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Safety of intra-articular gold micro-implants in horses – a randomized, blinded, controlled experimental study

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Abstract

Arthrogenic pain is a common problem in equids. Frequently used treatments such as systemic NSAIDs or intra-articular steroids can lead to severe side effects if used repeatedly. Gold has been used since ancient times to treat a variety of conditions and has anti-inflammatory and immunomodulating properties. Results from clinical and in vitro studies suggest that local gold might provide a safe and effective alternative to alleviate articular pain. In particular, gold micro-implants have been proposed to this end, but it is unknown if and how healthy joints react to this treatment.

The aim of this study was to evaluate the safety of gold micro-implants (Berlock®-Micro-Implants) mixed with hyaluronic acid and injected into the middle carpal joint of 9 healthy horses. Each horse was treated in one carpus and sham-treated on the contralateral.

Lameness, carpal temperature increase, swelling and reactions to joint palpation were observed in both treated and sham-treated carpi in the first week following needle arthroscopy and treatment. Significant differences between treated and sham-treated carpi were found only for mechanical nociceptive thresholds 4 days after treatment. Higher thresholds were found in treated joints compared to sham-treated joints. None of the outcome measures selected in the present study indicated systemic or local adverse effects specifically attributed to gold micro-implants.

In the absence of systemic and local adverse reactions to gold micro-implants, the results of the present study support future clinical trials to test the pain-relieving efficacy of this treatment modality in sub-acute or chronic articular inflammatory processes in equine patients.

Key Words: gold; horse; intra-articular; micro-implants; Berlock®

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1. Introduction

Osteoarthritis (OA) is a common cause of pain and lameness in equids, it is a career limiting disease in both sport and leisure horses. The most frequently used treatments are oral or intravenous NSAIDS, or intra-articular steroids [1]. Despite their proven efficacy severe side effects might be encountered in case of prolonged therapy. This limits their applicability and safe alternatives are needed.

Gold has been used since ancient times for its anti-inflammatory and immunomodulating properties [2, 3]. Originally conceived as a way to continuously stimulate acupuncture points [4], periarticular gold implants, several millimeters in diameter, have been used to obtain long-lasting pain control in OA [5-7]. Experimental studies showed that gold ions, the active component in aurotherapy, are released from implanted gold by dissolucytosis, an extracellular process performed by macrophages. This can only occur when the implant is at least 20 µm in size as smaller particles are phagocytized and no gold ions are released [8, 9]. Gold micro-implants (20-40 µm) tested in vitro and in vivo in experimental animals have been patented for direct application in inflamed tissues, including joints (Berlock®-Micro-Implants (BMI), Goldtreat ApS) [10]. For administration, BMI are mixed with hyaluronic acid (HA) in order to guarantee an adequate distribution. The local release of gold ions, with their anti-inflammatory activity, is enhanced in inflamed tissue, thus providing the rationale for intra-articular use of gold implants in equine OA. To date, no safety data for intra-articular BMI application can be found in the literature. Prior to initiating clinical trials focusing on the pain-relieving efficacy of BMI in equine OA, the occurrence of major adverse reactions needs to be ruled out. Only then will it be acceptable to recruit and treat client-owned horses with BMI.

The aim of this study was to evaluate the safety of intra-articular BMI mixed with HA in healthy horses. In each horse, one middle-carpal joint was treated with BMI mixed with HA while the other was treated with HA alone as a control. In order to detect any potential systemic or local adverse effects arising from the BMI treatment, several outcome measures were evaluated over a period of 3 months.

2. Materials and Methods

2.1 Study design and ethical statement

The study, designed as a blinded, randomized, controlled, prospective experimental trial, was approved by the Cantonal Authority for Animal Experiments, Canton Bern and Vaud, Switzerland (BE105/12). All procedures were carried out under strict rescue analgesia regimen. If any horse showed signs of pain, reaching a score >9/36 on the composite pain scale (Appendix 1), or a lameness degree greater than 2/5 at any time during the study, further clinical evaluations were performed and analgesic treatment administered according to specific clinical signs. Flunixin (1 mg/kg IV) was administered in case of lameness (>2/5) and/or swelling (score ≥ 2) (Appendix 2). In case of persistent (>3 days) moderate or acute severe joint pain or swelling, joint flushing and intra-articular administration of morphine 0.1 mg/kg under general anesthesia was planned.

2.2 Animals

Nine healthy horses (8 females, 1 castrated male) belonging to the Swiss Institute of Equine Medicine (ISME) without any signs of carpal pathology were included in the study. Horses had a median age of 10.2 years (range 10-18) and a median bodyweight of 580 kg (range 498-645 kg). Breeds included 3 Franche Montagnes horses, 3 Swiss

Warmbloods, 2 Trotters and 1 Hannoverian (Table 1). Eight horses were stabled together in an open barn with access to an outdoor pen, they were fed 3 times a day with hay and the resting area was bedded with straw, the access to water was unlimited. The remaining horse was stabled individually in a box bedded with shavings and was exercised daily on a walker or through paddock-turnout. This horse was fed haylage three times a day, with ad libitum access to water.

2.3 Inclusion criteria

Prior to inclusion in the study all horses underwent full clinical and hematological examinations. Radiological evaluation of the carpal joints was performed, with three radiographs taken of both carpi (Dorsomedial-palmarolateral oblique, dorsolateral-palmaromedial oblique and flexed latero-medial). Only horses that had normal bloodwork, showed no radiological abnormalities, no lameness and no signs of pain were included in the study.

2.4 Outcome measures

Outcome measures, as detailed below, were recorded by 2 investigators blinded to the treatment (NM and SW), before the treatment and up to 3 months thereafter according to the schedule shown in Table 2.

2.4.1 Pain score

A modification of the multifactorial numerical rating composite pain scale published by Bussières et al. (2008) [11] was used to evaluate pain and guide administration of rescue analgesia (Appendix 1).

2.4.2 Lameness and symmetry index

Subjective lameness examination was performed with the horses trotting in a straight line on a loose rope on a hard, even surface. A score from 0 to 5 (where 0: clinically sound and 5: non-weight bearing lameness, details in Appendix 2) was attributed to each horse before and after 1 minute of carpal flexion. Objective lameness evaluation was performed using data collected from a poll-mounted three-dimensional accelerometer processed according to published algorithms [12, 13] using MatLab (The MathWorks, Inc.) by custom-made software (Equigait) [14]. The generated symmetry index was interpreted using previously established values for non-lame horses [15]. Symmetry indexes of below 0.82 and greater than 1.18 indicated right and left forelimb lameness' respectively.

2.4.3 Carpal variables

Joint swelling was scored using a 0-3 numerical rating scale (Appendix 2).

Carpal circumference was measured using a standard measuring band at the distal aspect of the accessory carpal bone.

Temperature of the carpus was quantified from frontal thermographic images of both carpi, collected with an infrared camera (InfraVet OptiRes D, VarioCAM, Infra Tech GmbH, Dresden, Germany) and analyzed with custom software (Exam Professional 5.8, InfraMedic GmbH, Germany). Horses were restrained in stocks for 30 minutes before the images were taken to avoid artifacts from ambient temperature changes and locomotion. The camera was positioned 1 m from the carpi for all readings. Temperature differences between the two carpi were calculated for each session. Differences of ≥1.25°C between the carpi were considered clinically relevant [16].

Reactions to palpation of the middle carpal and carpometacarpal joints were scored using a 0-3 numerical rating scale (Appendix 2).

Mechanical nociceptive thresholds (MNT) were determined using a calibrated hand-held algometer (FDN 200, Wagner Instruments, Greenwich CT, USA) with a rubber tip 1 cm^2 in diameter and a maximal capacity of 20 Kg. Pressure was applied perpendicular to the skin surface on 7 predetermined anatomical landmarks along the front limb and was immediately released when the horse showed first signs of discomfort (e.g. lifting of the limb, twitching of the skin) or the pressure applied had reached 12 Kg/cm^2 (cut-off value). A rate of pressure increase of approximately 2 $Kg/cm^2/s$ was used for each measurement. Selection of landmarks was based on a previous report [17] and modified to assure evaluation of the area of interest (Appendix 2). Landmarks were evaluated in random order twice; a mean threshold value was calculated for each landmark and used for analysis.

Passive joint range of motion was measured as described by Liljebrink and Bergh (2010) [18] using a calibrated goniometer which could be fixed in place and read afterwards using a standard set-square with integrated protractor.

Ultrasonographic evaluations of the synovial lining were performed by one of two examiners blinded to the treatment (SW, NM) using a portable ultrasound (M-Turbo; SonoSiteTM, Bothell, WA, USA) with a C11x/8-5 MHz linear transducer. Images of the joint-space between the radiocarpal bone and third carpal bone (dorsomedial) and the joint-space between the intermediate carpal bone and the third carpal bone (dorsolateral) were obtained in both B-Mode and Doppler-Mode. Each middle carpal joint was scored in accordance with the human sonographic definition of synovitis or synovial hypertrophy as per OMERACT 7 [19] on a 0-3 numerical rating scale. Scores were defined as 0: normal synovial lining; 1: mild hypoechoic synovial thickening; 2: moderate hypoechoic synovial thickening; 3: marked hypoechoic synovial thickening.

2.4.4 Needle arthroscopy

Needle arthroscopy was performed to allow thorough evaluation of the intra-articular architecture of both middle-carpal joints immediately prior to treatment administration and 3 months thereafter. Horses were premedicated with 0.03 mg/kg acepromazine IM (Prequilan, Arovet AG, Switzerland) and sedated with a bolus of 0.05 mg/kg methadone (L-Polamivet, MSD Animal Health GmbH, Switzerland) and 0.05 mg/kg romifidine (Sedivet, Boehringer Ingelheim, Germany) IV. Sedation was maintained by IV continuous rate infusion of 0.05 mg/kg/h methadone and 0.05 mg/kg/h romifidine in Ringer Lactate (Laboratorium Dr. G. Bichsel AG, Switzerland) titrated to effect. Following routine preparation for surgery, lidocaine (Lidocaine 2% Streuli, G. Streuli & Co. AG, Switzerland) was injected subcutaneously (2 ml) and intra-articularly (20 ml) to distend the joint during endoscopy. Subsequently, a semi-rigid, 1.2 mm (outer diameter), 10° optic needle endoscope with a working length of 100 mm (BioVision, Golden, CO) was used to evaluate the middle carpal joint and standard images were obtained. In case of abnormal findings, a thorough description of the visualized altered structures was provided. All examinations were carried out by the same experienced orthopedic surgeon (SW). Time to perform the procedure was recorded. Flunixine meglumine (1 mg/kg IV) (Fluniximin, Dr. E. Graeub AG, Switzerland) was administered postoperatively. The horses were confined to a box stall for 2 days before returning to their normal stabling and routine.

2.4.5 Blood and synovial fluid analysis

Venous blood was collected for routine hematology, biochemical evaluation and to determine serum amyloid A (SAA) concentration.

Synovial fluid samples were collected into plain tubes. An aliquot was diluted 1:20 in isotonic NaCl 0.9% and the number of nucleated cells immediately determined by a

blinded investigator using a Neubauer counting chamber on a Leica microscope with 40 x magnification. The remaining synovial fluid was centrifuged at 1200 g to pellet cells and cell-free supernatant frozen in 1 ml aliquots and stored at -80°C until further analysis.

Levels of IL-1β, TNF-α, Aggrecan Chondroitin Sulfate and Substance P in the synovial fluid were determined according to the manufacturers protocol using the following commercially available ELISA kits: GSI Equine IL-1β ELISA Kit- Synovial Fluid (Genorise Scientific, Inc, USA), GSI Equine TNF alpha ELISA Kit- Synovial Fluid (Genorise Scientific, Inc, USA), Aggrecan Chondroitin Sulfate 846 Epitope (CS846 ELISA, IBEX Pharmaceuticals Inc., Canada), Substance P EIA Kit (Cayman Chemical Company, Ann Arbor, MI).

2.5 Treatment

Each horse underwent bilateral carpal treatment on day 0: immediately following needle arthroscopy and synovial fluid sampling, one joint (randomly determined) was injected with 1 vial of BMI mixed with 2 mL HA (treated) while the other was injected with 2 mL HA alone (sham-treated) (Table 1).

One vial of BMI consisted of 10 mg of gold (approximately 36.000 particles, 20-40 µm in size). Prior to administration, hyaluronic acid (20 mg, Ostenil, Trb Chemedica S.A., Switzerland) was aseptically injected into the vial and shaken until the gold particles were adequately suspended. All horses were treated within 2 consecutive weeks.

2.6 Statistics

Analysis was performed with the statistical computing package R. Descriptive statistics were calculated for all outcome measures and medians and ranges reported. The data were analyzed at individual time points using a linear regression model and over time

using a linear regression model with horse specific random effects on the treatment difference. All models were stratified by horse to acknowledge that each horse was both treated and sham-treated and a two-sided alpha level of 0.05 for statistical testing was used.

3. Results

Main results are summarized in Table 3.

3.1 Pain score

No signs of pain were observed for any horse at any time point during the study. The highest score recorded on the composite pain scale was 3/36.

3.2 Lameness and symmetry index

Subjective and objective lameness examinations showed mild to moderate degrees of forelimb lameness in 5 out of 9 horses in the first week following treatment. Horses 3, 5, 6 and 7 showed lameness on the sham-treated limb while horse 4 was lame on the treated limb. However, only two of the horses (horse 4 and 5) needed to be treated with a short course of NSAID for lameness and swelling of the carpal and metacarpal region on days 4, 5 and 6. After 3 days, the treatment was discontinued as both swelling and lameness had resolved. On day 30 all horses were sound. On day 60, two horses (2 and 5) showed mild lameness, both on the sham-treated limb. A thorough orthopedic examination and diagnostic perineural blocks localized pain to the hoof in both horses. On day 90 all 9 horses were sound. Since the conclusion of the experimental phase 3 years ago, horses included in the study have been closely observed for 1 to 3 years. None of the horses was lame due to a clinical relevant carpal pathology.

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3.3 Carpal variables

Local swelling of the carpi was noticed in all horses at least during the first week after arthroscopy. Five horses showed a similar degree of swelling in both carpi; 2 horses (horses 4 and 5) showed more swelling in the treated carpi whilst in a further 2 horses (horses 3 and 8) the sham-treated carpi were more swollen.

Compared to baseline on average all horses had an increase in carpal circumference in the first week after treatment in both carpi. Although overall this appeared more pronounced in the sham-treated joints, there was no statistically significant difference compared to the treated joints and all carpi were within 0.5 cm difference in circumference 90 days after treatment.

Five of the 9 horses showed no significant temperature difference between the treated and sham-treated carpi at all times. In horse 1 the treated carpus had a relative increase of 3.5°C on day 30. Horse 3 had a higher reading in the sham-treated carpus on day 4 (10.25 °C) and day 6 (4.6 °C), as did horse 8 on day 6 (2.8 °C) and horse 6 on day 90 (1.8 °C).

In 2 horses (horses 1 and 2) palpation of the carpi was not painful at any time. Horses 8 and 9 were scored 1 at a single time point, whereas horses 5 and 6 were scored 1 repeatedly on both carpi. Horses 3 and 7 were scored 1 or 2 on palpation of the sham-treated carpus and horse 4 at palpation of the treated carpus at several time points during the first 14 days.

There were no significant differences in MNTs between treated and sham-treated limbs except for landmark 3 on day 4: treated joints had statistically significant higher MNTs than sham-treated joints (P=0.009) (Fig.1).

Six out of 9 horses showed no significant changes in range of motion during the 90 day period. Two horses showed a temporary, reduced range of motion compared to baseline, one in the treated limb (horse 4) and one in the sham-treated limb (horse 3), which had returned to baseline by day 90.

Three out of 9 horses showed no ultrasonographic joint changes. Horse 7 had a score of 1 in the left middle carpal joint before treatment only. Horses 2, 8 and 9 showed a score of 1 on both treated and sham-treated carpi at different time points and horses 4, 5 and 6 had a score of 1 on the sham-treated carpus at different time points. No horse had ultrasonographic changes on day 90.

3.4 Needle arthroscopy

In 4 joints of the 18 joints examined on day 0 clear images could not be captured due to hemorrhage. On day 0 a small full thickness lesion was noted on the weight bearing surface of the fourth carpal bone in horse 8 and of the second carpal bone in horse 9. Both defects were unchanged at the second arthroscopy (day 90). Time to perform the procedure ranged between 30 and 90 minutes (average 63.3 minutes) on day 0, and between 30 and 55 minutes (average 38.9 minutes) on day 90. At 90 days, gold particles could be seen in the region of the lateral palmar intercarpal ligament of the left middle carpal joint in horse 9.

3.5 Blood and synovial fluid analysis

Compared to baseline, hematology, serum chemistry and SAA values showed no significant difference throughout the study. No values were outside the normal range at any time point.

Cell count in all synovial fluid samples was below 100 cells/μl at all time points, therefore differentiation was not performed as inflammatory effusion was not suspected. For CS846 and Substance P all values were within the normal range, while for IL-1β and TNF-α all values were below or within the normal range. No differences between treated and sham-treated joints were found.

4. Discussion

The current study aimed to evaluate the safety of intra-articular gold micro-implants mixed with HA in horses. Nine healthy experimental horses without any signs of pathology in the carpal region had their middle carpal joint unilaterally treated with BMI mixed with HA while the contralateral side was sham-treated with HA alone.

Our findings indicate that no major clinically relevant differences between the treated and the sham-treated joints were detected during the whole study period for any of the outcome measures evaluated.

In order to recognize as many potential adverse effects of intra-articular BMI on joint homeostasis, a large number of outcome parameters were included. Several diagnostic imaging techniques lend themselves for this purpose in vivo. We chose ultrasonography for the serial evaluation of the synovial membrane, as this method is easy to perform, non-invasive and provided a real time detection of pathological intraarticular changes [20]. Furthermore, needle arthroscopy was chosen for real-time macroscopic assessment of the target structures as it has been proposed as a minimally invasive technique allowing direct visualization without the need for general anesthesia. A thin

needle endoscope was used to visualize the middle carpal joints in standing horses as previously described [21, 22]. The findings of the present study question just how minimally invasive this procedure is, as a relatively high incidence of lameness, swelling and increased local sensitivity to palpation and pressure were observed during the first week following surgery. Despite the fact that optimal intra-operative sedation and analgesia was provided and that the smallest endoscope available was used, adequate patient cooperation was vital for surgery to take place without complications. Complications (intra-articular hemorrhage, articular and periarticular trauma) resulting in the signs observed in the first week following surgery, were more frequent in those horses that were least cooperative during the procedure. For future application, a mechanical fixation system might help to keep the correct limb positioning over time, with the carpus slightly lifted and flexed. Furthermore, well instructed personnel should be available to optimize the procedure while reducing risks of trauma.

The administration of HA in combination with BMI represents a further risk factor for side effects of treatment. Occasional adverse reactions to synthetic HA ("joint flare"), lasting 3 to 5 days, have been described 1 to 3 days after administration in horses and humans [23, 24]. As the viscosity of HA was needed to provide adequate distribution of the gold microimplants [10] in the treated joints, HA (and not saline) was also used as the control in the sham-treated joints. Hyaluronic acid as a confounding safety factor could therefore be largely excluded.

Significant differences between treated and sham treated limbs were found only for mechanical nociceptive thresholds as measured by algometry. Higher thresholds were found in the treated carpi than in the sham-treated carpi on day 4, on landmark 3, corresponding to the dorsal aspect at the mid-body of intermediate carpal bone. Testing of

mechanical nociceptive threshold is a method commonly used to assess local sensitivity to mechanical stimulation in humans [25]. Using a calibrated spring-loaded instrument it is possible to quantify the pressure necessary to evoke pain when applied to a predefined anatomical landmark. Higher thresholds correspond to lower pain responses. In an experimental model of carpal osteoarthritis, mechanical nociceptive threshold testing of the carpal region has been previously validated in horses and shown to be useful in differentiating between affected and non –affected limbs as well as to quantify postoperative nociceptive sensitization [17]. In the present study, lower baseline threshold values in the carpal area were found compared to the previously published reference values; this discrepancy can likely be explained by the decreased rate of pressure increase applied (approximately one fifth of the previously adopted rate in the current study). This rate was chosen as the operator (NM) was able to provide more reliable results. Similar rates have previously been described in human studies [25, 26].

In addition to the higher MNTs found in the treated carpi, other local variables, such as temperature, swelling, reaction to palpation and carpal circumference showed a similar clinical trend, with lesser adverse reactions being detected in the treated limbs. This may be interpreted as early postoperative analgesic and/or anti-inflammatory effect of gold in the treated joints. Further clinical studies are necessary to confirm this clinical impression.

Besides the clinical outcome measures, a panel of synovial molecular biomarkers, each reflecting specific intra-articular processes, was selected to assess articular health in the present study. The pro-inflammatory cytokines TNF-alpha and IL-1β together with Substance P, a neuropeptide known to accompany articular pain and inflammation, would be expected to increase in the face of BMI induced articular inflammation [20, 27]. In

case of direct or indirect damage from BMI to cartilage matrix homeostasis, the epitope 846 of chondroitin should also increase, reflecting enhanced aggrecan turn-over [28, 29]. No increases were noted in this study. Arthrocentesis was first performed 7 days after treatment in order to avoid further mechanical insults to the operated joint and possible partial removal of gold particles. The results of routine joint fluid analyses at 4 time points throughout the study showed no clinically or statistically significant differences between the treated and the sham-treated carpi and no deviations from baseline values. The absence of increase in cell counts and of catabolic, pain and inflammatory biomarkers observed in this study indicates that BMI did not adversely affect synovial fluid homeostasis in healthy equine joints.

Although gold particles were seen arthroscopically in 1 horse at 90 days the distribution of potentially toxic particles was not further evaluated. Previous studies [30] investigating the permeability of synovium in healthy and diseased human knees showed that synovial micro-vessels are permeable to a molecule radius of up to 1.75-40 nm. While gold particles 1-2 nm in size have been shown to be cytotoxic [31], no toxic effects on vital organs could be demonstrated after intra-articular injection of 13 and 50 nm gold particles in an arthritis model in rats [32]. In the latter study, joint swelling and histological changes of the joint decreased significantly when treated with repeated nano-gold injections. As nano-gold particles are promptly phagocytized by macrophages repeated administration is necessary to obtain a persistent anti-inflammatory effect [8]. In contrast, one single injection of micro-gold particles (as in the case of BMI) is expected to allow permanent release of gold ions, thus establishing longer-lasting local therapeutic levels [8, 10, 33].

The gold particles used in the present study had a diameter of 20-40 μm and were injected into healthy joints. Migration outside of the joint and a systemic effect are therefore

unlikely. Furthermore, the low concentration of gold ions potentially leaving the joint [8] is not expected to cause systemic toxic effects. No changes in SAA concentrations, a reliable and very sensitive marker of systemic inflammation and infection [34, 35] were seen over the study period. As the first blood sample for SAA determination was collected 7 days following treatment, we might have missed acute changes of this parameter.

General signs of pain were monitored by use of a multidimensional pain score, previously validated in an equine model of experimentally induced arthritis [11] and slightly modified for the purposes of the present study. The highest score attributed to any horse at any time point was 3/36, indicating minimal or no systemic pain signs throughout the study attributable to systemic adverse reactions of BMI. These findings support the absence of both systemic and extensive local inflammatory effects after BMI injection.

The present study was limited to a follow up period of 90 days. This duration was considered sufficient for adverse reactions to BMI treatment to become manifest. Furthermore, all horses included in the study have been under continuous supervision since the end of the study. They have been regularly reevaluated for a minimum of 1 year up to 3 years and none of the horses ever showed lameness due to a carpal pathology. Ex-vivo histological articular tissues examination would have provided stronger evidence of safety, but would have needed a terminal study design. A further limitation of the present study is the low number of subjects included. Indeed, in order to conclusively demonstrate absence of adverse effects in studies on medications or implants for human use, considerably higher subject numbers are required.

While a higher number of horses would have been helpful to draw more reliable conclusions, the robust study design and the fact that each horse acted as its own control make it unlikely that a higher number of horses would have altered the results.

5. Conclusion

Over the primary study period of 90 days we were unable to determine systemic or local detrimental effects of a single administration of gold micro-implants injected with HA into the middle carpal joint of healthy horses. Given the absence of acute adverse findings during the primary study period as well as during the long-term clinical follow-up of 1-3 years, the results of the present study indicate that the treatment is safe and thus support the performance of clinical trials testing efficacy in established sub-acute or chronic inflammatory processes.

Declarations

List of abbreviations

OA: Osteoarthritis **NSAID**: non-steroidal anti-inflammatory drug; **BMI**: Berlock®-Micro-Implants, **HA**: hyaluronic acid; **MNT**: Mechanical nociceptive threshold; **SAA**: serum amyloid A; **IL-1β**: interleukin 1 beta; **TNF-α**: tumor necrosis factor alpha

Ethics approval

This study was approved by the by the Cantonal Authority for Animal Experiments, Canton Bern and Vaud, Switzerland (BE105/12).

Availability of data and material

The dataset supporting the conclusions of this article is included within the article and its additional files.

Additional files

Additional file 1 (.pdf): Appendix 1, Composite Pain Scale

Additional file 2 (.pdf): Appendix 2, Local Outcome Measures and Lameness data collection sheet

Competing interests

The Berlock®-Micro-Implants (BMI) and the hyaluronic acid used in this study were sponsored by GOLDTREAT ApS, Denmark. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Authors contribution

SW, SR, VG and CS conceived the study, participated in its design and coordination and reviewed the manuscript. NM, SW and AR were responsible for the clinical care of the horses and for data collection. SK and NM carried out the laboratory analysis and both interpreted the data obtained. NM and CS drafted the manuscript. All authors have read and approved the final manuscript.

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Figures Legend

Figure 1: Box and whiskers plots representing the medians (midline), 75% and 25% percentiles (upper and lower quartiles, respectively) and ranges (upper and lower whiskers)of mechanical nociceptive thresholds (MNTs) measured on the dorsal aspect of the mid-body of the intermediate carpal bone (landmark 3) of treated joints (dark grey) and sham-treated joints (white). * Significant difference between treated and sham-treated joints were detected 4 days after treatment administration.

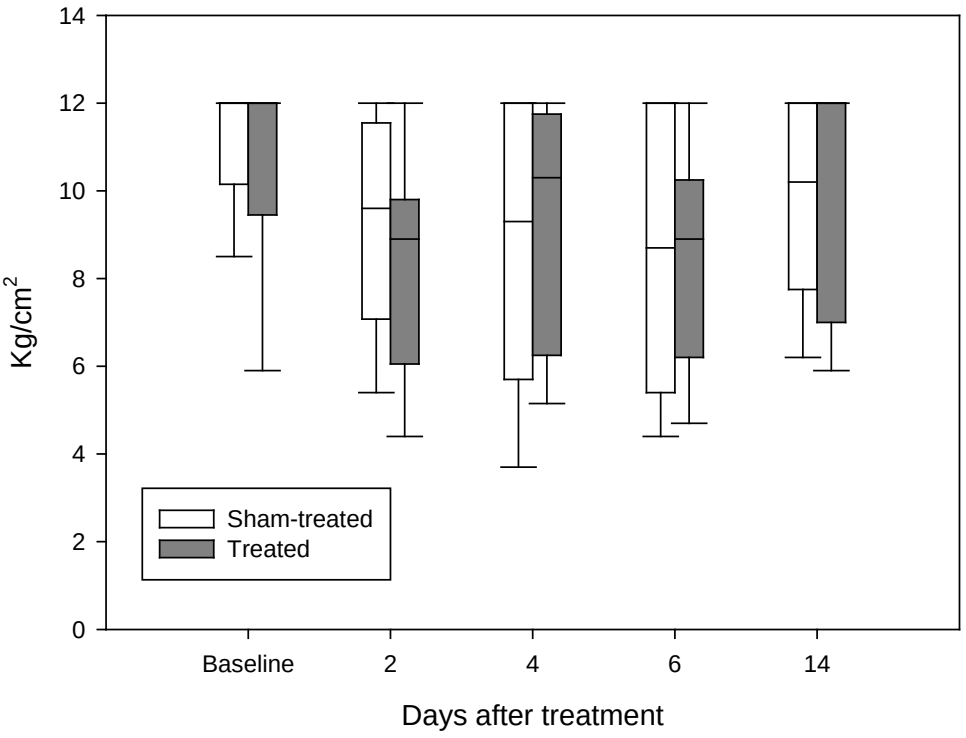

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Table 1: Demographic data and random treatment assignment

Horse	Breed	Age (years)	Gender	Body weight (kg)	Treatment	
1	Hannoverian	19	gelding	600	sham-treated	treated
2	Trotter	10	mare	498	sham-treated	treated

3	Franche Montagnes	10	mare	617	treated	sham-treated
4	Franche Montagnes	11	mare	615	treated	sham-treated
5	Trotter	10	mare	547	treated	sham-treated
6	Swiss Warmblood	13	mare	615	sham-treated	treated
7	Swiss Warmblood	11	mare	583	treated	sham-treated
8	Swiss Warmblood	14	mare	618	sham-treated	treated
9	Swiss Mountain Horse	10	mare	604	treated	sham-treated

Breed, age, gender and body weight of the horses included in the study and their treatment. Treated: Berlock® Micro-Implants mixed with hyaluronic acid; Sham-treated: hyaluronic acid alone

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Table 2: Outcome parameters assessment schedule

Study Phase	Study Day	Week	CPS	LLP	BS	SFS	US	RX	NA	T
Pretreatment	-14 to -7	-1/-2	x	x	x			x		
Treatment	0	0				x	x		x	x
Follow-up	1	1	x							
	2		x	x						
	3		x							
	4		x	x						
	5		x							
	6		x	x						
	7		x		x	x	x			
	14	2	x	x						
	30	4	x	x	x	x	x			
	60	8	x	x						
End of Study	90	12	x	x	x	x	x	x	x	

CPS: Composite Pain Score; LLP: Lameness and local pain assessment, see Appendix 2; BS: Blood sampling; SFS: Synovial Fluid sampling; US: Ultrasound examination; RX: Radiography; NA: Needle Arthroscopy; T: intra-articular treatment (Berlock® Micro-Implants mixed with hyaluronic acid) or sham-treatment (hyaluronic acid alone)

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Table 3: Main results

		Baseline	Week 1	Day 14	Day 30	Day 60	Day 90
MNT 2 treated	(kg/cm^2)	10.9 (7,12)	9.5 (4.9, 11.3)	10.3 (6,12)	10.6 (7.8,12)	10.6 (6.2,12)	11.7 (6.7,12)
MNT 2 sham	(kg/cm^2)	10.5 (6.9,12)	9.1 (5.2,11.5)	10.3 (7.2,12)	10.6 (8.9,12)	11 (8.9,12)	11.1 (6.9,12)
MNT 3 treated	(kg/cm^2)	12 (5.9, 12)	9.4 (4.7,10.4)	12 (5.9,12)	11.3 (7.9,12)	11.8 (7.8,12)	10.3 (6.3,12)
MNT 3 sham	(kg/cm^2)	12 (8.5, 12)	8.5 (4.9,12)	10.2 (6.2,12)	10.9 (8.6,12)	12 (9.4,12)	10.6 (7.7,12)
MNT 5 treated	(kg/cm^2)	11 (7,12)	9.5 (5.6,12)	9.2 (6,12)	10.3 (5.8,12)	10.6 (7.6,12)	10.7 (5.7,12)
MNT 5 sham	(kg/cm^2)	10.5 (5.3, 12)	8.7 (5,11.6)	9.7 (6.7,12)	10.4 (8,12)	11.1 (9.1,12)	10 (6.8,12)
Carpal flexion treated	(°)	26 (16,35)	23 (17,40)	22 (14,39)	22 (15,43)	21 (16,37)	20 (17-23)
Carpal flexion sham	(°)	26 (16,34)	21 (15,30)	22 (13,33)	22 (14,30)	21 (17,27)	20 (14,25)
Circumference treated	(cm)	31 (29,34)	32 (30,35)	32 (30,34)	31 (29,34)	32 (29,34)	31 (30,34)
Circumference sham	(cm)	31 (30,33)	32 (30,34)	32 (30,34)	31 (29,33)	31 (30,34)	32 (30,34)
Symmetry Index	(score)	1.01 (0.9,1.22)	1 (0.7,1.75)	1.09 (0.65,1.65)	0.87 (0.75,1.27)	1.1 (0.52-1.37)	1 (0.87,1.18)
Aggrecan treated	(ng/mL)	9716 (5476, 20629)	8220 (5226,21569)		6229 (3416,13156)		8439 (3149,30277)
Aggrecan sham	(ng/mL)	6276 (5340, 24079)	8734 (4816,50844)		10775 (5798,37405)		8400 (3252,15263)
IL-1 beta treated	(ng/mL)	0.126 (0.09, 0.168)	0.128 (0.079,0.192)		0.105 (0.066,0.161)		0.109 (0.066,0.154)
IL-1 beta sham	(ng/mL)	0.128 (0.061,0.186)	0.105 (0.09,0.211)		0.132 (0.107,0196)		0.087 (0.063,0.198)
TNF-alpha treated	(ng/mL)	0 (0, 0.091)	0 (0,0.298)		0 (0,0.271)		0 (0,0.23)
TNF-alpha sham	(ng/mL)	0 (0,0.069)	0 (0,0.301)		0 (0,0.338)		0 (0,02)
Substance P treated	(pg/mL)	7 (6,9)	8 (5,18)		8 (5,14)		7 (4,16)
Substance P sham	(pg/mL)	8 (5,13)	8 (5,18)		7 (6,12)		6 (5,18)

Median values and ranges for the main outcome parameters at baseline, week 1, day 14, 30, 60 and 90 after treatment (Berlock® Micro-Implants mixed with hyaluronic acid)/sham treatment (hyaluronic acid alone). Mechanical Nociceptive Thresholds (NMTs) are reported for landmark 2, 3 and 5. No significant differences ($P<0.05$) were found at any of the reported time point between treated and sham treated joints.