Equine DSC Therapy of Musculoskeletal Conditions: an Ultrasonographic Evaluation

Research Thesis

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by

Logan Scheuermann

The Ohio State University
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Project Advisors:
Dr. Ian Hamilton, Department of Evolution, Ecology and Organismal Diversity
Dr. Alicia Bertone, College of Veterinary Medicine

Abstract

Musculoskeletal diseases are among the most common disorders that affect horses, and current methods to treat these disorders can be invasive and dependent on the severity of the injury. The use of mesenchymal stem cells (MSCs) may provide an effective and non-invasive method to treat equine musculoskeletal disorders. An experiment was conducted to analyze the safety of treating equine musculoskeletal disorders with an intra-articular injection of a novel stem cell product. To measure the safety of the product, the edema elicited by the injection of the product was measured using ultrasonographic images. These images were taken before the injection and seven and fourteen days after the injection. To measure edema at the three time points, three soft tissue measurements were taken for horses affected by tendonitis/desmitis and four measurements were taken for horses affected by osteoarthritis at each time point. Edema that the intra-articular injection of the stem cells may have elicited was resolved and no longer statistically significant within the first week after injection. The transient edema observed suggests that the intra-articular injection of the stem cell product is a safe option for treating equine musculoskeletal disorders.

Introduction

Musculoskeletal disorders such as osteoarthritis (OA), tendonitis, and desmitis are among the most common ailments that affect race, working, and pleasure horses [1-5]. The effects of the methods currently used to treat musculoskeletal injuries (e.g., non-steroidal anti-inflammatory drugs, tendon splitting, polysulfated glycosaminoglycan, etc.) depend on the severity of the injury and tend to lead to a high rate of reinjury [1,4]. Furthermore, treating these disorders with current methods also tends to involve invasive procedures and anesthesia, which present additional disadvantages [6].

Conversely, the use of MSCs is a marginally invasive treatment that has shown promise in many different species [2,4,6,7]. The potential for MSCs to differentiate into different tissues such as bone, cartilage, and tendon is well known [4,6,8,9]. MSC differentiation is highly regulated and dependent on extracellular stimuli such as growth factors and extracellular matrix molecules [10]. In a Caprine model, the systemic delivery of bone marrow derived MSCs by intra-articular injection utilized extracellular stimuli in injured joints to stimulate tissue regeneration [8]. This suggests that intra-articular stem cell injections may have a therapeutic affect on OA. In both horses and Sprague Dawley rats, intra-articular MSC injections have also been associated with a reduced rate of superficial digital flexor tendon reinjury and a reduced degeneration in cruciate ligaments [3,6,11]. The direct intra-articular injection of stem cells has shown modest efficacy in equine joint injury models, which may be associated with the ability of stem cells to home in on affected tissues [12,13]. While the intra-articular delivery of bone marrow derived mesenchymal stem cells shows promise, the harvesting of the cells can be invasive.

Dental stem cells (DSCs) are easily accessible MSCs derived from dental pulp and they provide a potential non-invasive source of pluripotent stem cells [14,15]. DSCs are able to proliferate more rapidly than bone marrow stem cells and DSCs are considered pluripotent because they have shown the ability to differentiate into distinct tissues [14,16]. The use of deciduous teeth offers a readily available, non-invasive, and reliable source of allogeneic stem cells that

could be used to treat equine injuries [14,15].

The intra-articular injection of allogeneic stem cells may be a safe and effective option to treat equine musculoskeletal injuries [13]. However, several studies have shown mixed results about negative effects of allogeneic cell injections such as the presence of edema and inflammatory responses [11-13]. In a pilot OA preclinical DSC study using an allogeneic pulp cell product (EqPCP), there was swelling of the joint on days 1, 2, 7, and 14 as detailed by joint circumference and ultrasonographic image measurements [AL Bertone, unpublished data].

The purpose of this study was to determine if edema elicited by the intra-articular injection of EqPCP persists up to 7 or 14 days after injection, using the same novel EqPCP as the pilot study. We hypothesized that any edema caused by the EqPCP injection would not be statistically significant 7 or 14 days after the intra-articular injection. Edema was not thought to be significant one to two weeks after injection because studies on larger scales than the pilot study have shown swelling to peak at 48 hours after intra-articular injection of allogeneic stem cells [13].

Materials and Methods

Ultrasound images were obtained of the joints or tendons/ligaments from forty horses affected by OA or tendonitis/desmitis, respectively, that met criteria for enrolment in the study (lameness score of 2 to 4 on AAEP scale; lameness for at least 4 weeks; and one or more additional clinical sign of tendonitis, desmitis, or OA). The standing ultrasound images were longitudinal and obtained directly over the site of intra-articular injection. An ACVR Diplomate and student selected images that consistently reflected the injection site at the affected joint, tendon, or ligament from 0, 7, and 14 days after an injection of either EqPCP or the carrying vehicle without EqPCP.

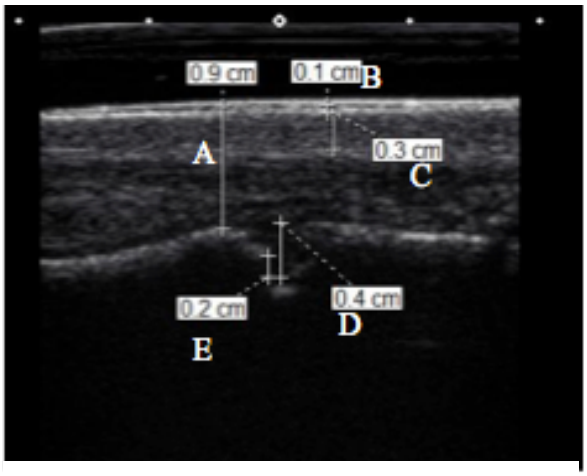


Fig. 1. Representative ultrasonographic image of the four measurements ([A] bone to skin depth, [B] subcutaneous tissue depth, [C] thickness of overlying tendon, [D] bone to joint capsule depth starting from [E] a constant depth and width) that were taken for horses affected by OA.

The images containing joints affected by OA were analyzed for edema by measuring the depth from bone to skin, to represent total soft tissue depth; depth from bone to joint capsule margin, to represent the joint fluid; thickness of the tendon overlying the joint; and depth of subcutaneous tissue (Fig. 1). The images containing tendonitis/desmitis were analyzed for edema by measuring the depth from bone to skin, to represent total soft tissue depth; thickness of the injured tendon/ligament; and depth of the subcutaneous tissue (Fig. 2). If the same measurement increased between time points (e.g., an increase in tendon thickness from 0 to 7 days after injection), it is indicative of an increase in edema. To obtain consistent depth measurements of the joint capsule, the measurements at all time points began at a constant depth below the bone and a constant thickness between the bones. The measurements were carried out through eFilm Workstation[1], a program that digitally

[1] Merge Healthcare Incorporated, 350 N. Orleans Street, Chicago, IL 60654

measures selected areas on ultrasound images. eFilm Workstation was chosen because it is able to automatically adjust the measuring scale based on the depth that the ultrasound image was taken at. This automatic adjustment ensures accurate and consistent measurements.

The measurements were entered into Microsoft Excel and separated into groups of horses affected by OA and tendonitis/desmitis. The OA and tendonitis/desmitis groups were then separated further based on measurement type (bone to skin, tendon/ligament, subcutaneous tissue, etc.). Within each measurement type, the data was then split into control and treatment groups. In order to account for varying bone to skin depths of affected areas between horses, changes in depth/thickness (between 7 and 0 days after injection and 14 and 0 days after injection) were converted to percent difference changes to standardize the data. Percent changes were then added together for each horse to yield percent differences in the total measured soft tissue depth between days 7 and 0 and days 14 and 0 (e.g., for a horse affected by OA, percent changes in subcutaneous tissue depth, overlying tendon width, and joint fluid depth were added together). To assess the significance between treatment and control groups, a two-factor repeated measure analysis of variance (ANOVA) was performed using Microsoft Excel. The data met the assumptions for two-factor repeated measure ANOVA as the Q-Q plots were linear, Levene's tests were not significant, and Mauchly's tests were also not significant. A P-Value of less than 0.05 was significant for all tests and analyses.

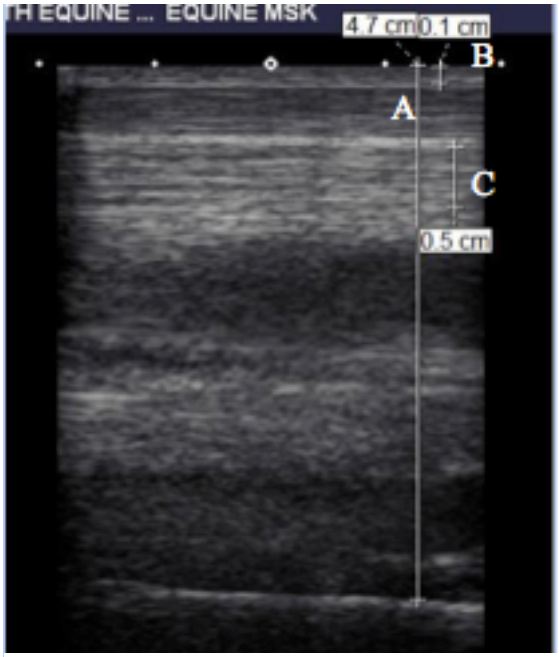


Fig. 2. Representative ultrasonographic image of the three measurements ([A] bone to skin depth, [B] subcutaneous tissue depth, [C] thickness of injured tendon/ligament) that were taken for horses affected by tendonitis/desmitis.

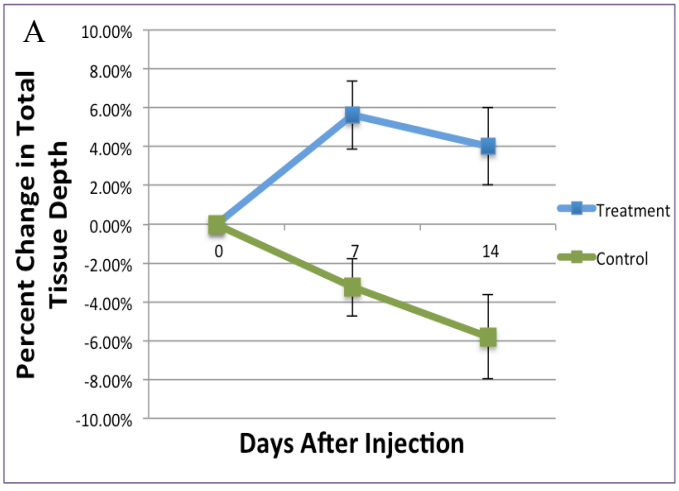
Results

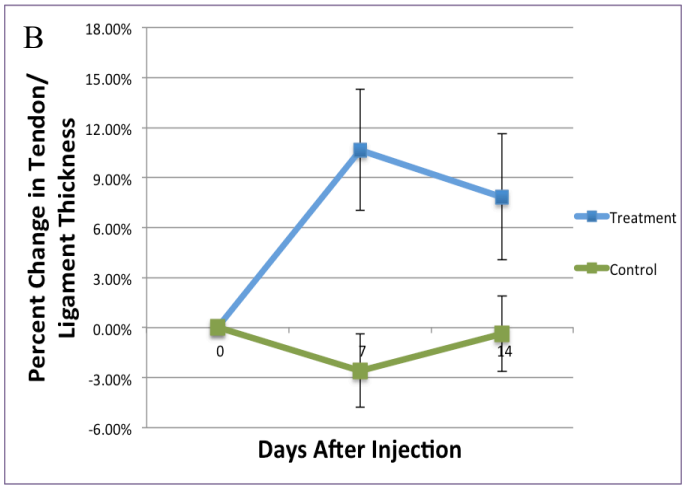
Seven and fourteen days after intra-articular injection of EqPCP, no significant differences in edema were measured between treatment and control groups at the injection site (Table 1). Although not statistically significant differences, in certain measurements, percent change increases in tissue depth/thickness were observed for horses that received an injection of DSC compared to the control groups in both the OA and tendonitis/desmitis groups (Fig. 3A-F). All of the changes in depth/thickness peaked seven days after the intra-articular injection of EqPCP.

Table 1. Two Factor Repeated Measure ANOVA Data

	F	Numerator Degrees of Freedom	Denominator Degrees of Freedom	P Value
Subcutaneous Tissue Depth – Tendonitis/Desmitis Group				
Days After Injection	0.2108	2	54	0.8106

Treatment/Control	0.3148	1	54	0.5771
Interaction	0.1743	2	54	0.8405
Tendon/Ligament Thickness – Tendonitis/Desmitis Group				
Days After Injection	0.0026	2	54	0.9974
Treatment/Control	12.2575	1	54	0.0009
Interaction	0.0996	2	54	0.9054
Total Soft Tissue Depth – Tendonitis/Desmitis Group				
Days After Injection	0.0145	2	54	0.9856
Treatment/Control	1.4100	1	54	0.2403
Interaction	0.1118	2	54	0.8945
Subcutaneous Tissue Depth – Osteoarthritis Group				
Days After Injection	0.0299	2	54	0.9706
Treatment/Control	4.2884	1	54	0.0432
Interaction	0.0448	2	54	0.9562
Overlying Tendon Thickness – Osteoarthritis Group				
Days After Injection	0.6108	2	48	0.5471
Treatment/Control	11.7625	1	48	0.0013
Interaction	0.2130	2	48	0.8089
Joint Fluid Depth – Osteoarthritis Group				
Days After Injection	0.0278	2	54	0.9726
Treatment/Control	1.2334	1	54	0.2717
Interaction	0.0050	2	54	0.9950
Total Soft Tissue Depth – Osteoarthritis Group				
Days After Injection	0.0275	2	54	0.9729
Treatment/Control	0.4583	1	54	0.5013
Interaction	0.0285	2	54	0.9719





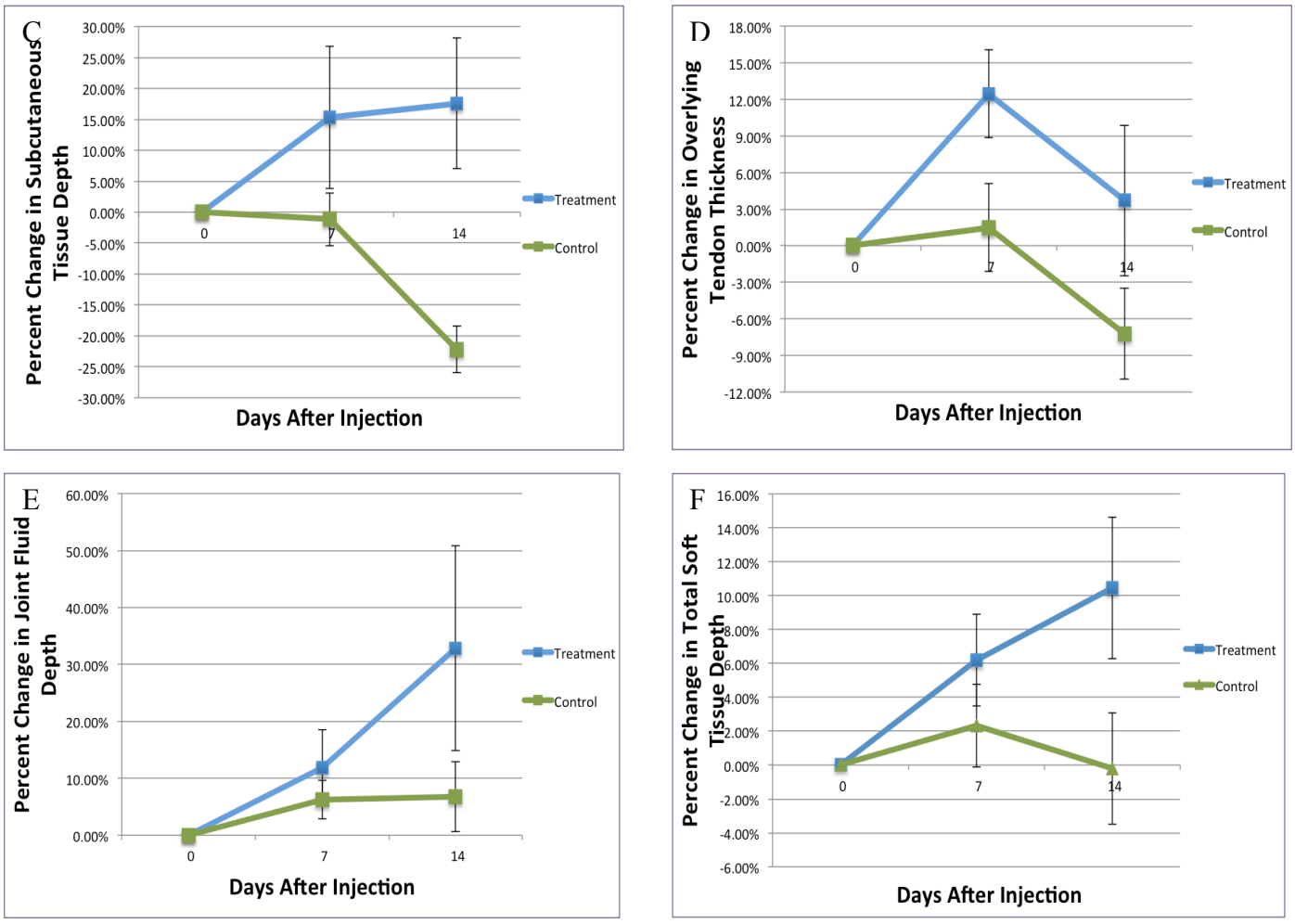


Fig. 3. Change in depth/thickness of tissue at 0, 7, and 14 days after DSC (treatment) and vehicle (control) intra-articular injection. (A) Total soft tissue depth of tendonitis/desmitis horses, (B) Tendon/Ligament thickness of tendonitis/desmitis horses, (C) Subcutaneous tissue depth of tendonitis/desmitis horses, (D) Overlying tendon thickness of OA horses, (E) Joint fluid depth of OA horses, (F) Total soft tissue depth of OA horses.

Discussion

This study showed that the intra-articular injection of EqPCP, a novel DSC product, did not cause edema that persists for at least one week after injection. The current study contradicts the findings from the pilot preclinical study in which edema persisted over the joint injection site for at least 14 days after DSC injection (AL Bertone, unpublished data). The disparity in edema measured in the two studies may be a result of the differences in horse enrollment number. The small sample size (6 horses) of the pilot study may have lead to a misrepresentation of the edema caused by the injection of EqPCP, while the larger size (40 horses) of the current study may resolve this issue.

While the results from this study are contradictory to those of the pilot study, they confirmed previous findings that edema caused by an intra-articular injection of MSCs is transitory [11,13]. It is also expected that a single dose of an anti-inflammatory drug or band-aging the site of MSC injection after administration would resolve the short-term edema. Both of these practices are typical in clinical settings but were not used in the current study.

A lack of edema one week after injection is important because it supports the use of EqPCP to safely treat OA, tendonitis, and desmitis. By safely treating musculoskeletal disorders, this stem cell product may be able to prove its efficacy as a minimally invasive treatment option. If effective, this product could find a niche in veterinary medicine as safe technique to treat several of the most common equine injuries [1,4,6]. To better understand the safety and potential edema at the EqPCP injection site, additional studies are needed to evaluate edema during the first week after the injection.

In conclusion, the intra-articular DSC injection did not cause edema that persisted for at least one week after treatment at the injection site. This result supports previous findings that intra-articular MSC injection is a safe option for treating musculoskeletal disorders.

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