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Operative Techniques in

Sports Medicine

Equine Models for the Investigation of Mesenchymal Stem Cell Therapies in Orthopaedic Disease

Aimée C. Colbath, VMD, MS, DACVS,[*] David D. Frisbie, DVM, PhD, DACVS, DACVSMR,[*] Steven W. Dow, DVM, PhD, DACVIM,[†] John D. Kisiday, PhD,[*,‡] C. Wayne McIlwraith, BVSc, PhD, DACVS, DACVSMR,[*] and Laurie R. Goodrich, DVM, PhD, DACVS[*,‡]

Mesenchymal stem cells (MSCs) have emerged as a promising treatment for orthopaedic disease. Well-established equine models of posttraumatic osteoarthritis, focal cartilage healing, and tendonitis provide a platform for testing safety and efficacy of biologic therapies such as MSCs in a species with naturally occurring disease. Horses routinely experience similar conditions that mirror human musculoskeletal injury, including osteoarthritis, meniscal injuries, and Achilles tendinopathy, which provide relevant clinical models for therapeutic interventions. The use of MSCs in equine models of osteoarthritis and focal cartilage healing has yielded encouraging results. When MSCs have been used in equine models of tendonitis or tendonosis, most clinical and experimental studies have been consistently positive. Currently, the relationship among MSC lifespan, persistence within the injured site, administration methods, and treatment efficacy remains unclear, resulting in widespread interest in cell tracking. We conclude that equine models of musculoskeletal disease can provide important preclinical insights into the likely efficacy and mechanisms of activity of MSCs for the treatment of human orthopaedic injuries.
Oper Tech Sports Med 25:41-49 Published by Elsevier Inc.

KEYWORDS Stem cells, equine, orthopaedic disease, models

Introduction

Originally, the primary therapeutic activity of mesenchymal stem cells (MSCs), which exhibit pluripotent differentiation capacity, was considered to be through participation in local tissue regeneration.[1] However, the current dogma suggests that the primary mechanisms of action of MSCs are the paracrine secretion and cell-to-cell interactions, leading to stimulation of host innate healing mechanisms.[2] This article focuses on the importance of equine musculoskeletal disease models, which relate to human disease and what has been learned to date from the use of these models regarding the efficacy and mechanisms of MSC therapeutics.

The Horse as a Model for Orthopaedic Disease in Humans

Small laboratory animals have been used extensively to test MSC use for the treatment of musculoskeletal disease.[3-5] Certainly, a great deal has been learned about cellular therapies from rodent models, but rodents are considered anatomically inferior to equine models in their cartilage thickness, joint size, and joint forces.[6,7] In addition, equine models of tendonitis have been proposed as superior to small animal models because the equine superficial digital flexor tendon (SDFT) is functionally very similar to the human Achilles tendon.[8,9]

*Orthopaedic Research Center, Colorado State University, Fort Collins, CO.
†Center of Immune and Regenerative Medicine, Colorado State University, Fort Collins, CO.
‡Department of Clinical Sciences, Colorado State University, Fort Collins, CO.
This work was completed at the Orthopaedic Research Center, Colorado State University.
Address reprint requests to Laurie R. Goodrich, DVM, PhD, DACVS, Orthopaedic Research Center, Colorado State University, 300 West Drake Rd, Fort Collins, CO 80525. E-mail: laurie.goodrich@colostate.edu

http://dx.doi.org/10.1053/j.otsm.2016.12.007
1060-1872//Published by Elsevier Inc.

Further, equine experimental studies of musculoskeletal disease may use a strenuous, controlled exercise regimen, with horses being trained to exercise on a treadmill to standardize postinjury activity providing more accurate prognostics of healing and reinjury rates.[10-13] Therefore, equine musculoskeletal models fill an important role as a preclinical model, transitioning promising therapeutics from small animal models into an equine model with greater clinical translation.

Horses as athletes, also provide a source of naturally occurring disease including articular cartilage trauma, osteoarthritis (OA), meniscal injury, osteochondritis dessicans, tendon injury, and ligament injury allowing for both clinical and experimental disease models.[14-17] The ability to logistically handle equine research studies are limited in the United States and Europe and may result in greater costs than that of rodent studies. However, the ability to use current imaging modalities and provide a level of exercise similar to a human athlete in a species with naturally occurring disease provides unique preclinical testing that is essential to enable translation to human trials. Large animal orthopaedic research centers, such as the Colorado State University Orthopaedic Research Center, are well equipped for research studies involving horses, and costs associated with animal procurement are considered reasonable for long-term pivotal preclinical testing.

Specifically, experimental studies using horses as a model for posttraumatic osteoarthritis (PTOA) and focal articular cartilage defects provide multiple objective criteria for evaluation that include both symptom and disease modification. The horse joint is a good model for the human joint owing to the size, volume of synovial fluid, and cartilage thickness.[18] The large amount of synovial fluid allows for sequential arthrocentesis, which is particularly helpful when monitoring the joint's response to treatment.[18] For example, Ardanaz et al[19] used sequential arthrocentesis to demonstrate that repeated intra-articular administration of allogeneic MSCs did not elicit increased joint inflammation. Likewise, Williams et al[20] used repeat arthrocentesis to document the anti-inflammatory effects of allogeneic MSCs in a lipopolysaccharide model of joint inflammation. The available volume of synovial fluid in the equine joint provides enough sampling quantity for sequential tests of total protein, nucleated cell counts, cytokines, and biomarkers.[21,22]

Pain may be graded subjectively by equine veterinarians who are adept in subjective musculoskeletal examination but objective pain evaluation is also common place through the use of force plates or inertial sensor systems or both.[23] In addition, joints may be evaluated by multiple imaging modalities including radiographs, computed topography, and magnetic resonance imaging (MRI) and monitored by repeat arthroscopic evaluation.

Grossly, the articular surface is subjectively assessed for abnormalities and routinely coupled with histologic grading of joint tissues. In 2010, McIlwraith et al published an OARSI histologic grading system for experimental models of OA and cartilage degradation. This system outlined a microscopic scoring system for histologic analysis of chondrocyte necrosis, complex chondrone formation, fibrillation or fissuring, focal cell loss, and safranin O/fast green staining in addition to macroscopic scoring of erosions.[24] Other published grading systems for joint injury evaluation in human, equine, and other model systems, include the ICRS visual histologic assessment scale, the O'Driscoll scoring system, the modified O'Driscoll scoring system, and the system of Pineda et al.[25-28] Histologic grading scales remain variable among studies, thus confounding attempts to directly compare study results.[29] In addition, postmortem infrared assessment of the joint surface is a promising new technology for monitoring cartilage surface abnormalities including subtle cartilage fibrillation (Drs Markus Wimmer and David Frisbie, unpublished data).

Experimental studies of tendonitis may be evaluated with much of the same objective and subjective criteria including lameness examinations, gait analysis, and imaging. Histologic analysis may be used to identify scar tissue or assess fibril diameter, collagen fiber organization, inflammatory infiltration, and lesion progression.[30-32] Imaging modalities include ultrasound, elastography, contrast computed tomography, and MRI. In addition, tenoscopy may also be performed in areas where there is surrounding tendon sheath if sequential gross anatomical monitoring is desired.

Equine Posttraumatic Osteoarthritis Model

Equine in vivo models of joint disease include PTOA and models for focal cartilage defects. A PTOA model has been well described in the middle carpal joint of horses.[7,10,12,13,33] This model, through the creation of bone and cartilage debris as well as an osteochondral fragment, results in secondary OA that mirrors clinical disease (racing thoroughbred and quarter horses) and can be effectively monitored by radiographs (Fig. 1).[34] The model has been used to test multiple treatments including steroids, hyaluronic acid, and culture-expanded MSCs and shock wave therapy.[10,12,13,33,35,36] In addition to radiographic assessment, monitoring may include lameness examinations, gait evaluation, synovial fluid analysis, follow-up arthroscopy, and histology, MRI, and computed tomography.

Experimental OA models have generated conflicting results after intra-articular MSC administration. For example, a model of amphotericin B–induced OA in donkeys showed clinical and radiographic improvement when MSCs were administered intra-articularly.[37] In contrast, in the earlier described PTOA model, intra-articular MSCs resulted in no change in clinical outcome, histologic scores, or gross appearance but did cause a decrease in PGE_2 within OA joints.[10] It would be presumptive to directly compare the results of such grossly differing models. Amphotericin B creates a severe, long-lasting lameness through the exposure of a chemical that is foreign to the joint. In contrast, the carpal model of PTOA creates a long-term joint insult, resulting in a slow onset of OA, which is arguably more clinically realistic. It is, however, reasonable that intra-articular MSCs may result in a more potent effect in the more severe model of amphotericin-induced OA but overreaching to directly compare results.

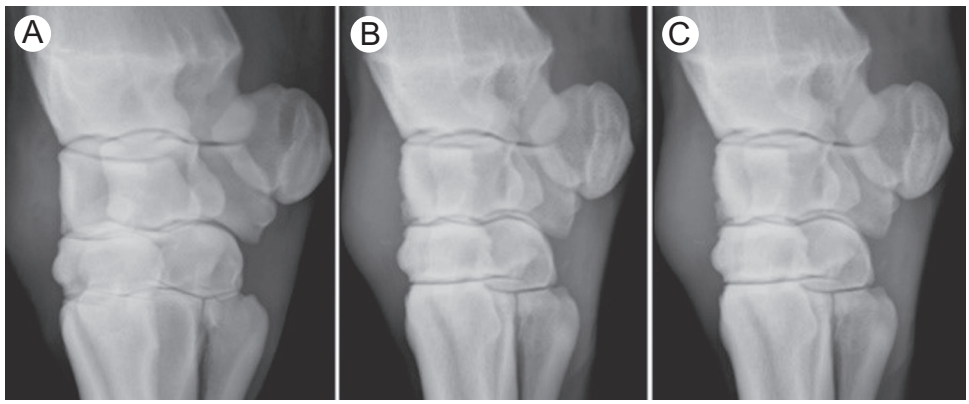

Figure 1 Dorsolateral-palmaromedial oblique radiographic images obtained 71 days after experimental induction of posttraumatic osteoarthritis of the carpus. (A) No evidence of osteophyte formation is seen at the site of a surgically induced osteochondral fragment. (B and C) Osteophyte formation is evident at the site of the surgically induced osteochondral fragment. (Adopted with with permission from Carvalho et al.[32]).

MSCs have become a popular treatment for musculoskeletal disease in the horse, and clinical studies have focused on their intra-articular use in animals with naturally occurring joint injury. A large retrospective study was performed by Broeckx et al,[38] which evaluated the use of autologous MSCs, allogeneic MSCs, or chondrogenically primed MSCs for the treatment of degenerative joint disease. In 165 cases, only 3 instances of joint inflammation postinjection ("joint flares") were observed. Overall, 78% of horses treated with MSCs and 86% of horses treated with chondrogenically induced MSCs returned to work. Interestingly, the study seemed to reveal a difference in efficacy depending on the joint treated; horses injected with chondrogenic-induced MSCs for pastern, fetlock, and coffin joint OA had a higher percentage of returning to work. In contrast, the treatment of stifle disease with chondrogenically induced MSCs resulted in a decrease in the rate of return to work.[38]

Although Broeckx et al[38] found little advantage for using MSCs in the osteoarthritic stifle. The treatment of meniscal disease appears particularly promising based on an experimental model by Murphy et al[39] reporting evidence of meniscal regeneration and significant dampening of the progression of OA in a sheep model of induced OA initiated by medial meniscectomy.[39] Similarly, Ferris et al[15] published encouraging results for intra-articular MSCs as a complementary therapy to stifle arthroscopy for the treatment of equine meniscal disease with 75% of horses returning to work. Further in vivo, controlled, experimental research is necessary to understand the benefits of MSCs and their effect on cartilage, meniscus, and synovial membrane in joint inflammation and OA with and without surgical intervention.

Equine Models of Focal Cartilage Healing

In a well-accepted and frequently used equine focal cartilage healing model, a critically sized cartilage defect is created on the non–weight-bearing portion of the stifle (the equivalent of the knee joint in humans) (Fig. 2). One to two defects can be created in each lateral (single) or medial (up to 2 defects) trochlear ridge, allowing the horse to serve as its own internal control.[40-43] Monitoring may include, as mentioned previously, lameness examinations, gait evaluation, imaging, synovial fluid analysis, follow-up arthroscopy, and histology.

Using the equine model of focal cartilage healing, MSCs have been administered in scaffolds to retain the cells within sites of injury. Examples of scaffolds include bacterial cellulose, collagen-based scaffolds, and fibrin glue.[40-43] Cartilage defects treated with MSCs imbedded in a fibrin scaffold resulted in significantly improved 30-day arthroscopic and chondrogenesis scores but no significant difference between experimental and control lesions was found in the long term (8 months).[29] When MSCs were instead combined with platelet-enhanced

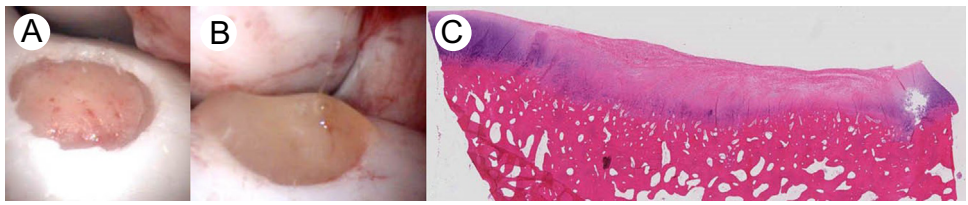

Figure 2 A model of focal cartilage healing using the trochlear ridge of the stifle. (A) A defect is created in the trochlear ridge. (B) The defect is filled with fibrin. (C) At 1 year, histopathologic analysis using H&E staining shows filling of the cartilage defect with fibrocartilage. H&E, hematoxylin and eosin. (*Courtesy*: Dr L. Goodrich) (Color version of figure is available online.).

fibrin scaffolds in a lateral trochlear ridge defect of the stifle joint, ectopic bone formed in the repair tissue, which resulted in a thinner repair tissue when compared with cell-free scaffolds alone.[44] These findings indicated that MSCs should not be combined with platelet-enhanced fibrin scaffold for cartilage regeneration owing to the risk of bone formation.[44] In contrast, when autologous, culture-expanded chondroprogenitor cells were implanted in fibrin scaffolds, improved cartilage repair tissue was observed when compared with culture-expanded allogeneic chondroprogenitor cells or fibrin scaffold alone.[45] Although, chondroprogenitor cells may differ significantly from MSCs, the study suggests further investigation into using cells within a scaffold.[45]

In a recent study, when culture-expanded MSCs were not implanted in a fibrin scaffold, but given freely into the joint, MSC treatment did not affect clinical scores. However, on gross and arthroscopic assessment, stem cell treatment did improve the firmness of the repair tissue and immunohistochemical analysis revealed greater aggrecan content compared with the control repair tissue within the defects.[46] Likewise, in 33 cases of clinical stifle injury, intra-articular administration of MSCs yielded improved outcome vs historical controls.[15]

The current literature suggests that free intra-articular MSC treatment without securing the cells within a scaffold, appears promising and deserves an ongoing experimental consideration. Additional studies are essential before firm recommendations can be made regarding scaffold-imbedded MSCs for the treatment of cartilage injuries. Research questions include the effect of scaffolds on viability of MSCs, the quality of the resultant repair tissue, and also how scaffolds affect the ability of MSCs to migrate and interact with the local joint environment.

Equine Impact Models of OA

Impact models of joint injury have been developed. An impact model of stifle cartilage degeneration and OA was first described by Bolam et al,[47] in which adult horses were subject to arthroscopically induced impact injury to the medial femoral condyle. This injury resulted in microscopic and macroscopic articular cartilage lesions, decreased GAG content in the cartilage and an increase in lameness.[47] In addition, a single contusive impact to the palmar aspect of the metacarpus has been suggested as a model for metacarpophalageal joint OA.[48] Although this model resulted in a decrease in GAG content within the cartilage and an increase in cartilage oligomeric matrix protein in the synovial fluid, macroscopic lesions were variable and palmar osteochondral disease did not develop.[48] In addition, arthroscopically created metacarpophalangeal osteochondral fragments have been used to create a model of early PTOA in the horse.[49] Furthermore, researchers at Cornell University are currently investigating a new equine model of human ankle PTOA using a single impact to the equine talus (Dr Lisa Fortier, unpublished data). Impact models may provide important insight into PTOA; however, these models are in their infancy and researchers have yet to examine the efficacy of intra-articular MSC administration in these models of OA.

Equine Models of Tendonitis

Models of tendonitis are characterized by their method of lesion induction, as either enzymatically induced or mechanically induced injury. Mechanical models of SDFT injury were first involved in the surgical removal of a window of tendon,[50] which has since been replaced by a model in which a central column of tendon is removed.[51] Other methods of tendon injury have included transcutaneous radiofrequency coblation,[52] or burr-induced mechanical injury.[53] Lesions may still be detected grossly, using MRI imaging, and with histopathologic staining (Fig. 3, unpublished data) and ultrasonographic imaging (Fig. 4, unpublished data) 12 months after mechanical disruption of the SDFT. Models of enzymatic digestion are dominated by direct injection of collagenolytic enzymes.[54,55] To improve injury consistency, Watts et al[56] recently used a model of injectable collagenase gel that produced more consistent lesions when compared with collagenase alone.

Most studies induce lesions in forelimb SDFTs. A recent study by Estrada et al[53] demonstrated that forelimbs heal differently than hind limbs, using a model in which SDFTs were injured using a synovial resector. Therefore, consideration must be given to the location of injury when assessing experimental and clinical results.

MSCs have been tested in multiple equine models of tendonitis and have also become a popular clinical treatment for naturally occurring tendonitis and desmitis. Most studies using experimentally induced lesions as well as those using horses with naturally occurring tendonitis report success.[17,30-32,57] In a study of enzymatic injury using collagenase gel, Carvalho et al[32] found that adipose-derived MSCs in platelet concentrate showed histologic and ultrasonographic improvement compared with a saline control, including superior collagen fiber organization and decreased inflammatory infiltrate. In contrast, Canaglia et al[30] found no appreciable difference in collagen fibril diameter after intratendon injection of MSCs in a model of mechanical tendonitis created by a synovial resector.

Five studies have sought to evaluate the effect of MSCs in race horses with naturally occurring tendinopathy.[17,31,57-59] These studies describe significant clinical success with 77%-98.2% of horses returning to racing.[31,58,59] However, perhaps, the most encouraging statistic is a significant decrease in reinjury rate. A study by Godwin et al[31] administered MSCs to 141 horses with chronic tendinopathy with a reinjury rate of only 27.4%, representing a significant improvement when compared to previous studies of medical management with reinjury rates of 42.5%-53%.[31,60,61] Smith et al[17] found improved histologic scoring including GAG content, DNA content, vascularity, and cellularity in tendons of chronically injured horses when the animals were treated with autologous bone marrow–derived MSCs. Lastly, Ricco et al[57] administered allogeneic adipose-derived MSCs concurrently with platelet-rich plasma to 19 horses with naturally occurring SDFT injury, and found a reinjury rate of 10.5% with 89.5% of horses returning to their previous level of competition.

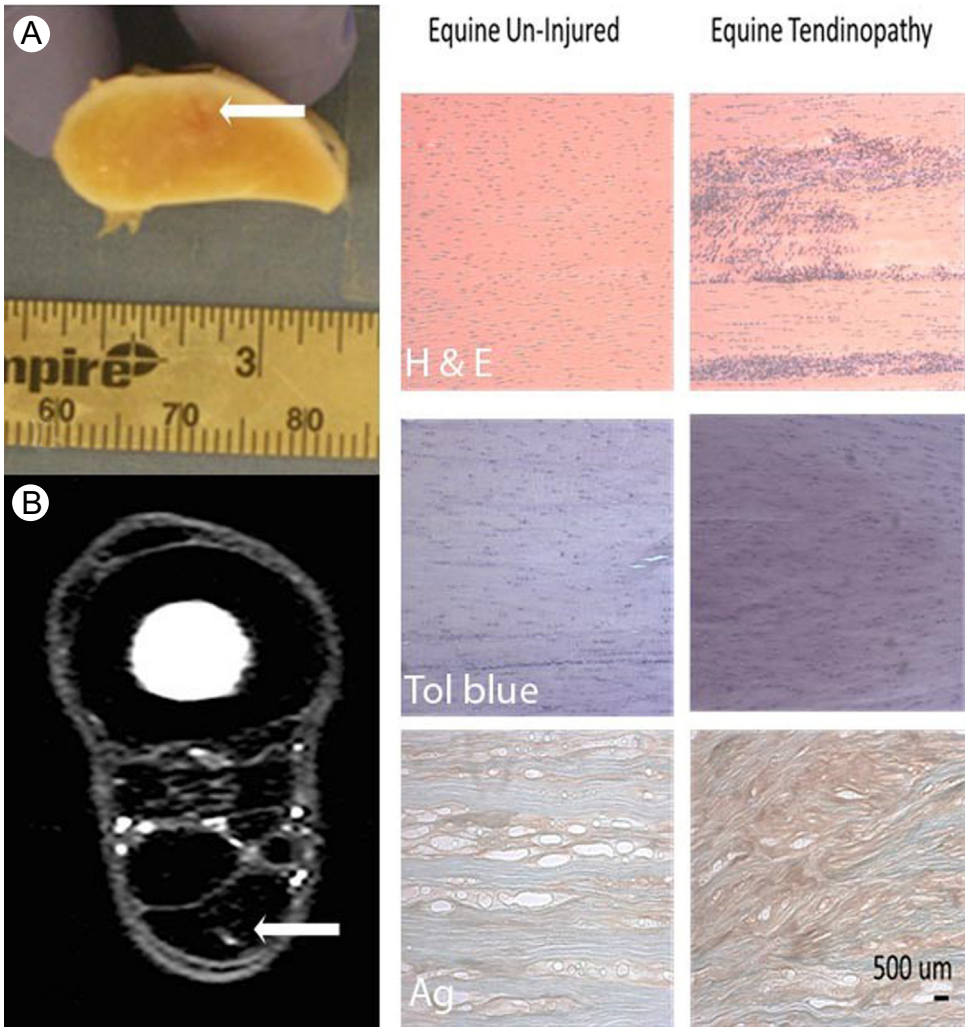

Figure 3 Mechanical model of superficial digital flexor tendinopathy. Samples of superficial digital flexor tendon (SDFT) 12 months after mechanical disruption. (A) The cross section of the superficial digital flexor tendon shows a gross lesion (white arrow). (B) Magnetic resonance imaging (trans FSE PD) shows a corresponding area of increased signal (white arrow). (C) Stained histopathological sections of superficial digital flexor tendon (H&E, hemotoxylin and eosin; tol blue, toluidine blue; Ag, aggrecan), demostrating the difference between normal tendon and 12 months after mechanical disruption (equine tendinopathy). (*Courtesy*: Drs S. Johnson and D. Frisbie) (Color version of figure is available online.).

Localizing MSCs to Sites of Musculoskeletal Injury

Although MSCs appear to aid in soft tissue and cartilage repair, it is unclear how exactly these effects are mediated. As researchers focus on paracrine secretion and the influence of MSCs on the endogenous repair processes,[2] investigators have sought to track MSCs after injection to determine the final location of the cells and their overall survival in tissues. Investigators have attempted to evaluate various routes of administration, including intravenous, intralesional, intra-arterial regional limb perfusion and intravenous regional limb perfusion (under tourniquet application). In naturally occurring tendon injuries, 24 hours after administration of MSCs, not surprisingly injected MSCs are more likely to be found in tendon tissues when the cells were administered intralesionally, whereas few cells were found at the site of the tendon lesion when the cells were administered intravenously.[62]

To track MSCs, the cells can be labeled using technetium-99m and tracked using nuclear scintigraphy or labeled with superparamagnetic iron oxide (SPIO) nanoparticles for analysis by MRI.[63-65] In addition, Burk et al[66] has described labeling of cells with Molday ION Rhodamine B labeling, thereby allowing the cells to be tracked both with MRI and flow cytometry. Finally, cells may be marked with green flourescent protein (GFP) for histologic analysis.[67]

An initial study injected GFP-labeled MSCs into a lesion created in the SDFT. The study found some GFP-labeled cells at the lesion site 10 and 34 days postinjection within the tendon lesion, demonstrating persistence of MSCs within the lesion.[67] Unfortunately, the use of GFP labeling requires euthanasia of the horses for subsequent tissue collection;

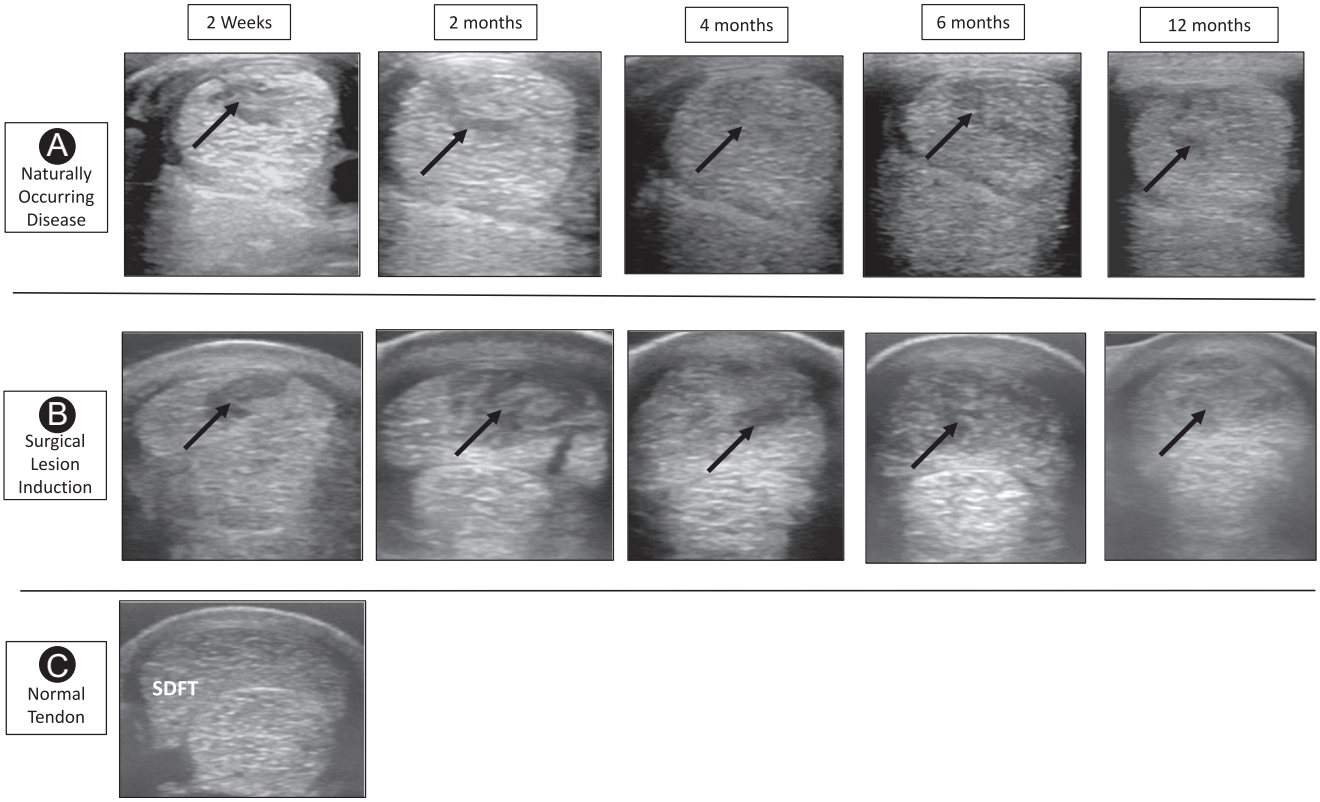

Figure 4 Longitudinal ultrasonographic evaluation of naturally occurring tendinopathy (A) and surgically induced tendinopathy (B). Normal, uninjured tendon for comparison (C). Note the diffuse enlargement of the SDFT with dark core-like lesions (arrows) in both types of injury. In both naturally and experimentally induced disease, the dark, core lesion expands in size from 0-2 months, then is gradually filled with tissue in the later phases of repair. (Courtesy: Drs S. Johnson and D. Frisbie).

therefore, quantitative measurement cannot be performed over time. In contrast, newer methods of labeling allow MSC tracking in live horses. A study using horses with naturally occurring tendon disease and technetium-99m-labeled MSCs reported only 24% of the injected cells present intralesionally at 24 hours.[62] Studies using SPIO labeling in naturally occurring tendinopathy were able to detect umbilical cord-derived MSCs within lesions in 5 of 7 horses for 8 weeks,[68] and adipose-derived MSCs in 4 of 4 horses for 9 weeks.[63] Although these studies are focused on determining cell location and survival, it is still unclear exactly how long MSCs need to survive within a lesion or whether cells even need to remain at the site of injury to stimulate joint or tendon healing.

Clinical Considerations

MSCs may be derived from several sources in horses, including bone marrow, adipose tissues, and umbilical cord tissues. Adipose-derived and bone marrow–derived MSCs are the most thoroughly investigated in the horse.[19,69-72]

Bone marrow can be easily procured in the horse, and may be obtained in a standing, sedated animal from the sternum or ilium. It has been shown that only a small volume (~5 mL) of bone marrow is necessary to maximize the yield of MSCs (compared with 50 mL), with additional bone marrow simply diluting the sample.[73] Isolation of bone marrow–derived MSCs may be performed by ficoll-gradient emulsification or by relying on the plastic-adherent properties of the cells.[74] Recently, several studies have compared MSCs derived from ilial bone marrow vs sternal bone marrow; the harvest sites produce minimal differences in growth potential in young horses.[70,73] In addition, no differences were detected in phenotype.[70] Chondrogenic potential has been reported to be modestly higher for MSCs of ilial origin, or not significantly different between the two sites of harvest.[69,73] However, another study reported no differences in chondrogenic phenotype between the 2 sites of harvest.[70] Finally, Delling et al[75] found that as horses age, bone marrow aspirate harvested from the ilial tuberosity has decreased proliferation compared with sternal aspirates. Until additional studies provide further insight into the importance of harvest site, clinician preference may dictate the location of bone marrow harvest.

Clinically, adipose tissue is typically taken from the tail base or head in the horse, although cosmetics or scarring is suboptimal. Isolation of adipose-derived MSCs is possible from 3-5 g of fat after mechanical and enzymatic digestion.[76] Adipose-derived MSCs, like bone marrow–derived MSCs are known to express mesenchymal cluster of differentiation (CD) markers CD90, CD44, and CD29 but lack major histocompatibility complex class II and CD34.[77]

After initial isolation, culture practices between bone marrow and adipose tissue are similar. Current practices most commonly rely on tissue culture plastic as a substrate for

proliferation. Once cells are 70%-90% confluent, cells can be dissociated from plastic adherence and replated for further expansion. In general, flow cytometric analysis of equine MSCs reveals consistent positive expression of CD90 and CD44 and the lack of CD34 expression.[78,79] Cell phenotype and surface marker expression is known to change after several passages, potentially signaling a loss of "stemness."[78,79] Therefore, most experimental studies use cells at the third or fourth passage. Earlier passage cells may be less uniform, and may not provide large enough numbers for experimental use.

In vitro, equine MSCs derived from bone marrow appear to have greater chondrogenic potential when compared with MSCs derived from adipose tissue.[80] In addition, researchers have found that bone marrow–derived MSCs appear superior in their osteogenic potential compared with adipose-derived MSCs in horses.[81,82] Furthermore, a study by Frisbie et al[10] found that bone marrow–derived MSCs produced a greater reduction in PGE_2, and adipose-derived stem cells caused an increase in the inflammatory cytokine tumor necrosis factor alpha when the cells were used in a model of PTOA.

In contrast, in an in vivo model of meniscal damage, adipose-derived stem cells appeared to provide an equivocal alternative to bone marrow–derived MSCs.[69] Other cell sources, such as synovial membrane–derived MSCs may provide advantages for joint-based therapies, but are not yet extensively studied in the horse.[83] Burk et al[84] investigated the gene expression of tendon markers from different MSC sources including bone marrow–derived MSCs, adipose-derived MSCs, umbilical cord tissue–derived MSCs, and MSCs from umbilical cord blood; the study concluded that adipose-derived MSCs may be more appropriate for the treatment of tendon lesions as they demonstrate the highest expression of collagen 1A2, collagen 3A1, and decorin. The earlier outlined literature suggests bone marrow–derived MSCs may be more appropriate for cartilage or bone healing, and adipose-derived MSCs may be more phenotypically appropriate for tendon lesions. However, further studies comparing the efficacy of MSCs derived from different sources will be necessary to clarify the best MSCs for various musculoskeletal conditions.

Regulation of Stem Cell Therapeutics in Animals by the Food and Drug Administration and Center for Veterinary Medicine

In 2015, the Food and Drug Administration issued a guidance (Guidance 218) regarding the evaluation of cell-based products in animals owned as pets or for show or competition, including horses. This guidance excludes animals reared intentionally for research purposes; therefore, the guidance specifically affects clinical research scenarios (clinical trials). The guidance defines cell-based products as "articles containing, consisting of, or derived from cells that are intended for implantation, transplantation, infusion, or transfer into an animal recipient." The Food and Drug Administration categorizes cell-based therapies into 4 different groups—xenogenic, allogeneic, autologous type I, and autologous type II. Autologous stem cells owing to their processing requirements are considered autologous type I; this group is defined by greater than "minimal manipulation."

An investigational new animal drug application (INADA) is required for research in client-owned animals. Thus, clinical investigation in animals with spontaneous diseases entails significant organization and paperwork including record keeping and monitoring. Before submission of an INADA, it is recommended that investigators submit a fee waiver request. A fee waiver can be granted if the fee is considered "a barrier to innovation". The INADA requires annual updates and renewal of fee waivers. Further information may be found at http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM405679.pdf.

Conclusions and Recommendations

The use of cellular therapies involving MSCs for the treatment of musculoskeletal diseases in humans has expanded rapidly in recent years. However, there are a number of key unanswered questions regarding the optimal application of MSCs for these conditions. The equine model of musculoskeletal injury can, therefore, play an important role in addressing some of these questions. In particular, this model may be useful in determining the optimal cell delivery and scaffold material (treatment of cartilage lesions), the most effective cell source and cell numbers to inject (tendon and cartilage injuries), and the best way to precondition MSCs before intra-articular or intratendon injection. Controlled experimental trials are necessary to further elucidate the mechanisms of MSCs action and to further define their efficacy. Equine models of musculoskeletal disease provide effective and realistic models for investigating cellular therapies, and can play an important role in helping to advance the field of regenerative medicine.

Acknowledgments

The authors would like to thank Dr Sherry Johnson for supplying the data and images for Figure 3.

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