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A Novel Treatment Combination of Sildenafil, Mepivacaine and Glucose with Disease Modifying Properties, In a Pony with Lameness Associated Osteoarthritis: A Case Report

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Abstract

Today no biomarkers reflecting joint tissue destruction or Disease-Modifying Osteoarthritis Drugs (DMOADs) are available on the market. This case report decscribes a new pharmaceutical drug combination consisting of Sildenafil, Mepivacaine and Glucose (SMG) used for intra-articular treatment of lameness associated osteoarthritis of the right distal interphalangeal joint in a nine-year-old Swedish pony. The pony had a history of moderate lameness during five months and were treated with intra articular corticosteroids three times without any recovery of lameness (visit 1-5). Clinical lameness examinations, including intra articular anaesthesia, radiological examination, standing low-field MRI and quantification of biomarkers for cartilage and bone degradation ($COMP^{156}$ and BGN^{262}) in synovial fluid and serum was performed over a period of 24 months post intervention.

At inclusion (visit 6) the pony showed lameness and levels of synovial fluid $COMP^{156}$ was 98 µg/ml and serum 6.0 µg/ml and serum BGN^{262} 1124 ng/ml. Intra-articular treatment with SMG was performed twice with 4 weeks interval. One month after the first injection (visit 7), the horse was non lame and the concentrations of $COMP^{156}$ was remarkable reduced to 17.6 µg/ml in the synovial fluid. The MRI data indicated improved subchondral bone sclerosis and healed bone inflammation. The horse remained sound and the longitudinal follow up of $COMP^{156}$ and BGN^{262} levels in serum declined during the post-treatment observation period of 24 months. Intra articular treatment of SMG injection was well tolerated, as no clinically adverse effects were observed

Keywords: Lameness • Osteoathritis • DMOAD • Biomarkers • Pharmaceutical treatment

Introduction

Osteoarthritis (OA) is a chronic progressive inflammatory joint disease that leads to severe joint pain and loss of joint mobility in human and animals [1]. The pathogenesis is described as slow and insidious [2]. The disease progresses over time with repeated intense inflammation, articular cartilage and subchondral bone destruction and reduced function [3].

Today there are no Disease-Modifying OA Drugs (DMOADs) available for use in human or veterinary medicine. A DMOAD would typically recede inflammation and pain by simultaneous deceleration in cartilage, bone and synovium destruction [1]. Early diagnosis of OA is crucial in providing an effective treatment, so that it can be monitored both clinically and molecularly to minimize the associated inflammation and pain.

Based on our preclinical research, we have successfully identified three different substances, which when used in combination, targets and reduces inflammatory activity at the cellular level [4,5]. The first component is an anaesthetic agent (bupivacaine or mepivacaine). Clinically used doses of the same produce effective analgesia, by blocking voltage-gated Na+ channels [6]. At far lower concentrations, the blocking of Na^+ channels do not appear. Instead, it promotes cellular communication of Ca^{2+} ions between supporting cells, which form large cellular networks, an important process for the cellular metabolic functions [7]. The second substance is sildenafil, a potent and selective Phosphodiesterase-5 (PDE-5) inhibitor [8]. PDE inhibitors act as anti-inflammatory agents by raising cyclic GMP, a potential tool in diseases where inflammation plays a central role [9]. This drug is used in clinical treatment of several diseases pertaining to its anti-inflammatory properties [8]. However, we have shown that in much lower concentrations than clinically used, this substance stimulates Na^+/K^+-ATPase and interacts with ATP, which result in a better Ca^{2+} signalling through the cellular network coupled cells [10].

The third substance is glucose. High concentrations of glucose seem to be crucial when used together with anti-inflammatory substances [11]. Thus, the combination of these three substances (SMG), exert anti-inflammatory effects in network coupled supporting cells [4,12].

Native Cartilage Oligomeric Matrix Protein (COMP) and Biglycan (BGN) is well known to play a structural and functional role in cartilage and bone homeostasis [13,14]. We have previously defined a soluble neo-epitope of COMP ($COMP^{156}$) in synovial fluid and serum that can reflect early matrix degradation in equine OA articular cartilage [15,16]. Biglycan fragmentation has been shown to reflect the subchondral bone environment and the biomarker BGN^{262} correlates with OA associted subchondral bone sclerosis [17].

Additionally we have performed a randomized triple-blind clinical trial in Standardbred trotters with lameness-associated osteoarthritis. The horses

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were treated with Sildenafil, Mepivacaine, Glucose (SMG) twice intra-articularly with 14 days in between. Celeston® bifas® was used as a positive control. Biomarkers reflecting articular cartilage (COMP[156]) and bone degradation (BGN[262]) were used to monitor the drug efficacy and safety [15,17]. The total follow up period was 60 days [18].

The presented case report describes the use of a novel SMG treatment in a nine-year old pony with lameness associated OA, unresponsive to three intra-articular injections of corticosteroids (Celeston® bifas®). The concentration of COMP[156] in synovial fluid was used to determine the treatment efficacy and COMP[156] and BGN[262] in serum was used to monitor long-term follow up (24 months) post intervention. The pony had a high concentration of the biomarkers in synovial fluid and serum at inclusion, which reflected the ongoing OA associated articular cartilage and subchondral degradationt.

Case Presentation

A nine-year-old Swedish Pony (mixed-breed, gelding) competing in jumping was diagnosed with chronic OA associated lameness in the Distal Inter Pahalangeal (DIP) joints of right front leg and did not respond to treatment of intra-articular injections of Celeston® bifas® at three occasions. The failure to respond to conventional treatment enabled an attempt for a trial treatment with the novel SMG drug. The follow-up period was set to 24 months. The pony was examined and treated at Hallands Djursjukhus, Kungsbacka Equine clinic, Gothenburg, Sweden. The owner signed an Informed Consent. An ethical permission for serum and synovial fliud collection was approved by the Ethics Committee, Uppsala, Sweden (D.nr: 5.8.18-02896/2018).

Clinical lameness evaluation

The clinical lameness evaluation consisted of, subjective lameness grading at walk and trott on straight line on a hard surface and when the horse was lunged. The veterinarian watches for signs of pain, weight shifting or irregular movement. The lameness was graded on a scale of 0–5, (0=sound, 5=non-weight bearing) [19,20] (Table 1). The grade set as the horse lunged on a hard surface in trott to the right, was used to compare the grade of lameness between visits. A diagnostic intra-articular anaesthesia (10 mL Carbocain® (mepivacaine hydrochloride, AstraZeneca, Södertälje, Sweden) was used (visit 1) to locate the joint responsible for the pain. The grade of lameness was evaluated 10 minutes post-injection and was considered positive when an 80–100% decline in lameness was evident.

Radiographic examination

The following radiological examinations were performed.

Visit 3: Phalanges joints of the right and left front legs were radiographed in lateromedial, dorsopalmar projections.

Visit 4: Navicular bones of right and left front legs (65 ° dorso-palmar).

Visit 8: Phalange joints of the right front leg were radiographed in dorsomedial-dorsolateral and dorsolateral-dorsomedial projections.

Standing low-field MRI evaluation

Standing MRI (Hallmarq, Standing Equine MRI) of the phalangeal joints of the right front leg were performed at visits 5, 12 and 15 by Ugglarp Gård Hästklinik, Evidensia, Sweden and the MRI images were read by Lucidity Diagnostics, Germany.

Sampling of synovial fluid and serum

Synovial fluid samples were collected from the DIP of right front leg at visit 6 and 7 and centrifuged at 5700 g for 15 min. Supernatant aliquots were frozen initially at -20 ℃ for one day before transferred to the -80 ℃ freezer for further analysis. Blood samples were collected at visit 6, 7, 8, 9, 10, 11, 13, 14 and 16 and stored at room temperature for 90-120 min and the centrifuged at 5700 g for 10 min. The serum was separated and frozen initially at -20 ℃ for one day before transferred to the -80 ℃ freezer for further analysis.

ELISA measurement

The quantification of COMP[156] and BGN[262] was performed with custom made ELISAs and has previously been developed and validated for horses [15-21]. There was not enough synovial fluid for running of BGN[262] analysis. All samples were run in duplicates and the concentrations were normalised to a control sample that was run in each plate.

Intra-articular treatments

At visit 1, 2 and 3 the pony was treated intra-articular with 1 ml Celeston® bifas® (betamethasone) in the distal interphanageal joint of the right front leg. At visit 6 and 7 the pony was treated intra-articular with 5ml SMG consisting of Carbocain® (mepivacaine hydrochloride, 20 mg/ml) solution, (AstraZeneca, Södertälje, Sweden), Revatio® (sildenafil, 0, 8 mg/ml) solution (Pfizer, Bruxelles, Belgium) and glucose solution 50 mg/ml (Braun Melsungen, Melsungen, Germany) mixed in sterile water (Braun Melsungen, Melsungen, Germany) in the ditsal interphalangeal joint of the right front leg. The formula concentrations of the individual drug components are patented (Patent application number 185133-3).

Past history, outcome and follow-up

The overwiev of visits, intervention and results are displayed in (Figures 1, 2a-d and Table 2).

In February 2018, the pony became lame and was diagnosed with a hoof abscess (right front limb). The lameness did not improve and a lameness evaluation was performed two months later (visit 1).

Visit 1: April 9, 2018: At the clinical examination the pony was found to be in an overall good condition without evidence of lameness in walk, no swelling of the limbs and a negative reaction on hooftest. At trot on a straight line, the pony displayed lameness grade 1 in the Right Front limb (RF) and grade 3 on lungeing to the right on hard surface and grade 3 after low flexion test. Diagnostic anesthesia of the DIP (RF) reduced the lamenens with 80%. The pony was diagnosed with joint inflammation in the DIP (RF) and was treated with 1 ml Celeston® bifas® intra-articular and Meloxicam orally for 10 days. The pony had boxrest for one day followed by rest in a small paddock and hand walked.

Visit 2: April 25, 2018: The pony had improved with no lameness when trotting on a straight line but showed lameness grade of 1.5 from RF on lungeing to the right. The pony was treated with 1 ml Celeston® bifas® intra-articular in the DIP (RF) joint and recommended similar aftercare as for visit 1.

Visit 3: May 16, 2018: One month later, the pony still showed lameness grade of 1.5 from the RF on lungeing to right and was treated once more with

Table 1. A grading system of lameness described by Dr Ross[3] and Dr Dyson[6].

Lameness grades from 0-5 are based on observation of the horse at a trot in hand, in a straight line, on a firm or hard surface.
0 = Sound
1 = Mild lameness observed while the horse is trotted in a straight line.When the lame forelimb strikes, a subtle head nod is observed; whenthe lame hindlimb strikes, a subtle pelvic hike occurs. The head nod and pelvic hike may be inconsistent at times.
2 = Obvious lameness is observed. The head nod and pelvic hike are seen consistently and excursion is several cm.
3 = Pronounced head nod and pelvic hike of several cm are noted. If the horse has unilateral singular hindlimb lameness, a head and neck nodis seen when the diagonal forelimb strikes the ground (mimicking ipsilateral forelimb lameness).
4 = Severe lameness with extreme head nod and pelvic hike is present. The horse can still be trotted, however.
5 =The horse does not bear weight on the limb. If trotted, the horse carries the limb (horses that are nonweightbearing at the walk or whilestanding should not be trotted).

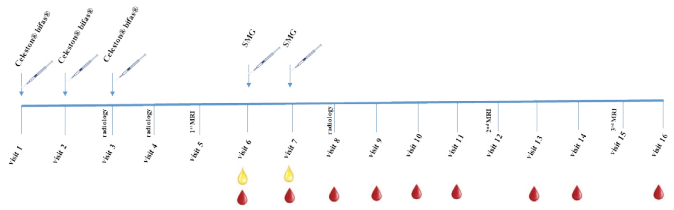

Figure 1. Schematic case overview with treatments, clinical examinations, radiology and MRI examinations. The pony received Celeston® bifas® at visit 1, 2 and 3 and SMG at visit 6 and 7 (SMG= sildenafil + mepivacaine + glucose).

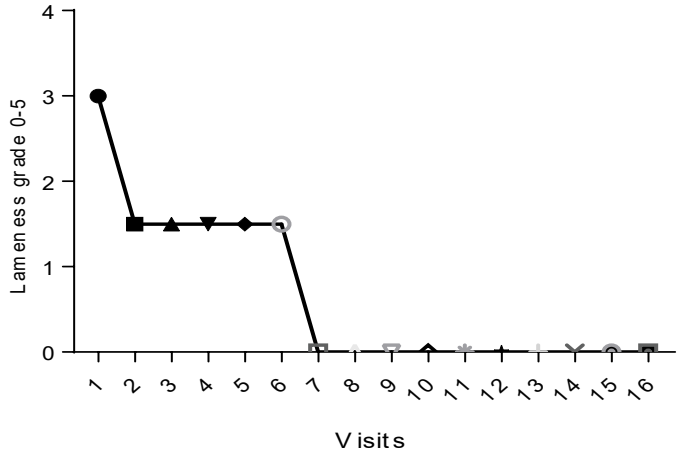

Figure 2a. Evaluation of clinical lameness (grade 0-5) pre- and post- intervention. The pony received Celeston® bifas® at visit 1, 2 and 3 and SMG at visit 6 and 7. (SMG= sildenafil + mepivacaine + glucose).

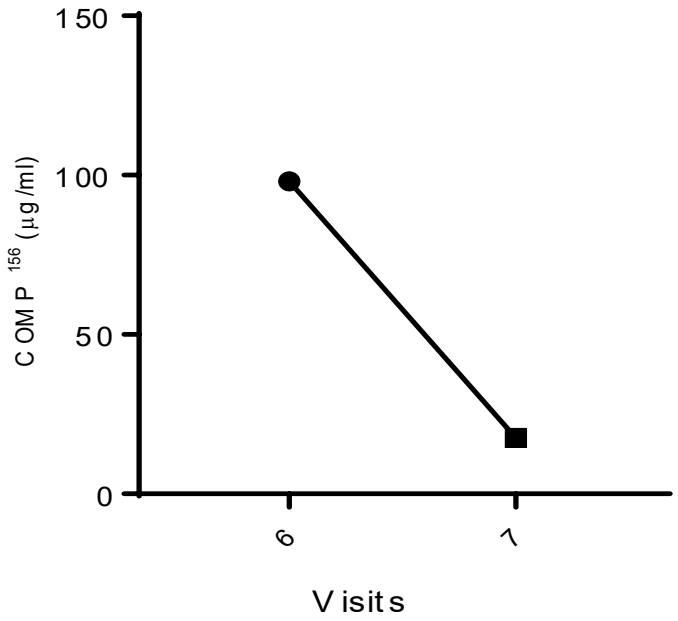

Figure 2b. Concentration of COMP[156] (µg/ml) in synovial fluid at visit 6 (June 29th) (first treatment with SMG) and at visit 7 (July 23rd) (second treatment with SMG). (SMG = sildenafil + mepivacaine + glucose).

1 ml Celeston® bifas® intra-articular in the DIP joint. Radiological examination was performed of the right and left three phalanges joints of the front legs (latero-medial, dorso-palmar projections) with no radiological findings.

Visit 4: May 30, 2018: There was no improvement from last visit and radiographic examination of the navicular bones of both front legs were performed (65° dorso-palmar), with no findings present.

Visit 5: June 26, 2018 MRI examination no 1: The pony was sent for standing low-field MRI examination of the distal phalangeal joints (hoof/pastern) of the right front leg. The MRI findings showed mild irregularities and loss of signal intensity of the hyaline cartilage medial in the Distal Interphalangeal (DIP) joint. Moderate subchondral bone sclerosis disto-medial in the second phalanx. Radiological Diagnoses/Interpretation from the MR-Images highly suggests presence of defects in the articular cartilage medially in the DIP joint. The findings are likely consistent with a mild chronic osteoarthritis of the DIP joint, even if the limited specificity of low-field MRI for cartilage evaluation is taken into consideration. There was a mild sclerotic bone remodeling in the subchondral bone plate of the second phalanx. There were no additional significant lesions in the fetlock region.

Visit 6: June 29, 2018 Treatment with SMG: After 2.5 months of treatment with corticosteroids, front leg lameness grade 1.5 (RF) was still present when lungeing to right on hard surface. The lameness had not improve after treatment with Celeston® bifas® and the decision to test the novel drug combination of SMG was taken. At the time COMP[156] concentration in synovial fluid was 98 µg/ml and serum 5.9 µg/ml (Figures 2 b-c and Table 2). The BGN[262] concentration in serum was 1124 ng/ml (Figure 2d and Table 2). After treatment the pony was box rested the first day and then hand walked or allowed to rest in a small paddock.

Visit 7: July 24, 2018 Treatment with SMG: The pony was sound on clinical lameness examination without evidence of lameness in walk, trot or lungeing to the right on hard surface. The horse was treated intra-articular with SMG and the COMP[156] concentration in synovial fluid was 17.7 µg/ml and serum 5.6 µg/ml. The BGN[262] concentration in serum was 1492 ng/ml. The pony was allowed one day of box rest and then to walk in a small paddock and ridden at walk for 30 min per day.

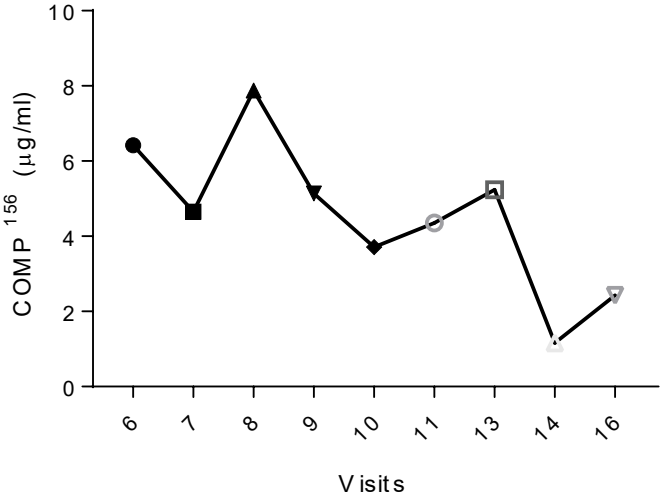

Figure 2c. Concentration of COMP[156] (µg/ml) in serum at visit 6 (June 29th) and after treatment visit 7 (July 23rd) and thereafter at additional 7 visits from August 2018 to May 2020 (visit 16).

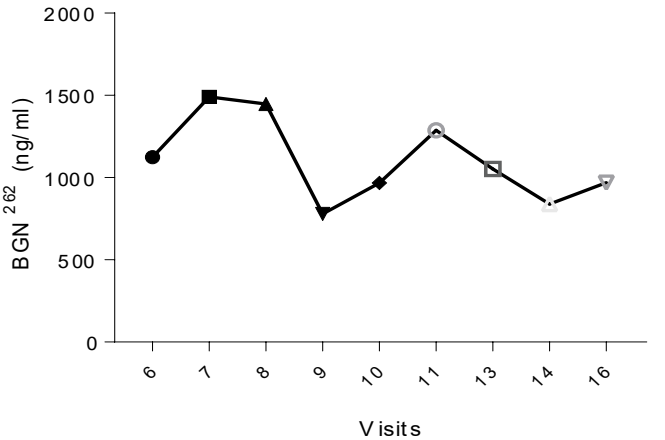

Figure 2d. Concentration of BGN[262] (ng/ml) in serum at visit 6 (June 29th) and after treatment visit 7 (July 23rd) and thereafter at additional 7 visits from August 2018 to May 2020 (visit 16).

Table 2. Case report overwiev with treatments, clinical examinations, radiology, sample collection and MRI examinations. (SMG=Sildenafil, Mepivacaine, Glucose), (SF=Synovial Fluid).

Visit	Date	MRI, Radiology	Treatments	Lamness Grade	SFCOMP^{156}ug/ml	Serum COMP^{156}ug/ml	Serum BGN^{262}ng/ml
visit 1	April 9, 2018	-	Celeston® Bifas®	3	-	-	-
visit 2	April 25, 2018	-	Celeston® Bifas®	1.5	-	-	-
visit 3	May 16, 2018	Radiology	Celeston® Bifas®	1.5	-	-	-
visit 4	May 30, 2018	Radiology	-	1.5	-	-	-
visit 5	June 26, 2018	MRI no 1	-	1.5	-	-	-
visit 6	June 29, 2018	-	SMG	1.5	98	5.9	1124
visit 7	July 23, 2018	-	SMG	0	17.7	5.6	1492
visit 8	August 8, 2018	Radiology	-	0	-	10.7	1448
visit 9	August 24, 2018	-	-	0	-	7.00	780
visit 10	September 5, 2018	-	-	0	-	5.0	968
visit 11	September 26, 2018	-	-	0	-	9.7	1288
visit 12	October 26, 2018	MRI no 2	-	0	-	-	-
visit 13	December 13, 2018	-	-	0	-	12.2	1053
visit 14	February 14, 2019	-	-	0	-	1.9	840
visit 15	April 4, 2019	MRI no 3	-	0	-	-	-
visit 16	May 13, 2020	-	-	0	-	4.6	969

Visit 8: August 8, 2018: The pony was sound on clinical lameness examination. Radiological examination of DIP of the right front leg reviled no radiological findings.

Visit 9-11, 13 (see Table 2)

Visit 12: 26 October, 2018; MRI-examination number 2: Interpretation from the MRI examination suggested a mild subchondral bone sclerosis disto-medial in P2. A minimal subchondral bone remodelling disto-lateral in P2 was suspected, but, no evidens of active inflammation in the bone was present. The minimal irregularity of the articular cartilage medially in the DIP joint appeared slightly smaller than at the previous MRI examination. However, it could be caused by a different position of the limb during the scans or by mild variations in the orientations of the MR-Images.

Visit 14: February 14, 2019: The pony was sound on clinical lameness examination. Serum COMP^{156} concentration was 1.9 µg/ml and BGN^{262} 840 ng/ml and the pony gradually went back to work and during the spring of 2019 (less than 12 months after the first SMG treatment in July 24th) it began jumping obstacles and during the summer it was back to same competition level as before the lameness start in February 2018.

Visit 15: April 4, 2019, MRI examination number 3: Interpretation from the MRI examination suggested a small irregularity of the the articular cartilage medially in the DIP joint with a suspicion of a partial or full-thickness lesion in that area, no signs of inflammation of the bone.

Visit 16: May, 2020: The pony went through a pre-purchase examination including a full clinical lameness evaluation including flexion tests. No signs of lameness could be seen and the serum concentration of COMP^{156} was 4.6 µg/ml and BGN^{262} was 969 ng/ml.

Study limitations

Low-field MRI has a very limited sensitivity and specificity for evaluation of hyaline cartilage. When the horse is moving that could cause different position of the limb during the scans or by mild variations in the orientations of the MR-Images. At the MRI examinations small irregularity of the hyaline cartilage was found throughout the scans with a suspicion of a partial or full-thickness lesion in that area. The described findings do occasionally show up in sound horses and may or may not be of clinical significance in this case. Therefore false positive findings are possible. It is recommended that in case of lameness an intra-synovial anesthesia is needed to confirm the clinical significance. As the pony was non-lame at the time for scan 2 and 3 and the cartilage biomarker COM^{156} was steady reduced in serum this was not done and the findings may not be of clinical significance.

Results and Discussion

This case study reports a new pharmacological drug combination, which is effective in relieving a nine-year-old Swedish pony from chronic OA associated lameness. The results are the first to show treatment efficacy not only on clinical lameness but also on the concentrations synovial fluid COMP^{156}, a novel neo-epitope biomarker associated with cartilage matrix degradation.

Initially, the pony displayed lameness grade 3, with pronounced head nod on lungeing to the right on hard surface. The pony did not show full recovery (lameness grade 1.5) after three intra-articular treatments with Celeston® bifas®.

After 2,5 months of lameness with three treatments of corticosteroids, the pony was still lame from its right front leg and with extremly high COMP^{156} concentrations in the synovial fluid of the distal interphalangeal joint, i.e., 98 µg/ml (reference range for a healthy horse is set to 16.5 ± 5.9 µg/ml) [15] indicating severe cartilage matrix degradation. After the first treatment with SMG the concentrations of COMP^{156} in synovial fluid, dramatically declined to 17.7 µg/ml. Moreover, the pony was non lame at clinical examination one month after the treatment and remained sound during the follow up period.

Drugs currently available in the market such as NSAIDs, hyaluronic acid and corticosteroids primarily target joint inflammation and subsequent pain. However, these drugs neither inhibit the catabolic events causing disease progression, nor stimulate the cartilage matrix synthesis. Several side effects namely flares, joint infections and poor pain-relieving effect, have been associated with the treatments [22].

The serum COMP^{156} concentration was 5.9 µg/ml prior to first intervention with SMG. Four weeks later it was 5.6 µg/ml. Serum was sampled at seven time points during the 22 month follow up period and during the last 15 months and the values were reduced corroborating healthy values [16].

The drug combination acts by targeting and restoring the cell-to-cell signalling of chondrocytes, osteocytes and synovial cells in the joint [23,24]. The drug combination with low concentrations of an anaesthetic agent and sildenafil was previously reported to restore elevated intracellular Ca^{2+} release and down regulates the inflammatory factors expressed in equine OA chondrocytes [4]. Interestingly OA chondrocytes consume more glucose than healthy chondrocytes since the glucose transporter proteins are downregulated which in turn disrupt the glucose balance [11]. The calculated sildenafil concentration obtained in a horse after one injection to the joint is ultralow and inhibition of PDE-5 cannot be significantly detected [25]. The

effect we aim for is a normalization of the intracellular Ca^{2+} transport of the OA chondrocytes, which differs from the normal pharmacological effect of the drug i-e-., PDE-5 inhibition.

On MRI examination, no clear improvement of the articular cartilage was observed. The reduced concentrations of COMP[156] in synovial fluid and serum and the clinical lameness evaluation post intervention indicated no lameness and a reduced cartilage degradation, which together indicates a healthy joint. However, in a pilot study in natural occurring cartilage defect associated with OA it was found that if an abnormal contour was seen in the articular cartilage, cartilage damage is likely to be present [26].

At the first MRI examination a moderate subchondral bone sclerosis with mild sclerotic bone remodeling in the subchondral bone plate was found. At that timepoint the levels of BGN[262] was 1124 ng/ml in serum. At MRI examination two a mild subchondral bone sclerosis with a minimal subchondral bone remodelling but, no evidens of active inflammation in the bone was present. The BGN[262] was was decreased to 1053 ng/ml in serum. At the third MRI examination no signs of inflammation of the bone was found and the levels of BGN[262] was further decreased to 969 ng/ml in serum. The results indicate reduced subchondral bone degradation post intervention. This data suggest that the levles of BGN[262] reflects the subchondral bone remodelling in consistent with previous report by [17], whereas the BGN[262] was prominent stained in subchondral bone sclerosis associated with moderate OA compared to healthy joints. No adverse effects of either injection with SMG were observed post intervention.

Conclusion

The results of SMG treatment in a pony with chronic OA associated lameness showed a fast reduction in lameness as well as decreased levels of COMP[156] in synovial fluid. The medical history showed that the pony did not respond to three intra-articular treatments with Celeston® bifas®. The prompt improvement in lameness grade following SMG intervention, a novel drug combination of sildenafil, mepivacaine (both components in extremely low concentrations) and glucose and the reduced biomarker levels in synovial fluid and serum implicate slowing down the disease progression. With the aid of sensitive and specific biomarker for cartilage and bone destruction, we present the possibility of measuring the treatment efficacy. Preventing progression of chronic OA by the new drug combination is an extremely novel disease-modifying outcome. Intra articular treatment of SMG injection was tolerated well, without any clinically adverse effects post intervention.

Consent

Written informed consent was obtained from the horse owner for publication of this case report and any accompanying images.

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References

1. Makarczyk, Meagan J., Qi Gao, Yuchen He and Zhong Li, et al. "Current models for development of disease-modifying osteoarthritis drugs." *Tissue Eng Part C Methods* 27 (2021): 124-138.
2. Van, Weeren, P. René and Janny C. de Grauw. "Pain in osteoarthritis." *Vet Clin* 26 (2010): 619-642.
3. Goldring, Mary B. and Miguel Otero. "Inflammation in osteoarthritis." *Curr Opin Rheumatol* 23 (2011): 471.
4. Hansson, Elisabeth and Eva Skiöldebrand. "Bupivacaine in combination with sildenafil (Viagra) and vitamin D3 have anti-inflammatory effects in osteoarthritic chondrocytes." *Curr Res Pharmacol Drug Discov* 2 (2021): 100066.
5. Hansson, Elisabeth, Ulrika Björklund, Eva Skiöldebrand and Lars Rönnbäck. "Anti-inflammatory effects induced by pharmaceutical substances on inflammatory active brain astrocytes—promising treatment of neuroinflammation." *J Neuroinflammation* 15 (2018): 1-13.
6. Olschewski, Andrea, Matthias Wolff, Michael E. Bräu and Gunter Hempelmann, et al. "Enhancement of delayed-rectifier potassium conductance by low concentrations of local anaesthetics in spinal sensory neurones." *Br J Pharmacol* 136 (2002): 540-549.
7. Block, Linda, Per Jörneberg, Ulrika Björklund and Anna Westerlund, et al. "Ultralow concentrations of bupivacaine exert anti-inflammatory effects on inflammation-reactive astrocytes." *Eur J Neurosci* 38 (2013): 3669-3678.
8. Rotella, David P. "Phosphodiesterase 5 inhibitors: Current status and potential applications." *Nat Rev Drug Discov* 1 (2002): 674-682.
9. Peixoto, Christina Alves, Ana Karolina Santana Nunes and Ana Garcia-Osta. "Phosphodiesterase-5 inhibitors: Action on the signaling pathways of neuroinflammation, neurodegeneration and cognition." *Mediators Inflamm* 2015 (2015).
10. De, Santana Nunes, Ana Karolina, Catarina Rapôso and Ulrika Björklund, et al. "Sildenafil (Viagra®) prevents and restores LPS-induced inflammation in astrocytes." *Neurosci Lett* 630 (2016): 59-65.
11. Rotter Sopasakis, Victoria, Ruth Wickelgren, Valentina Sukonina and Camilla Brantsing, et al. "Elevated glucose levels preserve glucose uptake, hyaluronan production and low glutamate release following interleukin-1β stimulation of differentiated chondrocytes." *Cartilage* 10 (2019): 491-503.
12. Hansson, Elisabeth and Eva Skiöldebrand. "Anti-inflammatory effects induced by ultralow concentrations of bupivacaine in combination with ultralow concentrations of sildenafil (Viagra) and vitamin D3 on inflammatory reactive brain astrocytes." *Plos one* 14 (2019): e0223648.
13. Cui, Jiarui and Jiaming Zhang. "Cartilage oligomeric matrix protein, diseases and therapeutic opportunities." *Int J Mol Sci* 23 (2022): 9253.
14. Miguez, Patricia A. "Evidence of biglycan structure-function in bone homeostasis and aging." *Connect Tissue Res* 61 (2020): 19-33.
15. Skiöldebrand, E., S. Ekman, Lillemor Mattsson Hultén and E. Svala, et al. "Cartilage oligomeric matrix protein neoepitope in the synovial fluid of horses with acute lameness: A new biomarker for the early stages of osteoarthritis." *Equine Vet J* 49 (2017): 662-667.
16. Ekman, Stina, A. Lindahl, U. Rüetschi and A. Jansson, et al. "Effect of circadian rhythm, age, training and acute lameness on serum concentrations of Cartilage Oligomeric Matrix Protein (COMP) neo-epitope in horses." *Equine Vet J* 51 (2019): 674-680.
17. Adepu, Saritha, Stina Ekman, Jakob Leth and Ulrika Johansson, et al. "Biglycan neo-epitope (BGN[262]), a novel biomarker for screening early changes in equine osteoarthritic subchondral bone." *Osteoarthr Cartil* 30 (2022): 1328-1336.
18. Skiöldebrand, E., S. Adepu, C. Lützelschwab and S. Nyström, et al. "A randomized, triple-blinded controlled clinical study with a novel disease-modifying drug combination in equine lameness-associated osteoarthritis." *Osteoarthr Cartil Open* (2023): 100381.
19. Bassage, L. H. and M. W. Ross. "Diagnosis and Management of Lameness in the Horse." Diagnostic Analgesia. 2nd (edn), WB Saunders, Philadelphia, United States (2011).
20. Dyson, S. "Can lameness be graded reliably?." *Equine Vet J* 43 (2011): 379-382.
21. Adepu, S., M. Lord, Z. Hugoh and S. Nyström, et al. "Salivary biglycan-neo-epitope-BGN[262]: A novel surrogate biomarker for equine osteoarthritic sub-chondral bone sclerosis and to monitor the effect of short-term training and surface arena." *Osteoarthr Cartil Open* 5 (2023): 100354.

22. Oo, Win Min. "Prospects of disease-modifying osteoarthritis drugs." *Clin Geriatr Med* 38 (2022): 397-432.

23. Skiöldebrand, Eva, Anna Thorfve, Ulrika Björklund and Pegah Johansson, et al. "Biochemical alterations in inflammatory reactive chondrocytes: Evidence for intercellular network communication." *Heliyon* 4 (2018).

24. Hansson, Elisabeth and Eva Skiöldebrand. "Coupled cell networks are target cells of inflammation, which can spread between different body organs and develop into systemic chronic inflammation." *J Inflamm* 12 (2015): 1-11.

25. Santillo, Michael F. and Mapa ST Mapa. "Phosphodiesterase (PDE5) inhibition assay for rapid detection of erectile dysfunction drugs and analogs in sexual enhancement products." *Drug Test Anal* 10 (2018): 1315-1322.

26. Van Zadelhoff, Claudia, Tobias Schwarz, Sionagh Smith and Antoine Engerand, et al. "Identification of naturally occurring cartilage damage in the equine distal interphalangeal joint using low-field magnetic resonance imaging and magnetic resonance arthrography." *Front Vet Sci* 6 (2020): 508.

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