasma, TGF-β1 bound abundantly to induced A2M, very little to native A2M, and moderately to A2M's intermediate form.[46] Most often, these interactions are initially noncovalent and reversible but become covalent over time.[47] Under conditions of inflammation, powerful oxidants, such as hypochlorite, are generated, which can induce A2M conformational modifications and differentially regulated cytokine binding.[30] Hypochlorite-induced dissociation

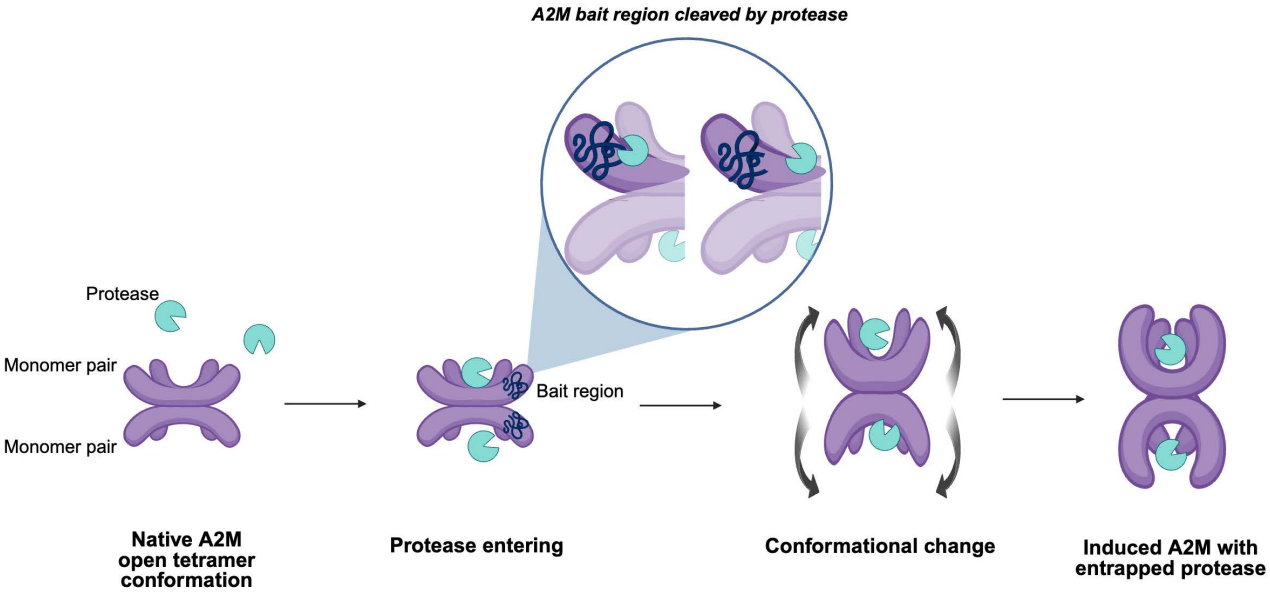

Figure 1—Schematic illustration of the bait-and-trap mechanism of alpha-2-macroglobulin (A2M). Alpha-2-macroglobulin in its native state, with 2 monomer pairs forming a tetramer and 2 cavities for protease entry. Inside, the protease cleaves the bait region. This induces a conformational change, with the monomers retracting to entrap the protease.

of native A2M into 2 dimers increases binding to IL-6 and TNF-α while abolishing the molecule's bait-and-trap action.[28,30] Further demonstrating A2M's critical role in the innate immune response, it has been shown to directly bind human complement protein.[48] Though primary investigations of this mechanism are more limited, it is postulated that under conditions of high protease activity, A2M rapidly clears cytokines from circulation via these direct interactions.

Anti-inflammatory signaling by A2M occurs both extracellularly and intracellularly. Treatment of chondrocytes in culture with fluorescently labeled recombinant A2M has shown that the macromolecule can be detected intracellularly; in cultures stimulated with IL-1β, the proportion of intracellular A2M was 57% compared to an unstimulated control (25%), indicating that A2M uptake may be associated with local IL-1β presence. The same study[36] used immunoprecipitation and western blot to demonstrate intracellular A2M binding of IL-1β, and double-labeling immunofluorescence analysis showed A2M/IL-1β, providing further evidence that A2M not only enters chondrocytes but binds IL-1β. Though not demonstrated in this study, others have reported that IL-1β binds covalently to the induced form of A2M, and this complex has been found in human plasma.[23] Alpha-2-macroglobulin's direct binding of IL-1β in chondrocytes implies its potential to alter multiple downstream catabolic pathways.

Regulation of Gene Expression

Alpha-2-macroglobulin has also been shown to regulate gene expression in joint tissues, modulating both the expression of proinflammatory and cartilage-protective genes as reflected by mRNA levels measured via quantitative real-time PCR. Alpha-2-macroglobulin treatment resulted in decreased TNF-α, MMP-1, MMP-3, and MMP-13 expression both in chondrocyte culture[35,36,38] and in the cartilage of collagen type II (COLII)-induced arthritis mice.[38] In the same models, chondroprotective genes contributing to the normal cartilage extracellular matrix, COLII and aggrecan (ACAN), were upregulated compared to untreated controls.[35,36,38] These results were corroborated in 3 different studies[34,39,49] utilizing a rat ACL transection (ACLT) model, which revealed suppressed MMP-13 mRNA levels and enhanced COLII[34] and ACAN[34,49] levels in cartilage. In a chondrocyte cell line (without OA), A2M treatment also increased COLII and ACAN expression while increasing cellular proliferation and inhibiting apoptosis.[37] Furthermore, in the same study, proliferating cell nuclear antigen was implicated as a regulator of cartilage metabolism. Proliferating cell nuclear antigen has an essential role in DNA replication and was shown to be increased at both the gene and protein levels in A2M-treated chondrocytes.[37] An ACLT model was also used in the aforementioned study, but since the report does not describe which A2M variants were used for treatment, these results will not be discussed herein. In the synovium, A2M treatment has similarly been shown to downregulate TNF-α as well as IL-1β expression when examined in a minipig model of OA.[40]

To further investigate A2M's effects on the IL-1β pathway of cartilage catabolism in OA, studies have also sought to characterize alterations in nuclear factor κB (NF-κB) expression. Interleukin-1β mediates the activation of the transcription factor NF-κB.[50] Downstream, NF-κB–dependent signaling is critical in OA development, driving cartilage breakdown through the expression of MMPs, aggrecanases (ADAMTS-4 and -5), and inflammatory cytokines (IL-6 and TNF-α) and chemokines (C-C motif

chemokine ligand 2/monocyte chemoattractant protein–1 [CCL2/MCP1] and C-C motif chemokine ligand 5/regulated upon activation, normal T-cell expressed and secreted [CCL5/RANTES]).[51] Ultimately, via a positive feedback mechanism, IL-1β is further produced and secreted. Simultaneously, the same signaling pathway downregulates synthesis of COLII.[51] Thus, therapeutic intervention in this pathway could be potently anti-inflammatory. Sun et al[36] demonstrated that chondrocytes stimulated with IL-1β show maximal concentration-dependent NF-κB protein expression with 10 ng/mL IL-1β, and treatment with A2M resulted in decreased NF-κB protein levels. This decrease was dose dependent as evidenced by NF-κB decreases with increasing A2M concentrations. Sun et al[36] also demonstrated that A2M simultaneously reduced IL-1β protein levels in the same cells, concluding that A2M can decrease IL-1β along with its inducible mediator, NF-κB. Alpha-2-macroglobulin's downregulation of NF-κB was supported by gene expression analysis of the synovium of minipigs treated with IA A2M by the same group.[40] Collectively, these studies provide evidence that A2M's effects on gene expression are complex, altering multiple inflammatory cascades in the pathogenesis of OA.

Clinical Effects of IA A2M

There is paucity of literature reporting the clinical outcomes of patients and research subjects treated with IA A2M; however, murine and porcine studies have been performed along with a single published human clinical trial. A 2022 study by Wang et al[39] examined the effects of A2M in an ACLT model of posttraumatic OA using 12-week-old Sprague-Dawley rats (n = 80). The A2M injectate was prepared from human serum by first filtering for impurities using a 0.45-µm filter, followed by centrifugation in an ultrafiltered centrifugation tube with a filter membrane size of 100 kD (4 °C, 5,000 X *g*, 90 minutes). Animals were injected IA at days 3, 14, and 28 after ACLT with this preparation of A2M-rich human serum. In this study, sham plus saline and ACLT plus saline groups (n = 8 each) served as controls and were compared to ACLT + 10 µg/µL A2M-rich human serum and ACLT + 20 µg/µL A2M-rich human serum (all injections in 30 µL). At 56 days after ACLT, chondrocyte loss was shown to be reduced by treatment with both ACLT + 10 and 20 µg/µL A2M compared with ACLT alone as determined by toluidine blue staining. Moreover, Osteoarthritis Research Society International (OARSI) scoring in the ACLT + 10 and 20 µg/µL groups was significantly lower than in the ACLT plus saline group, indicating that cartilage degeneration is reduced with A2M treatment. Adverse effects of injection at any time point were not mentioned.

In a Yucatan minipig model of OA, similar clinical effects have been shown.[40] Modified IA drilling was used to induce stifle posttraumatic OA. Forty-eight minipigs (15 to 16 months old) were grouped as follows: mIAD plus saline, mIAD plus single A2M injection, mIAD plus 3 A2M injections, and sham control. Alpha-2-macroglobulin treatment was prepared using human-derived recombinant A2M in saline vehicle at a concentration of 2 mg/mL in a volume of 3 mL, and treatments were performed immediately after surgery and at 2 and 4 weeks postoperatively. Animals receiving a single A2M injection received treatment immediately after surgery, followed by saline injections at the subsequent time points. Fifteen weeks postoperatively, macroscopic cartilage assessment revealed that animals receiving 1 or 3 A2M injections had significantly smaller lesion areas compared to untreated mIAD animals. Microscopic cartilage damage, as assessed using OARSI scoring, was significantly lower in the groups treated with A2M compared to the untreated saline group. Synovial membrane scores (measuring hyperplasia, inflammatory cell infiltration, and vascularity) were significantly lower for animals receiving either 1 or 3 A2M injections compared to animals in the saline group, though there was no difference between scores for 1 or 3 treatments. This study also assessed gait changes, comparing preoperative gait (week 0) to postoperative gait at weeks 4, 8, 12, and 15 using a pressure-sensing walkway system. Animals receiving 1 A2M injection showed a significant decrease in the impulse ratio between weeks 0 and 8, and animals receiving 3 injections showed a significant decrease in stance time between weeks 0 and 4. These early gait changes for these groups normalized by 15 weeks and, per the authors, were not clinically meaningful gait changes in the group receiving 3 A2M treatments. Of note, in the animals receiving multiple injections, all minipigs experienced a limp after the second and third injections, which resolved within 3 to 5 days; however, limping was not captured by gait analysis because measurements were collected before all IA injections. The authors suggest that this could represent an immunological reaction to the human recombinant A2M used in this study, resulting in increased inflammation and pain.

Notably, in a 2022 study by Zhao et al,[35] pigs in an idealized ACL autograft reconstruction model treated IA with autologous A2M experienced gait improvement. Injectate in this study was prepared from the serum of each subject. Eighteen mature (18 months) female minipigs were randomized evenly into the following groups: idealized ACL reconstruction (IACL-R), IACL-R plus A2M-rich serum (A2MRS), or sham. After unilateral surgery of the right knee, IA injection of 2.5 mL A2MRS was performed at 2, 6, 14, and 29 days postoperatively, whereas animals in the sham and IACL-R groups received an equivalent injection of saline. Pigs were euthanized 3 months after surgery; however, gait data were collected preoperatively, at day 7 postoperatively, and every 15 days thereafter until the study's conclusion. Using a pressure-sensing walkway, it was demonstrated that the gait ratios of the left hind to the right hind limbs of A2MRS-treated animals were significantly lower than those of the IACL-R group. Furthermore, no biomechanical parameters, from day 30 to euthanasia, differed significantly from day 0 in the IACL-R plus A2MRS group. In the IACL-R group, this lack of difference was not seen until day 45, implying

an early improvement in gait with A2M treatment. Improvement in gait was corroborated by analysis of cartilage degeneration, which revealed microscopic OARSI synovium and cartilage scores that were lower in the IACL-R plus A2MRS compared to the IACL-R animals.

Alpha-2-macroglobulin has also been investigated in a murine model of rheumatoid arthritis (RA) for its potential to neutralize catabolic enzymes and prevent bone and cartilage damage in this chronic inflammatory joint disease. Li et al[38] used a COLII-induced arthritis (CIA) model of RA in 2-month-old DBA/1 mice (n = 60) to recapitulate the cell and humeral immunity characteristics of RA. Four groups were compared, plus a PBS vehicle–only group, with all injections performed in 10 µL: CIA + 1.2 µg A2M, CIA + 0.8 µg A2M, CIA + 0.4 µg A2M, and CIA + 10 µL PBS; in all groups, treatment injection was performed in the right ankle, and the left ankle was injected with PBS. Alpha-2-macroglobulin source and species were not reported in this publication. Intra-articular treatment with A2M began on day 36, when all CIA ankles showed significant swelling and redness but no bone damage on radiographs, and was continued weekly for 3 weeks. After day 43, significantly lower ankle thickness was observed in A2M-treated mice at all doses when compared to CIA plus PBS. Collagen type II–induced arthritis inflammation clinical scores, which characterize the amount of paw inflammation, swelling, and joint ankylosis (0 to 4 scale),[52] were also significantly reduced: mice treated with 1.2, 0.8, and 0.4 µg A2M had mean clinical scores of 2.2, 3.1, and 3.5, respectively, whereas mice treated with PBS had a mean clinical score of 3.9. Histological evaluation (day 57) revealed that all doses of A2M resulted in less synovial hyperplasia, more intact cartilage, typical bone organization, and uninflamed fat tissue compared to CIA plus PBS mice. This difference was also reflected in significant differences in OARSI scoring, with A2M-treated groups having mild degeneration and CIA plus PBS groups being scored as more severe.

There is a single double-blinded, randomized, controlled clinical trial that investigated the short-term clinical efficacy of IA A2M-rich PRP concentrate compared to conventionally prepared PRP and corticosteroids in patients with symptomatic knee OA.[41] Seventy-five subjects were randomized into 3 treatment groups: A2M-rich platelet-rich plasma concentrate (hereafter referrred to as A2M), conventionally prepared leukocyte-poor PRP, and methylprednisolone (MP). To prepare the A2M injectate, plasma was prepared from 120 mL of autologous whole blood, and an FC120 Alpha 2 Macroglobulin Kit (EmCyte Corp) was used to concentrate the protein fraction in a 5- to 10-mL injectate. Patients were followed for 12 weeks following a single injection, and improvement was assessed via patient-reported outcome (PRO) surveys. A significant improvement in score was observed in the A2M-treated patients by 6 weeks after injection for visual analog scale, Western Ontario and McMaster Universities Osteoarthritis Index, Knee Injury and Osteoarthritis Outcome Score, and Tegner Activity Scale scores. In comparison, patients in the PRP group showed no significant changes in outcome scores at 6 or 12 weeks after injection compared to baseline, and MP-treated patients showed improvement at 6 weeks in visual analog scale and Knee Injury and Osteoarthritis Outcome Score but not Western Ontario and McMaster Universities Osteoarthritis Index, with these scores commensurate to baseline at 12 weeks. Between the 3 treatment groups, there were no significant differences in baseline to 6- or 12-week change in PRO survey outcomes, meaning that improvement in PROs for the A2M group was not significantly different from improvement for the PRP or MP groups. The authors concluded that A2M showed comparable efficacy to PRP and corticosteroid treatment in cases of mild-to-moderate knee OA as evidenced by modest improvement in knee pain and function at 6 weeks after injection. It was also concluded that given its inferior cost-to-benefit ratio compared to PRP or MP, A2M may be less suitable for routine use in knee OA: the authors' reported price of a single A2M injection ranges from $2,000 to $5,000, whereas PRP ranges from $300 to $2,500 per injection compared to just $84 to $146 for corticosteroid treatment. This discrepancy in cost can be partially explained by the fact that neither A2M nor PRP are covered by insurance due to their regulatory status, and therefore this disparity may be less in veterinary patients where insurance policies and patient coverage vary.

There are no peer-reviewed publications that investigate A2M in the equine patient; however, it should be mentioned that there is a single retrospective clinical study[20] published as proceedings. One hundred eighty horses were treated once with IA Alpha2EQ prepared via the manufacturer's instructions and were presented for recheck examination within 8 weeks of injection. Data on diagnosis or clinical examination findings for these patients were not reported. Descriptive clinical parameter data, including joint effusion, lameness grade (American Association of Equine Practitioner scale), response to flexion test, and pain on palpation, were presented for the distal interphalangeal joint (n = 38 injections), tarsometatarsal and distal intertarsal joint (28 injections), and cervical dorsal articular facet joint (48 joints). Within the 19 horses receiving distal interphalangeal joint Alpha2EQ injections, 78.9% showed improvement in at least 1 clinical parameter, with both effusion and lameness grades decreasing by recheck (average initial effusion score, 2.3; postinjection score, 1.3; average initial lameness grade, 1.4; postinjection grade, 0.5). It should be noted that an average lameness score of 1.4 would indicate that the horses were mildly or inconsistently lame at baseline. Overall, 85.7% of horses receiving tarsometatarsal/distal intertarsal joint treatment showed improvement in flexion response. In horses receiving Alpha2EQ treatment of cervical dorsal articular facet joints, pain improved in 71.4% of cases; however, a validated pain scoring system for horses was not reported to be used in this study. There was 1 reported case of joint flare following injection, but further details are not provided. As acknowledged by the authors, due to its retrospective design, this work also does not include case control comparisons.

Due to the preliminary nature of this work, it should not be considered primary evidence for the mechanism, function, or efficacy of the Alpha2EQ product in the treatment of equine OA.

To date, a single study[19] has reported the concentration of A2M in the equine orthobiologic Alpha2EQ. Barot et al[19] used selected reaction monitoring mass spectrometry to measure a specific A2M peptide that was enzymatically liberated from A2M as a surrogate for the whole protein. They found the median concentration of A2M in Alpha2EQ prepared from 6 healthy adult horses to be 1.99 mg/mL (range, 1.76 to 2.66 mg/mL). This A2M concentration was reported to be significantly less than that of APS (4.08 mg/mL; range, 3.5 to 4.77 mg/mL).[19] The selected reaction monitoring mass spectrometry method used in this study does not yield information on which structural intermediates of A2M are present in a given sample, and therefore the bioactivity of A2M in Alpha2EQ and related orthobiologics (such as ACS and APS examined in this study) also remains unknown. Due to the complex nature of A2M as a bioactive component of orthobiologics, more research is needed to explore whole A2M structures, including tetramers and dimers, as well as A2M bioactivity in these IA products.

Similarly, substantial work must be done before clinicians will understand how A2M-containing orthobiologics are altered by the patient's physiologic state and disease status. Currently, there are no published guidelines for veterinarians in the selection of OA orthobiologics that consider these factors[4] and no studies that report how A2M concentration, structure, or biologic function in these products change with health or disease. The effect of prolonged aerobic exercise on A2M production in plasma has been investigated using matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) peptide mass fingerprinting in a limited group of endurance horses.[53] Alpha-2-macroglobulin was shown to increase 2.9-fold from baseline when plasma was examined at 24 and 48 hours after racing in 8 Anglo-Arabian stallions.[53] Though this finding may imply that A2M concentration in orthobiologics would increase with exercise or exertion, such conclusions should be drawn with extreme caution and with an eye to research examining these effects in a broader population. Conflicting data exist: in 10 Hanoverian crossbred and Belgian saddle horses engaged in active show jumping competition, plasma A2M measured using MALDI-TOF mass spectrometry did not change significantly at any time point following exercise.[54]

With regard to systemic health, it has been shown using western blot that A2M expression in the visceral adipose tissue of horses with insulin dysregulation (ID) is increased compared to those without ID.[55] Interestingly, A2M expression was not significantly upregulated in the SC adipose or skeletal muscle in the same horses[55]; as such, these results certainly build our foundational knowledge of A2M in the context of equine endocrinopathies but should not be used to draw conclusions about plasma A2M or its presence in the autologous orthobiologics prepared from ID horses. Since A2M is an acute-phase protein, its production by the liver in all mammalian species is reasonably affected by inflammatory conditions and diet. In studies involving rat[56] and human[57] subjects, it has been shown that both protein[56] and gluten[57] in the diet can alter A2M expression levels; more relevant dietary research will need to be conducted in the horse. Furthermore, A2M is a well-recognized serologic biomarker of liver fibrosis in human patients, with differential expression during disease progression.[58] These studies set the groundwork for future work to elucidate the multifaceted roles of systemic health in the preparation of orthobiologics for the equine athlete.

Conclusions

The anti-inflammatory properties of the A2M glycoprotein have led to its development as an orthobiologic for people[59] and horses, with evidence across species demonstrating its multifaceted effects on joint cells and tissues. Alpha-2-macroglobulin has multiple mechanisms of action that act to intervene at various points in the inflammatory cascade of OA, from entrapment of effector proteases to modulation of gene expression and interruption of proinflammatory positive feedback cycles. Its presence in mammalian blood makes it an ideal effector molecule for the generation of autologous blood-based therapies, and its well-characterized protein structure allows it to be readily expressed and acquired as a recombinant protein for research. There are, however, challenges to studying A2M at this time, including lack of a commercially available ELISA for its quantification in the horse.[18,60] While more work will be needed to fully characterize the commercial Alpha2EQ product for use as an OA treatment, A2M's ability to attenuate joint inflammation in other species motivates our investigation of the effect of Alpha2EQ in equine synovial fibroblasts. The companion original research paper describes the findings of our targeted transcriptomic analysis. This work provides the first, early evidence that Alpha2EQ modulates proinflammatory gene expression in vitro, making it a promising orthobiologic for the treatment of OA.

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