Evaluation of an intra-articular carboxymethylcellulose crosslinked hydrogel in horses with osteoarthritis

EVALUATION OF AN INTRA-ARTICULAR CARBOXYMETHYLCELLULOSE CROSSLINKED HYDROGEL IN HORSES WITH OSTEOARTHRITIS

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Abstract

Numerous treatment strategies have been proposed to prevent osteoarthritis or to minimize its clinical signs in sport horses. Carboxymethylcellulose (CMC), a derivative of cellulose, that belongs to the class of cellulose ethers, is utilized in veterinary medicine for diverse applications. This study aimed to to test the efficacy of a commercial CMC hydrogel in reducing lameness due to osteoarthritis. Sixteen horses were included in the treated group and fourteen in the control group. Horses in the treated group received an intra articular injection of CMC, while those in the control group received an intra articular injection of hyaluronic acid and 12 mg/joint of triamcinolone acetonide (TA). The horses were re-evaluated after 15, 30 and 90 days.

At 90 days post-baseline, horses of treated group presented a statistically significant treatment success rate (75%), while control group did not exhibit any statistically significant improvement (8%). The improvements of clinical lameness manifestation in horses treated intra-articularly with CMCpointed out a possible alternative in effectively and durably treating equine osteoarthritis.

Keywords: Intra-articular treatment, fetlock joint, lameness

1. Introduction

Osteoarthritis (OA) represents one of the most widespread and debilitating condition affecting horses, significantly impacting the economic viability of the equine industry [1]. A variety of treatment modalities including physical, biological and pharmaceutical interventions have been proposed to either prevent the onset of OA or to minimize clinical signs of pain (lameness), reduce joint deterioration, and extend the competitive career of equine athletes [2].

CMC is an anionic polymer compound with a molecular weight ranging from thousands to millions Da and is one of the derivatives of cellulose belongs to the class of cellulose ethers. Nowadays, it is utilized in veterinary medicine for diverse applications, such as lubricant, wound healing agent, and barrier material to prevent postoperative adhesions[3]. Due to its low toxicity and immunogenicity, alongside favorable biodegradability and biocompatibility [4], CMC gels have garnered extensive research attention, emerging as one of the most promising materials for use as drug carriers in clinical settings [5]. In human orthopedics, in vitro studies indicate that CMC enhances chondrocyte differentiation and promotes extracellular matrix synthesis [6].

This prospective open-label comparative clinical efficacy study was design to investigate the efficacy of a CMC crosslinked hydrogel compared with TA 12 mg/joint and sodium hyaluronate (HA) 20 mg/joint for managing naturally occurring fetlock joint lameness in horses. The underlying hypothesis was that there would be a significant difference between treatments throughout the duration of the study.

2. Materials and Methods

2.1 Ethical approval

The study obtained favorable approval from the ethics committee of the University of Bologna

Prot. n. 99874/2024.

2.2 Materials, tested product group (TPG)

The sterile, isotonic CMCgel for intra articular use was supplied by Innate s.r.l (Italy). The gel consists in an association of cellulose gum and croscarmellose (crosslinked) in a physiologic buffer.

The composition is: cellulose gum, sodium croscarmellose, sodium chloride, sodium phosphate monobasic dihydrate, dibasic sodium phosphate dodecahydrate, sodium hydrate 30% solution.

The pre-filled syringe contains 3 ml of product.

2.3 Materials, control group (CG)

12 mg/joint TA (Kenacort, Bristol-Myers Squibb S.r.l.) in combination with 20 mg/joint of high molecular weight HA (Alien, ACME S.r.l.). These two products are intended to be administered together in a single injection; however, their physicochemical stability has not been evaluated.

2.4 Methods

To be included in the study, horses were required to be at least 2 years old and weigh less than 650 kg. Horses should exhibit lameness originating from osteoarthritis (OA) affecting the forelimb fetlock joint. The lameness had to persist for a minimum duration of four weeks, in addition flexion test must be interpreted as positive as well as intra-articular diagnostic analgesia. Other inclusion criteria were the presence of radiological signs of OA of the affect joint, besides radiological confirmation that no osteochondral fragments or fractures were present.

Exclusion criteria comprised several conditions: horses displaying lameness attributed to joint disease affecting multiple joints, or presenting fractures or osteochondral fragments; those subjected to local or systemic administration of nonsteroidal anti-inflammatory drugs, glycosaminoglycans, hyaluronic acid, corticosteroids, or other antiarthritic drugs, shock wave therapy, acupuncture, or any homeopathic or oral supplements in the 30 days prior to the study. Additionally excluded were lactating or pregnant mares, as well as horses with signs of systemic diseases or infectious septic arthritis.

Post-inclusion removal from the study cohort could occur if any horse failed to adhere to inclusion criteria during the study period, specifically concerning compliance with prescribed treatments and durations or if serious adverse effects attributable to the drug manifested; or if the owner withdrew the horse from participation without necessitating justification.

Horses that met enrollment criteria received a lameness score according to the American Association of Equine Practitioners (AAEP) scoring system.

2.4.1 Patient management and welfare

The horse owner was required to consent to the study protocol and sign an informed consent agreement. All veterinarians involved were instructed on study protocol and standard operating

procedures by Quality Control group of Department of Veterinary Medical Sciences, University of Bologna (Italy). The horses involved in the trial remained under the care of their respective owners.

2.4.2 Visit protocol and interventions

Patients were screened for eligibility based on previously mentioned criteria. The veterinary investigators performed the examinations as follows: each horse was trotted on a loose lead both in a straight line and a circle. Upon identifying the affected limb, further examination for swelling, heat, and pain was performed to localize and score the lameness. A flexion test was performed. The examination proceeded if the investigator considered the findings consistent with fetlock joint disease. After this, mepivacaine 100 mg/joint was aseptically injected into the articular space of the affected joint. The horse was then reevaluated to assess any change in lameness severity. X-ray examination of the joint was then conducted.

The researchers randomly assigned patients to one of two groups using a mobile phone application system. Veterinarians involved in the study were not blinded to the treatment.

Depending on their group assignment, horses received either 12 mg/joint TA combined with 20 mg/jointof high molecular weight HA or CMC prefilled syringe as a single intra-articular injection via an 18–21 gauge needle after aspiration of 2-3 ml of synovial fluid.

Post injection, horses were hand-walk for days 1 to 5, up to 20 minutes on the walker for days 6 to 15, then from 16 to 20 if serviceably sound were allowed to jog up to a mile a day before returning to full work. The horses were re-evaluated after 15, 30 and 90 days at identical locations and surfaces to assess lameness degree and reaction during fetlock flexion tests.

2.5 Data analysis

No pre-study power calculations were used. Admission scores for lameness and reaction to fetlock flexion were compared between groups using non-parametric Kruskal-Wallis test. Success was defined as a complete resolution of the variable over time. Data were analyzed using Chi-Squared tests for statistical comparisons with significance set at $P.< 0.05$. and, point estimates for the difference in percentage of successful treatments and associated 95% Confidence Intervals.

3. Results

3.1 Recruitment

Horses were recruited from April 2024 to September 2024. Re-examination by the veterinarian was at a 15, 30 and 90 days after baseline.

3.2 Baseline data

Relevant baseline data for participating horses are provided in Table 1. Table 1 summarizes the baseline characteristics of the study population, including age, sex, body weight, affected joint, and initial AAEP lameness grade. No significant differences were observed between groups at baseline There were no statistically significant differences observed in any of the baseline parameters between groups.

A total of 30 horses were enrolled in the study, all of which met the inclusion criteria, with an age range of 3 to 12 years and a mean age of 7 years. No horses were excluded throughout the course of the study.

Only one horse in the TPG experienced an adverse reaction, specifically severe joint effusion was observed for a few days before regressing spontaneously.

	TPG	**CG**	
Number	16	14	P values (95% CI)
Age range (mean)	3-12 (6.7)	3-12 (7)	P>0.05
Thoroughbred (%)	7 (43,75%)	8 (57,14%)	P>0.05
Standardbred (%)	3 (18,75%)	0 (0 %)	P>0.05
Pleasure horses	6 (37,50%)	6 (42,86%)	P>0.05
Lameness range (mean)	1-3 (1,5)	1-3 (1,5)	P> 0.05
Reaction to fetlock flexion (mean)	0-2 (1,5)	0-2 (1)	P>0.05

Table 1. Pre admission variables per group

3.3 Outcomes

At 15 days, a significant higher proportion of horses in the CG were classified as treatment success rate for the considered variables (90%), whereas 20% of horses in the TPG group met the criteria for treatment success; however, this difference was not statistically significant when compared to the control group.

At 30 days, both groups show statistically significant treatment success rate (75%).

At 90 days post-baseline, TPG presented a statistically significant treatment success rate (75%), while CG did not exhibit any statistically significant improvement (8%).

To improve the clarity of treatment outcomes, clinical success rates at each evaluation point are summarized in Table 2.

Timepoint	TPG	CG
Day 15	20% (3/15)	90% (13/14)
Day 30	75% (12/16)	75% (10/14)
Day 90	75% (12/16)	8% (1/13)

Table 2. Clinical success rates at each follow-up point in the treated group (TPG) and control group (CG)

3.4 Adverse events

Only one horse in the TPG develop an adverse reaction that appeared to be possibly product-related, based on the timing of the event and clinical manifestations observed (severe joint effusion of the injected joint was observed for a few days), which subsequently regressed spontaneously. The incidence of adverse drug-related events was not statistically different between groups.

4. Discussion

This report describes the results of a randomized clinical trial involving 30 lame horses, aimed at evaluating the comparative clinical efficacy of CMC compared to 12 mg/jointTA combined with 20 mg/joint HA.

A significant proportion of the CG exhibited an immediate improvement in lameness and fetlock flexion, which was maintained for about 30 days before gradually diminishing. These results suggest a favorable initial clinical response within this patient population. It is likely that corticosteroids provide their potent anti-inflammatory and analgesic effects for a limited period of time following intra-articular injection; however, the incorporation of hyaluronic acid may confer additional advantages [7].

Conversely, the TPG horses displayed minimal improvement for the considered variables after 15 days, but then substantially improve after 30 days, with this improvement remaining stable for up to 90 days.

A possible explanation for this result is due to the fact that CMC serves as an effective filler for cartilage defects [6], but is subject to thixotropic behavior. Hydrogels composed of CMC can alter their mechanical properties temporarily under appropriate mechanical stimuli [8]. It is likely that factors such as load, body temperature and controlled exercise facilitate changes in viscosity, contributing to the progressive improvement of the lameness. CMC acts as a mechanical barrier, isolating the tissues from one another [9], and probably the key to improving this mechanical barrier lies in the thixotropic behavior. However, the fact that certain viscosupplementation devices required a period of time to exert their effects is not novel; in fact, this has already been demonstrated in horses for polyacrylamide gel [10,11].

Additionally, while the antibacterial properties of CMC have been proved [12], its anti-inflammatory effect, assessed by monitoring the release of IL-1, at 14 days, appears to be limited [6].

The observed delayed clinical response in the treatment group receiving CMC is consistent with the proposed mechanical and viscoelastic behavior of the hydrogel. Unlike corticosteroids, which exert a rapid anti-inflammatory effect through direct modulation of inflammatory mediators, CMC appears to act primarily as a mechanical barrier and joint lubricant, modifying the intra-articular environment gradually. This interpretation aligns with the temporal pattern observed in the present study, where horses in the CMC group showed minimal response at day 15, but significant and sustained improvement by day 90. Understanding this mechanism is crucial for guiding clinical expectations and suggests that when using CMC, follow-up periods should be sufficient to capture its full therapeutic potential.

The horses included in the TPG received a sterile, isotonic crosslinked CMC gel for intra-articular use. A study conducted by human medical researchers on in-vitro and in-vivo (rabbit knees) effects of a synthetic amidic derivative of CMC on chondrocyte regeneration offered promising outcomes [6]. In vitro results suggested that CMC ameliorated the chondrocyte differentiation as well as the synthesis of extracellular matrix components. In-vivo results led to the conclusion that CMC hydrogel stimulates cartilage healing [6].

Moreover, despite the rapid degradation rate typically observed with common hydrogel visco-supplementation when compared to cartilage regeneration, a cellulose derivative appears to mitigate these limitations due to its elevated polysaccharide molecular weight, which ensures reduced

degradability and consequently prolongs its intra-articular effectiveness [6]. While long-term studies to exclude further tissue degeneration are not available, a human surgical study [13] demonstrates that the use of intra-articular CMC -polysaccharide B following anterior cruciate ligament reconstruction showed superior results for pain control, hemarthrosis, and knee movement gain in the postoperative period when compared to patients from the non-treated group.

In the present study, the reason for using cross-linked CMC and not a polymerized CMC gel is predicated on the potential adverse effects of polymerization, which may destroy certain physiological characteristics of the hydrogel and suffer from several uncontrollable factors like temperature, pH and reaction time. In contrast, hydrogels prepared via cross-linking with a natural polymer exhibit good biodegradability and mild and controllable reaction conditions, which render this approach the most ideal and promising synthesis method for CMC gels [14].

The joint selected in this study is the fetlock joint, that is characterized as a highly loaded motion joint that frequently sustains injuries in athletic horses; thus, it serves as a representative model for common joint disorders in equines [15].

Radiographic imaging was employed exclusively to exclude cases involving fetlock joint diseases associated with osteochondral fragments or fracture-related complications from this study. Although other diagnostic methods (like CT or MRI) could enhance the classification of joint pathologies, the authors considered radiography sufficient to exclude from the study cases of OA clearly related or complicated by fractures or osteochondral fragment and therefore include just cases of OA mainly caused by inflammatory and degenerative joint diseases alone.

The selection of cases with a minimum lameness duration of four weeks helped empirically in excluding horses that might have suffered from an acute joint inflammation or mild synovitis. Consequently, this selection process focused on those cases that are more indicative of chronic conditions, consistent with some degenerative intra-article changing, confirmed by radiographic images. One of the main limitations of this study is the relatively small sample size (30 horses), which may limit the generalizability of the results. However, conducting clinical trials in equine medicine, especially with strict inclusion and exclusion criteria, and involving performance horses, poses logistical, ethical, and economic challenges that frequently restrict large-scale recruitment. Despite this limitation, the consistent findings across multiple time points strengthen the validity of the observations and suggest that even with a limited sample size, the treatment effect was robust and not likely due to random variation.

Another limitation of the present study is the relatively short follow-up period of 90 days. However, 90 days represents a commonly used endpoint in equine lameness studies to detect meaningful improvements or treatment failures, and it is relevant for informing decisions in clinical practice, particularly in performance horses.

An additional limitation is the lack of blinding for the treating veterinarians, which may introduce observer bias, despite the use of standardized clinical evaluation methods.

According to the findings of this study at 90 days post-initial treatment, horses in the TPG showed a significant clinical improvement, while the CG horses didn't demonstrate a clinically significant improvement. This observation raises the hypothesis that the previously observed benefits from intra-articular administration TA and HA may have diminished in efficacy within this latter cohort.

Further research should aim to validate these findings in larger, multicenter populations and to explore the long-term effects of CMC on joint preservation and function. Studies investigating synovial fluid biomarkers, imaging-based assessment of cartilage condition, and comparison with other emerging viscosupplementation agents would provide valuable insights.

5. Conclusions

While the data derived from this study are based on a limited number of horses and do not provide specific information about the effect of CMC hydrogel on equine joint biological components, nor its interactions and specific effect on inflammatory response and joint degeneration, they offer promising clinical results. The improvements of clinical lameness manifestation in horses treated intra-articularly with CMC pointed out a possible alternative in effectively and durably treating equine OA, encouraging further dedicated research and investigation.

6. Conflict of interest

No competing interests have been declared.

7. Source of funding

This work was supported by Innate s.r.l. (Italy) who supplied the tested product

8. Acknowledgements

We would like to thank Dr. Pierluigi Capodagli and Dr. Gabriele Rosati for the active patient recruitment.

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