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Operative Techniques in

Sports Medicine

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

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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]

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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.