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Stem Cells for Equine Joint Disease

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Bone marrow-derived autologous stem cells have been used in routine clinical practice with the promise of improving the return to function of severe joint injuries. This paper defines where the field is today. Author's address: Equine Orthopedic Research Center, Colorado State University, 300 West Drake Road, Fort Collins, CO 80523; e-mail: dfrisbie@colostate.edu. © 2011 AAEP.

1. Introduction

In the last decade, researchers have been looking into the use of stem cells to aid in various joint pathologies. These pathologies can loosely be broken down into three categories: (1) cartilage resurfacing, (2) osteoarthritis, and (3) damaged intra-articular soft tissue structures like the meniscus. It must be realized that joint disease usually has some component of all three categories. To date, the equine peer-reviewed literature has been focused on autologous cells for two main reasons: (1) the immunogenicity of allogenic equine stems cells has yet to be established in the peer-reviewed literature, and (2) the legality of the regulatory pathway for commercial use of allogenic cells is not established. Thus, this manuscript will focus on autologous cells.

2. Cartilage Resurfacing

Researchers from Cornell University have been assessing autologous stem cells mainly for cartilage resurfacing techniques. They have been using fibrin as a scaffold to hold the mesenchymal stem cells (MSCs) in cartilage defects. This is an elegant technique that, until recently, had required the MSCs to be prepared before the arthroscopy. This approach does have the disadvantage of leaving the surgeon uncertain of the need for the MSCs until diagnostic arthroscopy is performed. Thus, if a lesion is not identified, this approach may not be the best use of resources. It also requires the availability of some specialized equipment, mainly gas inflation. Although some clinical success has been reported by Wilke et al.,1 in a controlled experimental study using autologous culture-expanded cells, did not show long-term beneficial effects. Researchers at Colorado State University (CSU) Orthopedic Research Center have taken a different approach by injecting MSCs into the joint space without the use of a scaffold. This technique is employed 30 days post-diagnostic arthroscopy, thus obviating the need for preparing the MSCs before ascertaining the need for this treatment modality. The timing of treatment application was based on return to athletic use in a group of clinically treated horses that had an average 21-mo follow-up visit. In an in vivo study, the group at CSU was able to show long-term benefits. Specifically, a 1-cm,2 full-thickness medial femoral chondral lesion was created in both stifles of the study horses. The defects were debrided down to the level of the subchondral bone plate, and subchondral bone micro-fracture was performed. All medial femoral joints were treated with either hyaluronan (HA) or HA +
MSC directly injected into the joint space 30 days post-lesion creation; the treatment sides were randomly selected. After 1 yr of strenuous exercise, some softening of the microfracture (HA only) repair tissue was noted, but the repair tissue in the MSC-treated joint remained firm compared with a 6-mo time point. Furthermore, the degree of aggregan staining was improved in the MSC-treated repair tissue compared with microfracture alone at the 12-mo time point. Based on this work and the work of Lee et al., who also administered MSCs to the joint space in a porcine model, it seems that direct intra-articular administration of MSCs may have long-term advantages compared with delivery into fibrin for cartilage resurfacing techniques at this time.

It is notable that a recent paper by Fortier et al. shows significant gross and histologic improvements using bone marrow that was aspirated from the sternum and centrifuged to generate autologous bone marrow concentrate, which was held in the experimentally created defect after activation with thrombin forming a gel or glue. Fortier et al. report some uncertainty on what in the bone marrow concentrate is responsible for the exciting results (cells and growth factors), and more work will have to be done to elucidate this but will be worth additional discussion.

3. Osteoarthritis/Soft Tissue Structures

Early work using labeled MSCs has shown that they do have an affinity for damaged joint tissues, including fibrillated cartilage, and more recently, in vivo studies have confirmed their ability to localize and participate in the repair of damaged joint structures, including cruciate ligaments, menisci, and cartilage lesions. Some of the first work suggesting promise for the use of MSCs in treating arthritis was published by Murphy et al. in 2003. Although this goat study showed regeneration of the meniscus, its aim was to evaluate the in vivo effects of intra-articular stem cell therapy on decreasing the progression of osteoarthritis (OA). This study created an unstable joint model by removing the medial meniscus and transecting cranial cruciate ligament, leading to the subsequent induction of OA. Murphy et al. concluded that the decrease in OA seen in the study seemed to be secondary to the regeneration of the medial meniscal tissues, which was dramatic in seven of nine cases. However, the design of inducing OA did not lend itself to determining if the stem cells had a direct effect on the articular cartilage and/or progression of OA. A subsequent equine study used an osteochondral fragment with bone and cartilage debris to induce OA, and unlike the work by Murphy et al., the equine model used a stable joint. The goal of the study was two-fold: first, to evaluate the effect of bone-derived culture-expanded MSCs and adipose-derived stromal vascular fraction (SVF) on improving acute OA and second, to compare the two treatment modalities.

The results of this study indicated significant improvement in synovial fluid prostaglandin E2 (PGE2) levels in response to treatment with bone-derived cells, although significant differences were not shown in other key parameters (clinical lameness and radiographic, histologic, and biochemical parameters). The study also showed an increase in synovial fluid tumor necrosis factor (TNF) concentrations in response to adipose-derived cells, which would be interpreted in a negative light. The conclusion of the study is that nominal improvements in symptom or disease-modifying effects were seen with bone-derived stem cells. Furthermore, the administration of adipose-derived SVF up-regulated the production of proinflammatory cytokines. This stable acute OA model brings up questions about the use of MSCs for the treatment of OA that will need to be critically assessed through additional research and follow-up clinical results.

Trying to simulate the beneficial results seen by Murphy et al., researchers at CSU have been treating various clinical cases of joint disease that have arthroscopic confirmation using a combination of HA and autologous MSCs, with special interest in those diagnosed with meniscal injury. A 6-mo follow-up pilot project involving 15 cases showed promising results and led Frisbie et al. to expand the study into a multicenter trial. The results of this prospective multicenter trial were also very promising. Specifically, 39 cases were treated with intra-articular (IA) administration of autologous bone marrow-derived MSCs and had a mean follow-up period of 21 mo. It is noteworthy that cases selected for this trial were meant to have failed routine treatments, be moderate to severely affected, and have surgical confirmation of the diagnosis. Seventy-seven percent returned to some level of work. Thirty-six percent (14/39) returned to or exceeded their prior level of work, 36% (14/39) returned to work at a lesser level or required some level of additional medical treatment in the affected joint to maintain soundness, and 28% (11/39) did not achieve work status before follow-up. Stifle injuries comprised 29 of 39 cases, with 20 having a primary diagnosis of meniscal damage. Interesting differences were noted when these data were compared with published studies on meniscal damage with surgery alone. With surgery alone, grade 3 meniscal tears had a 0% and 6% return to work compared with the current study with MSCs and surgery having a 60% return to work. The intra-articular treatment with autologous cells had an 8% incidence of acute inflammation or flare, which given the concurrent administration of HA (12% flare incidence reported), would be expected or lower than expected incidence. Thus, in clinical cases of joint disease (especially the more severe), it seems that the addition of stem cells may improve the horse’s ability to return to work over surgery alone. The next logical step would be a placebo
controlled clinical trial; however, this is unlikely because of difficulties gaining owner compliance for such a study design, but a positive controlled clinical trial may be possible and shed more light on the most useful treatment applications for cell-based therapies.

4. Conclusion
In conclusion, the use of endogenous cells stimulated through subchondral bone microfracture seems to be the state of the art for equine cartilage resurfacing. It seems that this technique can be significantly augmented with the addition of MSCs to the joint space. There is evidence that MSCs do not work in all cases of stable OA, but we do not have enough information to make a definitive conclusion on all types of OA. Finally, there seems to be evidence that augmenting surgery with MSC administration improves the return to work of horses suffering from severe meniscal injury. In summary, it is early in the use of MSCs for the treatment of joint injury; however, it seems that they will provide benefit to our equine patients, but we have much to learn.

Dr. Frisbie is a shareholder in Advanced Regenerative Technologies (ART).

References