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Review Article

Intra-articular 2.5% polyacrylamide hydrogel, a new concept in the medication of equine osteoarthritis: A review

Aziz Tnibar*

The Equine Hospital, Jockey Club of Saudi Arabia, PO Box 26323, Riyadh, Saudi Arabia

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ABSTRACT

Recent clinical and experimental trials have demonstrated that intra-articular 2.5% Polyacrylamide hydrogel (PAAG) is highly effective (82.5% free of lameness horses at 2 year follow-up), lasting and safe for the treatment of equine osteoarthritis (OA). Over the last decade, intra-articular 2.5% PAAG has shown to be a potent and promising drug in the medication of OA in horses, as no other single medical treatment for OA has such prolonged efficacy. Most of these studies were presenting some limitations. Preliminary observations on the mechanisms of action of intra-articular 2.5% PAAG support a mechanical effect through integration into the synovial membrane, an increase in joint elasticity possibly reducing overall joint capsule stiffness, and provision of lasting viscosupplementation which contributes to protecting articular surfaces. In addition, no effects on pro-inflammatory cytokines have been observed. Studies also suggest that these positive effects occur in the absence of intra-articular neurotoxicity or fibrosis. The effect on the synovial membrane and joint capsule and the long-acting viscosupplementation represent new concepts in the management of equine OA.

Horse; Osteoarthritis, Medication, 2.5% polyacrylamide hydrogel

1. Introduction

Osteoarthritis (OA) is a common clinical problem in horses [1] and the most common joint disease resulting in chronic pain and physical impairments in humans and animals [1–3]. Surveys estimate that up to 60% of lameness problems in horses are related to OA [4,5], which can occur both early in the equine athlete's career or later in older horses [6].

OA refers to a clinical syndrome of joint pain accompanied by varying degrees of functional limitation [4]. The disease is caused by acute trauma, overload or repetitive stress and is characterized by several pathways of articular degeneration and regeneration. OA is characterized by chronic inflammation of the synovial membrane, progressive cartilage damage, remodeling of the subchondral bone, narrowing of the joint space, formation of marginal osteophytes which result in a loss of function of the joint [7,8].

Biomolecular research has examined the complex pathogenesis of OA at the molecular level. Initially interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a) are the most important inflammatory mediators, which are involved in the damage of hyaline cartilage. They initiate the synthesis of catabolic enzymes, such as matrix metalloproteinases, which cause degeneration of the structures described above [9]. Despite intensive ongoing research in the field of human and veterinary medicine, the knowledge about the exact pathogenesis of OA is limited [9].

The diagnosis of OA is routinely based on physical lameness examination and diagnostic analgesia. Diagnostic imaging methods include radiography, ultrasonography, magnetic resonance imaging, computed tomography and gamma scintigraphy. Additional information might be gained by the analysis of synovial fluid or serum and also by arthroscopic examination.

The medical treatment of OA in the horse is one of the most utilized therapeutic regimens in the equine practice. Once the diagnosis of OA is established, a variety of treatment options are available. Various nonsteroidal anti-inflammatory drugs can be used such as phenylbutazone, flunixin meglumine, ketoprofen, naproxen, and carprofen. The intra-articular medication is common practice, since high intra-articular concentrations of the therapeutic agent can be achieved and the risk of systemic side effects can be minimized [10]. In horses, the commonly used intra-articular joint medications to treat OA are corticosteroids (Triamcinolone,

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Animal welfare/ethical statement: All the studies reported in the review article were conducted under animal experiment permits.

* Corresponding author at: Aziz Tnibar, The Equine Hospital, Jockey Club of Saudi Arabia., PO Box 26323, Riyadh 11486 Saudi Arabia.
E-mail address: aztnibar@gmail.com

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Methylprednisolone, Betamethasone, Dexamethasone), hyaluronic acid and polysulfated glycosaminoglycan.

A recent development in the treatment of OA is the use of autologous, regenerative and innovative preparations to achieve restoration of articular cartilage [11]. The main medications used are autologous conditioned serum, platelet-rich plasma, mesenchymal stem cells and gene therapy.

Until recently, there was a lack of available effective long-term medication for OA with a long-lasting efficacy needed, as most of the available therapeutic options only provide short-term mild-to-moderate effects [3,5,12,13]. As part of the OA-complex, elastoviscosity of the synovial fluid is abnormally low [14], and thus viscosupplementation has been implemented as part of the treatment for OA in humans [15,16], and horses [5,12].

As mentioned previously, there are several classical medication options for OA [3,5,12,13], and reflects that the majority of these options do not provide long-lasting management of OA. However, over the last decade, 2.5% Polyacrylamide hydrogel (PAAG) was introduced and received considerable interest in equine OA therapy, backed-up by highly promising results in the medication of OA in humans [17–20].

2. 2.5% Polyacrylamide hydrogel[1]: chemistry and attributes

The 2.5% PAAG is a non-toxic and non-immunogenic biocompatible polymer injectable hydrogel consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide [21,22]. Biocompatibility within soft tissues (Urology, reconstructive surgery, ophthalmology) has been demonstrated [23–25]. Also, 2.5% PAAG is a non-particulate, stable, homogenous gel similar to sodium hyaluronate gel in overall structure and tissue compatibility [26], but with a longer-lasting viscous effect, as well as non-biodegradable and non-migratory [21]. The 2.5% PAAG has also proven to have an excellent safety profile in humans validated through over more than 20 years of use for the augmentation of connective tissues in both urology and reconstructive surgery [25,26].

3. Clinical studies of 2.5% Polyacrylamide hydrogel

Over the last decade, 2.5% PAAG has gained considerable interest in equine OA therapy [27–34]. Clinical trials have investigated the effect of 2.5% PAAG on improving clinical signs of OA in horses [27–33], and an experimental trial have investigated its effects on induced OA in goats [35,36].

In the first clinical trial using intra-articular 2.5% PAAG in 43 horses (Warmbloods: 70%, Racing breeds: 19%, other breeds: 11%), older than 2 years, with OA located within only one joint (Metacarpo (metatarso) phalangeal: 93%; one of the carpal: 7%), horses were followed-up at 1, 3, 6, 12, and 24 months. The confirmation of OA was based on clinical evaluation, lameness abolished after intra-articular anesthesia and imaging (Radiography). Lame horses with severe radiographic abnormalities were also included in the study. The study was designed as a prospective multi-center clinical study. Efficacy of the treatment was evaluated by lameness examination of the affected joint, including response to flexion tests. Lameness grading [37] was performed at baseline, and at 1, 3, 6, 12, and 24 months. All horses were clinically assessed under similar circumstances by clinicians (one per center) different from the one who had originally examined and treated the horse, and unaware of the identity of the horse and whether joints were treated or not at 1, 3, 6, 12, and 24 months post-treatment. Safety of the joint treatment was evaluated through recording of any adverse reaction following joint injection. All horses received only one injection of 2.5% PAAG during the study. The first published report of this trial was about the 6 months follow up results [27]. Before treatment, the proportion of horses with lameness score 1, 2, 3, and 4 was 27.3%, 33.3%, 33.3%, and 6.1%, respectively. The estimated lameness improvement at 1, 3, and 6 months was 81%, 88%, and 87%, respectively. At 6 months, approximately 79% of horses were free of lameness [27]. At 24 months follow-up, 82.5% of horses were free of lameness and no side effects were observed related to the treated joints during the study period [30]. In this study, there was a significant decrease in lameness score from baseline to 1, 3, 6, 12, and 24 months ($P < .0001$) and a significant positive association with joint effusion ($P < .0001$). Estimates for Odds Ratio (OR) revealed that the effect of treatment increased over time (OR for lower lameness scores from month 1 to 24, relative to baseline, increased from 20 to 58). There were some study limitations including a low number of horses, the fact that it was a prospective non controlled clinical study, and the subjective assessment of joint distension. This was a multi-center study, which represented another study limitation due to several clinicians involved in the study, and the potential for inconsistency in application of the lameness grading scale among the clinicians and within clinicians at different examinations.

Tnibar *et al.* [29] performed a controlled prospective non-randomized clinical trial for the efficacy of 2.5% PAAG in horses older than 2 years with OA located within only one fetlock joint. Fetlock pain was confirmed using intra-articular anesthesia. OA signs were detected using radiography and/or Magnetic Resonance Imaging (MRI). Forty lame horses were enrolled, 20 (Warmbloods: 80%, other breeds: 20%) in each group. An intra-articular injection was performed with either 2 ml 2.5% PAAG or 10 mg Triamcinolone acetonide + 20 mg sodium hyaluronate (TA-HA). A clinician, different from the one who had originally examined and treated the horse, and blinded to the treatment, assessed lameness at 1, 3, and 6 months post-injection. Efficacy of the treatment was evaluated by lameness examination of the affected joint [37], including response to flexion tests. Safety of the joint treatment was evaluated through recording of any adverse reaction following joint injection. At 1 month post-injection, 55% of the horses in the 2.5% PAAG were free of lameness versus 15% in the TA-HA group. At 3 months post-injection, 65% of the horses in the 2.5% PAAG were free of lameness versus 40% in the TA-HA group. At 6 months post-injection, 75% of the horses in the 2.5% PAAG were free of lameness versus 35% in the TA-HA group. This study demonstrated that 2.5% PAAG significantly improved OA clinical signs when compared to horses treated with TA-HA ($P = .001$). The main study limitations included the fact that it was a controlled but non-randomized clinical study with a low number of horses.

Janssen *et al.* [28] investigated the effects of intra-articular use of 2.5% PAAG as a treatment for OA of the distal interphalangeal joint in 12 horses (11 Warmbloods, 1 Pony). The diagnosis was based on clinical signs associated with distal interphalangeal joint OA, presence of lameness with a positive response to intra-articular anesthesia, and the presence of distal interphalangeal joint osteoarthritic signs on radiography and/or MRI. All the horses had been lame for at least three months prior to injection. All horses had previously been treated with TA and HA and/or autologous conditioned serum. An intra-articular injection of 2 ml of 2.5% PAAG was performed. The clinical investigation, treatment and follow-up were carried out by an experienced orthopedic surgeon. None of the horses developed side effects. At six months post-injection, 8 of 12 (67%) horses were free of lameness, two were improved and two were non-responsive. The main limitations of the study included the fact that it was a prospective non controlled study with a low number of horses.

Bathe *et al.* [31] performed a prospective study on 20 sport horses non-responsive to treatment for proximal and/or distal interphalangeal joint OA. Lameness was associated with OA, diagnosed by diagnostic analgesia and radiography and/or MRI. All horses were persistently lame after previous corticosteroid treat-

ment. The average length of lameness was >15 months and grade was 3 of 10 at day 0. All horses were injected with 1 ml of 2.5% PAAG intra-articularly. In total, 18 horses were available for follow-up at a median of 12 months later. This revealed 12 of 18 returned to full function, 3 of 18 returned to a lower level, and 3 of 18 failed to improve. One horse was treated twice and one horse had a transient adverse reaction. The study limitations include the lack of a control group, however each case could act as its own control, as conventional treatments has always failed, and the low number of horses.

Another study investigated the use of 2.5% PAAG for the management of joint lameness in flat racing Thoroughbreds [32]. Forty-nine flat racing Thoroughbreds with carpal or metacarpophalangeal joint lameness were treated with a single injection of 2 mL of 2.5% PAAG, at a single training facility. Horses were selected from those presenting for routine veterinary clinical examination for lameness, using a modified American Association of Equine Practitioners lameness scale formatted for the study with a positive response to intra-articular anesthesia. Horses were assessed at day zero and followed up at weeks 1, 4, 12, and 24, post injection. Post injection complications were recorded throughout the entire study period. This study concluded that 2.5% PAAG was safe and a practical first-line treatment option for lameness associated with the metacarpophalangeal and carpal joints in Thoroughbred racehorses. The percentage of horses free of lameness at 4 weeks, 12 weeks and 24 weeks post-injection was 43%, 67%and 65%, respectively. Results of the final statistical model showed a statistically significant improvement in lameness grades at weeks 1 ($P < .01$), 4 ($P < .001$), 12 ($P < .001$), and 24 ($P < .001$) when compared to week 0.There were some obvious limitations to the study including the lack of randomization, the lack of blinding, and the lack of controls which represents the main weakness of the study.

A prospective double-blinded positive control study was performed to compare the efficacy of 2.5% PAAG in the management of middle carpal joint lameness in Thoroughbreds against treatments of triamcinolone acetonide (TA) or sodium hyaluronate (HA) [33]. A total of 31 flat-racing Thoroughbreds with lameness (grade 1-3/5) localized to the carpus by intra-articular analgesia were selected. Horses were randomly assigned for intra-articular treatment with either 2 ml of 2.5% PAAG, 12 mg TA or 20 mg HA (followed by two further intravenous treatments of 40 mg, at weekly intervals in the HA group only), by a treating veterinarian. All horses were rested for 48 hours post-treatment and then re-entered an unaltered training regimen. Subsequent examinations at 2, 4, and 6 weeks were performed by a blinded examining veterinarian for all groups, while horses treated with 2.5% PAAG were monitored for 12 weeks for recurrence of lameness. Significantly more joints treated with 2.5% PAAG were free of lameness (83%) at 6 weeks compared to TA (27%; $P = .007$) and to HA (40%; $P = .04$). There was no significant difference between TA and HA groups at any time. All the joints treated within 2.5% PAAG that were free of lameness at 6 weeks (10/12) were still free of lameness at 12 weeks. In conclusion, treatment with 2.5% PAAG led to statistically superior results compared to TA and HA in the management of selected middle carpal joint lameness in flat-racing Thoroughbreds, with therapeutic effects persisting up to 12 weeks. Several study limitations were apparent in this study and include a relatively low numbers of horses, and short study duration. Pre-study power calculations were not used but the study design allowed for the study's continuation until a statistically significant effect was achieved. Outcome parameters, although based on clear scoring systems, were subjective.

A randomized controlled pilot study using an experimental OA model in the stifle joint (Transection of medial collateral ligament, bisection of medial meniscus and partial-thickness cartilage incisions of medial tibial plateau) in goats has shown that 2.5% PAAG significantly improved the lameness caused by OA, with 75% of the cases becoming sound by four months post-treatment evaluation [35,36]. The study limitations included the fact that it was a pilot study with a low number of animals. Non clinical results of this study will be presented in the section "Mechanisms of action of 2.5% Polyacrylamide hydrogel."

The following table summarizes the clinical studies performed with 2.5% PAAG in horses.

4. Safety of 2.5% Polyacrylamide hydrogel

There is evidence that 2.5% PAAG has proven to be safe, over 10 years of use for the treatment of clinical equine OA [27–33], and also in experimental OA in goats [35,36]. At 24 months follow-up, no side effects (Joint effusion, warm joint, lameness) were observed in the treated joints during the study period [30]. A controlled prospective study comparing 2.5% PAAG with TA combined with HA in horses with fetlock OA has shown no adverse-effects [29]. Another report has shown no adverse reactions in any of the 12 horses treated with 2.5% PAAG for distal interphalangeal joint OA [28]. In a study reported by Bathe et al. [27] involving 20 horses treated with 2.5% PAAG for proximal/distal interphalangeal joint OA, only one horse had a transient adverse reaction (Not specified by the authors) after two treatments. Another study has concluded that 2.5% PAAG is a safe and practical first-line treatment option for lameness related to the metacarpophalangeal and carpal joints in Thoroughbred racehorses [32]. In a prospective double-blinded positive control study performed to compare the efficacy of 2.5% PAAG in the management of middle carpal joint lameness in Thoroughbreds against treatments of TA or HA, none of the horses developed any adverse reactions to 2.5% PAAG [33]. A randomized controlled pilot study using an experimental OA model in goats has shown that no adverse reactions were seen following intra-articular injection of 2.5% PAAG [35,36].

However, as with any others product when used incorrectly (for example using a non-sterile technique) some safety concerns may arise. Products with 2.5% PAAG come in a sterile pre-loaded luer-lock syringe that reduces the risk of infection and increases safety even further.

Based on the available published studies, the complication rate for intra-articular injection of 2.5% PAAG is estimated to 0.004 % and the only reported complication was transient (Not specified by the authors).

5. Mechanisms of action of 2.5% Polyacrylamide hydrogel

Histopathological observations on joint tissue from horses [38] have demonstrated that a part of the 2.5% PAAG becomes integrated within the synovial membrane. To elucidate mechanisms of action of 2.5% PAAG in OA joints, a randomized controlled blinded study was conducted on an OA stifle model in goats [35,36]. This study was conducted involving goats with induced OA on the left stifle joint. OA was surgically induced by the transection of the medial collateral ligament, the bisection of the medial meniscus at its midpoint and partial-thickness incisions of the cartilage of the medial tibial plateau. Goats were allowed free exercise, and 3 months after surgery they were randomly divided into 2 groups: Treatment group which received 2.5% PAAG and control group which received saline solution. 2.5% PAAG and saline solution were injected intra-articularly (1 ml). All goats were videotaped on a treadmill for lameness examination. MRI was performed prior to surgery, as well as 3, 4, 5, and 7 months post-surgery. Seven months post-surgery, gross pathology and histopathology, including immunohistochemistry for nerve endings, were performed on both stifles. Joint capsule elasticity of the stifles was measured in both groups. Following euthanasia, a small piece from the lateral and medial sides of

the right and left stifle joint of each goat was removed for evaluation of joint capsule elasticity.

MRI showed reduction followed by stabilization of OA lesions (intra-articular bony and cartilaginous lesions) after 2.5% PAAG treatment [35,36]. At gross pathology, 2.5% PAAG was seen present within the joint cavity and adhering to synovial membrane. Histopathology showed that intra-articular 2.5% PAAG injection added to the thickness of the synovial membrane by allowing angiogenesis, collagen and synovial cell increase; 2.5% PAAG was integrated into the synovial membrane. The hyperplasia of the inner capsule and/or synovial membrane was more significant in the treated goats than in the control goats. In a histological report, a similar tissue reaction was seen in horses with osteoarthritic joints that were injected with 2.5% PAAG, including cases treated two years earlier with 2.5% PAAG [38]. From the goat model, nerve endings were seen in a similar pattern, whether the goats had had good or minor clinical results from the 2.5% PAAG gel injection or were in the control group (saline only). In all goats used for nerve staining, the nerves were intact with normal morphology and numbers, and no evidence of neurotoxicity was observed [35,36]. Joint capsule elasticity investigation showed that treated stifles had a higher elasticity when compared to control stifles [35,36]. By integrating the synovial membrane, which may probably decrease the joint capsule and the joint stiffness, 2.5% PAAG might relieve pain of the OA joint. This theory is supported by clinical observations in the clinical trials in horses [29,30], where OA joints that responded well to 2.5% PAAG also have resolution of the previous positive response to joint flexion.

This study presented preliminary observations regarding the mechanisms of action of 2.5% PAAG on OA joints [35,36]:

1 Histopathology and joint capsule elasticity suggest that 2.5% PAAG, by acting on synovial membrane (increasing joint capsule elasticity), may reduce overall joint capsule stiffness, a major source of pain in OA [39]. This represents a mechanical mechanism of action.
2 Gross pathology demonstrated that this gel was present within the joint cavity in all the treated animals protecting the articular surface and providing viscosupplementation. 2.5% PAAG is a non-degradable and highly viscous product [21] and thus might contribute to protecting the articular surface of an osteoarthritic joint, and hence it could reduce and stabilize the OA lesions. This represents a mechanical mechanism of action. In addition, histopathology demonstrated that 2.5% PAAG had no effect on articular cartilage and subchondral bone.
3 MRI and gross pathology revealed stabilization of OA lesions in 2.5% PAAG treated goats, possibly caused by 2.5% PAAG's high viscosupplementation and non-degradability.
4 No signs of intra-articular neurotoxicity or fibrosis were observed.
5 Intra-articular treatment with 2.5% PAAG did not have any influence on hematology, biochemistry, or acute phase proteins.

In addition, in a biomolecular study, equine synovial fluid was analyzed for cytokine and/or chemokine expression using ELISA method before and 6 weeks after intra-articular injection of 2.5 % PAAG as treatment for OA [40]. Ten adult horses with fetlock OA were included in the study. Two samples per horse were done, so in total n = 20 samples (>1 ml) of synovial fluid were collected from these joints. Samples were collected before and 6 weeks after intra-articular injection with 2 ml of 2.5 % PAAG. The study concluded that there was no evidence of significant elevation in any of the pro-inflammatory cytokines like IL-6 and IL-1 in the OA fetlocks treated with 2.5 % PAAG [40].

Fig. 1 presents the current knowledge of the mechanisms of action of 2.5% PAAG.

Precise characterization of the mechanism-of-action of 2.5% PAAG on OA joints has not yet been fully established; however, preliminary observations from the experimental study in goats [35,36] and horses [38] emphasize: 1- The mechanical nature of the mechanisms of action of 2.5% PAAG. 2- The major role of synovial membrane and joint capsule, as well as the long-acting viscosupplementation of 2.5% PAAG for the treatment of OA. In addition, no effects on pro-inflammatory cytokines have been observed [40]. These are new concepts in the treatment of OA.

6. Not all Polyacrylamide hydrogels are the same

There are several PAAG available, and although often considered to be the same material, there are clear differences in composition, manufacturing and injection technique, as well as abilities to interact with surrounding tissues [41]. These characteristics ultimately determine the safety and efficacy profiles of each gel formulation, which should therefore not be used interchangeably.

The 2.5% PAAG[1] is produced by a patented technology called In-line Cross-Linking Technology (ILX Technology), forcing water molecules between the cross-linked polymers of polyacrylamide (CAS No. 9003-05-8) that provides the gel with exceptional molecular stability and the ability to retain its viscoelastic properties *in situ*.

Having undergone extensive analysis for safety, efficacy, toxicology, and manufacturing, 2.5% PAAG was first approved by the New Zealand regulatory authorities in mid-2019 for veterinary treatment of joint lameness in horses, followed by Australia in 2020. In 2020, US FDA (Food and Drug Administration), has approved 2.5% PAAG as a medical device. In addition, FEI (Federation Equestre Internationale) has not included 2.5% PAAG on their controlled or prohibited substance list.

Less is known about the performance or safety of other PAAG products, but widespread and largely indiscriminate use of these products in some countries has caused serious long-term complications, mainly infection and granulomatous reactions [42,43].

Investigations into 4% PAAG hydrogel[2] [44] were based only on improvement in lameness score, as a measure of clinical success. This product is often mistaken for 2.5% PAAG, however, not only is the polyacrylamide concentration different but also the manufacturing process. Furthermore, 4% PAAG also contains silver ions, whose interaction within a joint is not known. In comparison, studies on 2.5% PAAG have consistently used 'complete resolution of lameness' as a measure of the primary outcome.

In addition, the 2.5% PAAG has been shown to be the most stable of the 11 PAAGs evaluated by Narins et al [41].

7. Practical tips for using 2.5% PAAG

Based on clinical experience with 2.5% PAAG and interpretation of the literature available, the following practical tips for using 2.5% PAAG are recommended:

- Indicated in early stage to chronic OA.
- 2.5% PAAG is a soft (low viscolastic) hydrogel, so it can also be injected using 18 to 20 gauge needles.
- Before using 2.5% PAAG, lameness must be abolished or significantly improved by intra-articular anesthesia.
- It is of paramount importance to inject this hydrogel into the articular space and not into the synovial membrane, or else the gel, unlike a fluid, will form a bulging into the synovial membrane and will not diffuse and act properly within the joint space.
- The recommended doses to treat osteoarthritic joints in a 500 kg horse are:

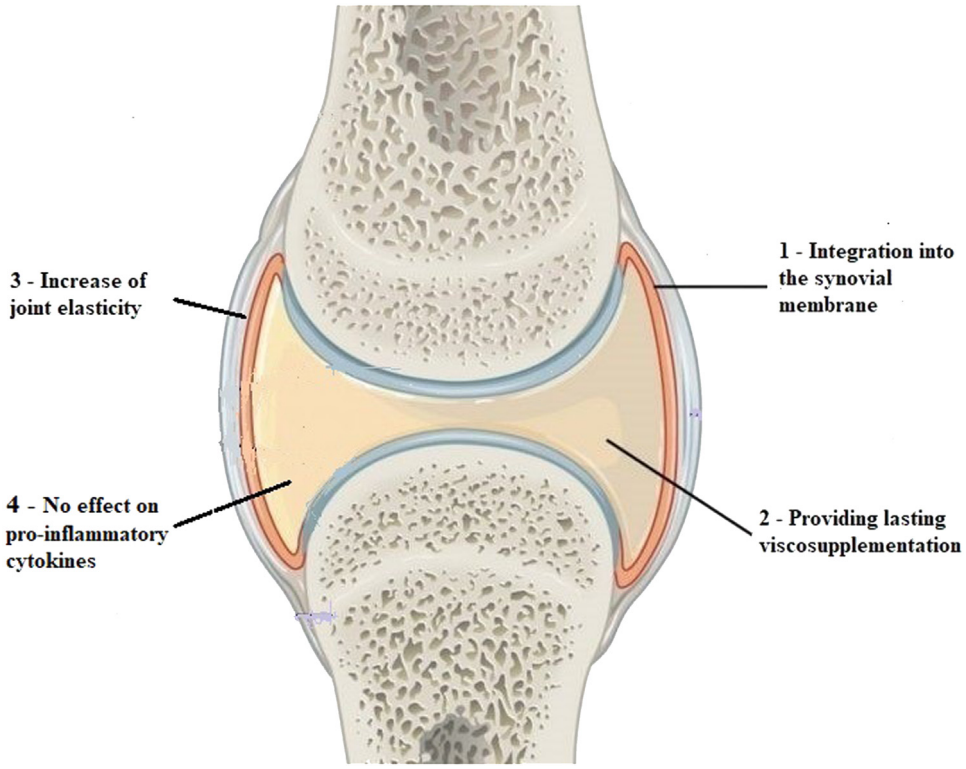

Fig. 1. Current knowledge of the mechanisms of action of 2.5% PAAG.

Joint	Recommended dose of 2.5% PAAG (ml)
Distal interphalangeal	2
Proximal interphalangeal	1
Metacarpo/Metatarso-Phalangeal	2
Antebrachiocarpal	2
Middle carpal	2
Elbow	2-3
Shoulder	2-3
Tarsometatarsal	1
Distal intertarsal	1
Talocrural	2-3
Femoropatellar	2-3
Lateral/Medial Femorotibial	2-3
Hip	2-3
Temporomandibular	1
Cervical facet joint	1
Thoracic/Lumbar joint	1

• Full response to the treatment begins as early as 1 week post-injection and may, in rare cases, need a few weeks to few months for a full response.

• If the diagnosis of OA is accurate and 2.5% PAAG is correctly injected, then only one injection is typically required. If there is no response to 2.5% PAAG, the diagnosis and/or the injected technique should be revised. It has been stated that the effect of 2.5% PAAG on OA might occur mainly during the first month after treatment and lasts and increases progressively until 6 months, with a stabilization between 6 and 24 months [30]. If there is an incomplete response to 2.5% PAAG, it is advised to wait for a repeated injection based on this statement.

• The horse should not have received any intra-articular medication within the two months prior to treatment with 2.5% PAAG.

• 2.5% PAAG should be administered alone. No study supports its administration in association with other intra-articular drugs.

• Post-injection management: In the vast majority of cases, one week of box rest and hand walking is required, and then horses can progressively resume their normal activity.

• Based on its mechanism of action, 2.5% PAAG is not listed as doping product to date.

8. From horses to humans

Building on the highly positive results of 2.5% PAAG for equine OA, it was suggested to study its effects in humans [17–20].

An observational proof-of-concept cohort study has been conducted at baseline and after 4, 7, and 13 months in order to establish an initial estimate of the effectiveness of intra-articular injections of 2.5% PAAG[3] for the treatment of knee OA symptoms in 84 patients (48 females) [17]. All the patients included in this study received intra-articular treatment of 3 ml 2.5% PAAG. The patients received up to two treatments within one month and attended clinical follow-up visits at 4, 7, and 13 months after the initial treatment. There were no restrictions regarding analgesics. There were statistically and clinically significant reduction in the WOMAC (Western Ontario and McMaster Universities Arthritis Index) pain after 4 months ($P < .0001$). Similar results were found in WOMAC stiffness, physical function, and WOMAC total [17]. Improvements were maintained throughout the observational period [17]. These results suggest beneficial effects from an intra-articular injection of 2.5% PAAG on knee OA symptoms, even long-term (1 year) [17]. This study has several inherent weaknesses. Firstly, the study was observational with no control group. Further, the reasons for the substantial amount of missing data were not documented. Also, information about the amount, type, dosage or frequency of analgesics taken by the participants during the observation period was not collected. Nevertheless, the results are encouraging as there are no treatments available with long lasting effects on knee OA symptoms.

Histological appearance of the synovial membrane after treatment of knee OA with 2.5% PAAG was reported in one case [45]. Nine months after treatment, biopsies showed the same type of synovial augmentation as seen in horses treated with 2.5% PAAG for OA [38].

The safety of intra-articular 2.5% PAAG for the treatment of knee OA symptoms was investigated in a retrospective case series with a long-term follow-up between 4 months and 7 years [18]. Inclusion criteria were painful knee(s) with confirmed radiological signs of OA. All the patients received intra-articular injection of 3 ml 2.5% PAAG into the knee joint cavity under ultrasound guidance. Each patient was interviewed and examined for evidence of adverse events. Of the 91 patients (46 females, 45 males) evaluated, the majority (73%) had not experienced adverse events or discomfort [18]. Patients reported mostly a sensation of distension (n = 15) and worsening of pain from treated knee (n = 7). Of the fifteen patients who experienced a sensation of distension of the knee joint after the treatment, in 14 (93%) this passed within days to weeks. To treat the knee pain, 2 cases received either analgesics or arthrocentesis. Neither intra-articular infections nor allergic reactions were reported [18]. This safety assessment study has several inherent weaknesses; especially the recall bias due to the retrospective nature of the adverse event reporting is a limitation. The authors concluded that this retrospective case series of patient-reported safety, clinical examination, and medical record reviews, found no significant incidence of adverse events or serious adverse events related to the intra-articular treatment with 2.5% PAAG for the relief of knee OA pain and disability [18].

Recently, a study was carried out and its primary objective was to evaluate the efficacy and safety of a single injection of 6 ml intra-articular PAAG over 52 weeks on knee symptoms in participants with moderate-to-severe knee OA [19]. Patients with symptomatic (WOMAC A1≥ 2/4 Likert) and radiographic (Kellgren-Lawrence grade 2 to 4) knee OA were consented into a prospective open-label study. Primary outcome of the study was the change in WOMAC pain subscale (normalized to 100) after 12 weeks. Secondary outcomes were WOMAC stiffness and function subscales, Patient Global Assessment of disease impact (PGA) and proportion of OMERACT-OARSI (Outcome Measures in Arthritis Clinical Trials-Osteoarthritis Research Society International) responders. Forty nine patients (31 females) received intra-articular 2.5% PAAG, with 48 and 46 patients completed the 12 and 52 weeks assessments respectively. There were statistically and clinically significant reductions in WOMAC pain after 12 weeks ($P < .0001$) that were sustained to 52 weeks ($P < .0001$). Similar benefits were found for WOMAC stiffness, function and PGA. After 12 weeks, 64.6% of the patients were OMERACT-OARSI responders, and this was maintained at 52 weeks [20]. No serious adverse events (mainly arthralgia and joint swelling) were seen in the initial 12 weeks with 2.5% PAAG [19]. No new adverse events were seen between 12 and 52 weeks. This study concluded that 2.5% PAAG can be delivered in a single 6 ml intra-articular injection and it suggests that the good clinical effects (significant reductions in WOMAC pain, WOMAC stiffness, function and PGA) at 12 weeks were maintained at 52 weeks in patients with moderate to severe knee OA. The main study limitations included the fact that it was a non-randomized, non-controlled clinical study with a low number of patients. In this study the authors used a single 6 ml injection, while comparable good clinical results were obtained in a previous study where the patients received up to two treatments of 3 ml within one month and attended clinical follow-up visits at 4, 7, and 13 months after the initial treatment [17].

In humans, the joints that have been injected with the 2.5% PAAG are: Knee, hip, elbow, metacarpo-phalangeal and interphalangeal in hands and feet, sesamoid-metatarsal and temporo-mandibular.

9. Discussion

Over the last decade, clinical trials have demonstrated that intra-articular 2.5% PAAG is highly effective, lasting and safe for the treatment of equine OA [27–33]. No other single medical treatment for OA has such prolonged efficacy.

Most of these clinical studies were presenting some limitations, and were either prospective non-controlled or controlled non-randomized studies with low number of horses [25–32]. Recently, a double blinded positive control study in horses demonstrated that significantly more joints treated with 2.5% PAAG were free of lameness (83%) at 6 weeks compared to TA and to HA [33], however the study duration was relatively short. Similar results have previously been reported in an international multi-center prospective non controlled study (82.5% free of lameness horses at two-year follow-up) [33]. A randomized controlled study using an experimental OA model in goats was performed however it was a pilot study with low number of animals. The appropriate choice in study design is essential for the successful execution of biomedical studies. All the studies with 2.5% PAAG are interventional studies (prospective) and are specifically tailored to evaluate direct impacts of this treatment on OA. Each study design has specific outcome measures that rely on the type and quality of data utilized. Additionally, each study design has potential limitations (controlled randomized study, number of horses, outcome parameters subjectivity...) that are more severe and need to be addressed in the design phase of the study. Further randomized controlled clinical studies need to further investigate the effect of this new technology on equine OA.

In horses, all the reported studies to date investigated the efficacy of 2.5% PAAG in naturally-occurring OA, as it was more applicable in the clinical setting than an experimental OA. However, evaluating 2.5% PAAG in an experimental OA study in horses (e.g., carpal chip model in horses) might contribute to a better understanding of the effect of this new medication on OA. Interest in developing OA models in the horse is driven as much by the clinical importance of the disease in this species as by its utility as a translational model for human disease. Both idiopathic primary OA and posttraumatic OA related to athletic use occur in the horse, and the challenges and expectations that exist regarding early diagnosis and the development of effective treatments that allow return to full function are similar to humans.

A systemic review and network meta-analysis assessing the effectiveness of HA and PAAG in horses with OA has concluded that PAAG is an effective alternative therapy, with a long period of action in reducing lameness in horses with OA [34].

To our knowledge, there is no evidence in the literature that is provided as drawbacks to using 2.5% PAAG in the treatment of OA in horses.

The 4% PAAG hydrogel[2] [44] is often mistaken for 2.5% PAAG, however, not only is the polyacrylamide concentration different but also the manufacturing process. Furthermore, 4% PAAG also contains silver ions, whose interaction within a joint is not known.

Two studies have compared 2.5% PAAG to steroids and sodium hyaluronate for the treatment of OA in horses. A controlled prospective non-randomized clinical trial has compared 2.5 PAAG with Triamcinolone acetonide and sodium hyaluronate for the treatment of OA in horses [29]. This study demonstrated that 2.5% PAAG significantly improved OA clinical signs when compared to horses treated with TA-HA. A recent prospective double-blinded positive control study has compared the efficacy of 2.5% PAAG in the management of middle carpal joint lameness in Thoroughbreds against treatments with steroids (Triamcinolone acetonide)

Table 1
Comparative table of clinical trials investigating the efficacy of 2.5% PAAG in horses.

Reference	Study design	N	Age range(year)	Type of activity (%)	Time point (month)	Joint (%)	Free of lameness (%)
Tnibar el al, 2012 [27]	Multi center prospective	33	2-15	-Sport horse: 65 -Racing 19 -Other: 16	1,3,6	Fetlock: 90 Carpus: 10	1 m: 3 m: 6 m: 70
Tnibar el al, 2015 [30]	Multi center prospective	43	2-15	-Sport horse: 65 -Racing 19 -Other: 16	1,3, 6, 12,24	Fetlock: 93 Carpus: 7	1 m: 59 3 m: 69 6 m: 79 12 m: 81 24 m: 82.5
Janssen et al, 2012 [28]	Prospective	12	4-14	-Sport horse: 92 -Other: 8	1, 6	Distal IP: 100	6 m: 67
Bathe et al, 2016 [31]	Prospective	20	NA	-Sport horse: 100	12	Proximal & Distal IP: 100	12 m: 67
De Clifford et al, 2019 [32]	Prospective	49	3-7	Racing TB: 100	0.25, 1, 3, 6	Carpus: 100	1 m: 43 3 m: 67 6 m: 65
De Clifford et al, 2021, [33]	Prospective double blinded positive controlled	31	2-6	Racing TB: 100	0.5, 1, 1.5, 3	Carpus: 100	1.5 m: 83 3 m: 83

Abbreviations: N, Number of horses; TB, Thoroughbred; IP, interphalangeal; m, month.

or sodium hyaluronate [33]. This study has demonstrated that treatment with 2.5% PAAG led to statistically superior results compared to triamcinolone and sodium hyaluronate in the management of selected middle carpal joint lameness in flat-racing Thoroughbreds.

Based on the highly positive results of 2.5% PAAG for equine OA, its effects were recently studied in humans and the results of the reported clinical studies are very encouraging [17–22].

To elucidate mechanisms of action of 2.5% PAAG in OA joints, to our knowledge, only few studies were conducted [35,36,38, 40]. Preliminary observations on mechanisms of action of 2.5% PAAG emphasize [35,36]: (1) The mechanical nature of the mechanisms of action (Integration into the synovial membrane, increase of joint elasticity possibly reducing overall joint capsule stiffness, providing lasting viscosupplementation which contributes to protecting articular surfaces). (2) The major role involving synovial membrane and joint capsule, as well as the long-acting viscosupplementation in the treatment of OA. (3) No effect on pro-inflammatory cytokines [40]. Studies also suggest that these positive effects occur in the absence of intra-articular neurotoxicity or fibrosis.

The effect on the synovial membrane and joint capsule and the long-acting viscosupplementation represent new concepts in the management of equine OA. However, to fully understand the mechanisms of action of this new technology, this area should be the focus of further studies.

Footnotes

1 Arthramid Vet, Contura International A/S, DK-2860 Soeborg, Denmark.
2 Noltrex Vet, Nucleous Provets, Kennesaw, GA 30144, USA
3 Arthrosamid, Contura International A/S, DK-2860 Soeborg, Denmark.(Table 1)

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