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Review Article

Clinical update on the use of mesenchymal stem cells in equine orthopaedics

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Summary

Stem cells have received much attention in recent times because of their potential to improve healing of othropaedic problems. This manuscript presents the genesis, issues and current state of stem cell treatment in equine medicine. Current literature supports the use of mesenchymal stem cells (MSCs) for treatment of orthopaedic problems.

Introduction

Stem cells continue to receive a great deal of scientific attention as well as coverage in the lay press. One of the many reasons for the attention derives from these cells having the potential to regenerate tissues without the production of scar tissue that is generally associated with healing processes.

This overview focuses on the clinical use of mesenchymal stem cells (MSCs) in horses and the justification for, and issues surrounding, their use. Many of the early reports used bone marrow as a source of these cells, but other sources have been more recently demonstrated. For example muscle, cartilage and adipose tissue all have been shown to contain multipotent MSCs.

Recovery techniques for mesenchymal stem cells

Isolation of MSCs from the marrow or digested tissue extracts is most commonly achieved by simple adhesion and proliferation of MSCs to tissue culture surfaces. This crude technique fails to ensure a homogenous population of MSCs because cells such as fibroblasts may likewise readily adhere and proliferate. While nonprogenitor cell contamination may be an expected outcome of the adhesion sorting technique, the extensive volume of literature detailing bulk multipotent behaviour of adherent MSC populations demonstrate the presence of a significant, if not a homogenous, MSC population. In fact near-homogenous MSC populations have been reported from adhesion sorting (Pittenger *et al.* 1999). Researchers are currently working on more rigorous methods of identifying stem cells through the use of cell surface antigens, such as cluster differentiation (CD) factors 34 and 44. There is still significant research to be done in this area, and a consensus on the exact antigen profile of an MSC has not been reached. Recent work has suggested that Ficoll separation improves the initial yield of mesenchymal stem cells but that this difference is not significantly different after the first passage (Bourzac *et al.* 2010).

Bone marrow vs. adipose tissue as a source of mesenchymal stem cells

Most of the research aimed at clinical treatments has been carried out using autologous MSCs, mainly from bone marrow (Muschler *et al.* 2004). Specifically, bone marrow derived stem cells have been used to generate bone, cartilage, tendon, ligament, meniscus, intervertebral disc, fat, muscle and nerve (Muschler *et al.* 2004). Because of the availability of adipose tissue, it too has received a fair amount of recent research as a source of MSCs (Zuk *et al.* 2001). Ease of collection procedure, number of stem cells recovered, capacity and efficiency to differentiate into various mesenchymal tissues, as well as morbidity associated with the collection procedure are all important points to consider when discussing bone-marrow versus adipose derived stem cells.

Because MSC treatments are being used from both fat and bone, it is important to be familiar with direct comparisons that have been published (Winter *et al.* 2003; Im *et al.* 2005; Kisiday *et al.* 2008; Noel *et al.* 2008; Vidal *et al.* 2008; Frisbie *et al.* 2009). A summary of the current evidence suggests that, while adipose derived MSCs have the ability to differentiate into musculoskeletal tissue, they appear inferior to bone marrow derived MSCs given the current understanding of differentiation conditions. Further, equine specific research also suggests this to be true (Kisiday *et al.* 2008; Vidal *et al.* 2008; Frisbie *et al.* 2009).

To date, a fair number of papers have explored the comparison of bone derived vs. adipose derived stem cells in musculoskeletal tissues. In the basic science arena, the preponderance of the work has determined a superiority of bone marrow when compared to adipose derived cells. In some cases, with the appropriate manipulation, adipose derived cells can begin to differentiate into musculoskeletal tissue in a similar fashion to bone derived MSCs but even then not superior to the bone derived cells (Hennig *et al.* 2007). Side by side comparisons have been completed in equine tissue and have concluded that, while adipose derived MSCs have

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the ability to differentiate into musculoskeletal tissue, they appear inferior to bone marrow derived MSCs given our current understanding of differentiation conditions (Kisiday *et al.* 2008; Vidal *et al.* 2008; Frisbie *et al.* 2009). It is important to note that these side by side comparisons were normalised for cell numbers to help ensure a fair comparison. In the current clinical setting, this is not the case because the number of MSCs contained in adipose derived stromal vascular fraction (2–4% of the nucleated cell count) is far less than that of treatments of bone derived culture expanded MSCs. Further, bone derived culture expanded cells affords the clinician to treat with cell numbers in the millions where current adipose derived sources do not culture expand the cells and therefore are providing the clinician with cell numbers only in the hundreds of thousands.

While definitive dose titrations are still needed, most *in vivo* work showing significant and/or promising results utilise MSC numbers in the millions. This suggests a possible sub-therapeutic number of MSCs with the current method of adipose stromal vascular fraction. The authors are unaware of published research that supports any difference in clinical improvement comparing culture expanded to nonexpanded cell populations. Although, a report in sheep suggests a significant increase in mononuclear cell infiltrate and CD34+ (MSC cell surface marker) cells are present if nonexpanded bone marrow aspirate is used compared to culture expanded bone marrow derived MSCs in a collagenase induced tendonitis model (Crovace *et al.* 2008).

Clinical use of mesenchymal stem cells in animals

Some of the first treatments to be termed 'stem cells' were really bone marrow aspirates that were taken typically from the sternum or ilium and then injected directly into the tendon/ligament lesion. While this treatment protocol gained some early traction, today few would consider this 'stem cell' treatment given that the concentration of stem cells is around 2000/ml and only a few mls are typically delivered. Despite the majority of research being conducted on bone marrow derived mature mesenchymal stem cells the first commercial product in the US was based on adipose derived stromal vascular fraction (AD-SVF) (Vet-Stem)[1] while, at the same time in the UK, bone marrow-derived cultured MSCs were developed for the commercial use (VetCell Bioscience Ltd)[2].

While to date the AD-SVF technique has been utilised widely in veterinary medicine, only 2 publications appear, both for the treatment of osteoarthritis in dogs, which has been a more recent target population (Black *et al.* 2007, 2008). These publications both show promising results and one is a randomised controlled study with n = 10. More recently 2 commercial companies (Advanced Regenerative Therapies [ART][3] and VetCell) have emerged from university research groups to provide a bone derived culture expanded source of stem cells. Smith and colleagues were integral in the formation of VetCell based on research at the Royal Veterinary College, London. This group has been very active in looking at the use of stem cells for treatment of superficial flexor lesions. They have treated over 1500 horses worldwide with a cohort from the UK followed up for more than 2 years and have shown more promising results as compared to historical controls. In a similar manner ART was founded out of work done at the Orthopaedic Research Center at Colorado State University. This group started looking at stem cells for the treatment of joint related issues in 2003 and has more recently amassed long-term follow-up (2005–2007) on both joint and tendon/ligamentous injury treated with bone derived culture expanded autologous stem cells. Randomised controlled studies have been published by this group both utilising *in vitro* (Kisiday *et al.* 2008) and *in vivo* (Frisbie *et al.* 2009) methods. Both have shown superiority of bone vs. adipose derived stem cells using equine tissue thus directing this groups approach to MSC clinical trials.

Stem cells for treatment of joint related disease in horses

Early work, using labelled MSCs, has shown that they do have an affinity for damaged joint tissue and more recent *in vivo* studies have confirmed their ability to localise and participate in repair of damaged joint structures, including cruciate ligaments, menisci and cartilage lesions (Agung *et al.* 2006). Most of the *in vivo* studies utilising MSCs has focused on meniscal repair, in some cases using MSCs in a carrier or scaffold while others utilise direct injection into the joint (Murphy *et al.* 2003; Izuta *et al.* 2005; Yamasaki *et al.* 2005). These studies have shown good support for use of bone marrow derived cells for treatment of meniscal damage. The degree of damage has ranged from experimental meniscal lacerations treated with bone marrow aspirates, separating and utilising only the nucleated cells (Abdel-Hamid *et al.* 2005), to total medial meniscectomy treated with injection of bone marrow derived culture expanded MSCs in goats (Murphy *et al.* 2003). With respect to cartilage healing, early work indicted that the use of MSCs deposited in a fibrin matrix would be useful in improving cartilage healing. Although a recent equine study demonstrated early benefit, no significant differences were noted when MSCs plus fibrin was compared to fibrin alone at 8 months (Wilke *et al.* 2007). Based on this work, it appears likely that modulation of the matrix or cells will need to be accomplished to observe long-term benefit of MSCs for cartilage repair.

The previously mentioned goat study, while showing regeneration of the meniscus, was aimed at evaluating the *in vivo* effects of intra-articular stem cell injection on decreasing the progression of osteoarthritis (OA) (Murphy *et al.* 2003). This study used a medial meniscectomy and cranial cruciate transection model to induce OA. The investigators concluded that the decrease in OA seen in the study appeared to be secondary to the regeneration of the medial meniscal tissues, which was substantial in 7 of 9 cases. However, the design of the study did not lend itself to determining if the stem cells had a direct effect on the articular cartilage and progression of OA. Thus, Frisbie *et al.* (2009) completed an equine study that used an osteochondral fragment with bone and cartilage debris to induce OA, unlike the study by Murphy *et al.* (2003), which relied on joint instability (medial meniscal model) to create secondary OA. The results of this study indicated significant improvement in synovial fluid prostaglandin E2 (PGE2) levels in response to treatment with bone derived cells. Also demonstrated was a negative response via an increase in synovial fluid tumour necrosis factor (TNF) concentrations in response to adipose derived cells.

The beneficial response seen with bone derived cells overall was interpreted as a nominal improvement in symptom or disease modifying effects (Frisbie *et al.* 2009). The results of this study and that of Murphy *et al.* (2003) suggest that the regeneration of the medial meniscus in the latter study may have in fact been the reason for less OA progression. Furthermore, these studies also suggest that MSCs by themselves do little to counteract the progression of acute OA mediated by enzymatic degradation and joint debris. It would appear modification of the MSCs is needed if they are to be

useful in treating the OA. Treatment timing in relation to the degree of pathology could also be a factor contributing to the insignificant results of the equine study. Specifically, because MSCs appear to have a tropism for damaged cells, including fibrillated articular cartilage, it may be that at Day 14 (day of treatment) the degree of fibrillation was not great enough for an effect of MSCs treatment to be realised. Evaluation in cases with more advanced fibrillation would need to be conducted to answer this question. Because significant improvement in acute OA could not be demonstrated following intra-articular treatment the authors have a dampened enthusiasm for the use of MSCs in clinical cases of acute OA. The authors concluded that the use of MSCs appears to be indicated with loss of soft tissue structures leading to instability, such as with meniscal damage, and have pursued this treatment modality clinically specifically in a multicentre trial.

The results of this prospective multicentre trial (Ferris *et al.* 2009) are promising. Currently 39 cases have been treated with IA administration of autologous bone marrow derived MSCs these cases have a mean follow-up time post treatment of 21 months. 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 (Ferris *et al.* 2009); 38% returned to or exceeded their prior level of work; 38% returned to work at a lesser level or require some level of additional medical treatment in the affected joint; and 28% (11/39) did not achieve work status prior to follow-up. Stifle injuries comprised 29 of the 39 cases.

This work is an extension of the study presented at the American College of Veterinary Surgeons Symposium in 2007 where there were 15 cases with 6 month follow-up and a 67% return to work. These data suggest further exploration of MSC for the treatment of joint related soft tissue pathology. This study has also evaluated the effect of the timing of treatment with respect to outcome and this suggested a superior long-term outcome when treatment was instituted greater than a month after diagnosis (Ferris *et al.* 2009). To the author's knowledge, this is some of the first work assessing outcome related to the time of injury, diagnosis and subsequent treatment with MSCs for joint disease.

Stem cells for treatment of tendon/ligament related disease

Some of the first published work assessing stem cells in the treatment of tendon disorders was conducted using adipose derived stromal vascular fraction (Nixon *et al.* 2008). This and subsequent work with bone derived MSCs (Schnabel *et al.* 2009) both demonstrated improved histological scores following treatment when compared to controls using a collagenase model of tendonitis. Fortier and Smith (2008) have shown some promising clinical results in national hunt horses treated with bone derived MSCs when compared to historical controls. The most recent assessment has assessed 25 National Hunt racehorses with naturally occurring superficial digital flexor tendon injury in an identical fashion to the data published for 17 conventionally managed National Hunt racehorses (Dyson 2004) re-injury rate over 2 years after a return to full work (Smith 2008). The re-injury rate for the MSC-treated horses was significantly lower (24%) than in conventionally managed horses (56%; $P < 0.05$). This apparent clinical benefit is being supported by preliminary data from an experimental study utilising naturally-occurring disease and comparing stem cell-treated cases with saline-injected controls, which is showing significant benefits in mechanical, organisational and compositional parameters 6 months after treatment with MSCs.

Ferris *et al.* (2009) have also completed a long-term follow-up study (average 21 months) on horses with soft tissue injury (tendon or ligament) treated with bone derived MSCs that indicated 85% (61 cases) returned to work with 51% returning or exceeded their previous level of work, 34% did return to work but at a lesser level and 15% had not returned to work at the time of follow-up. Similar promising results were published by Pacini *et al.* (2007) showing significant improvement in the ability of horses to return to racing following bone marrow derived MSC treatment when compared to a population of horses treated with conventional methods and similar rehabilitation protocols (Pacini *et al.* 2007). Specifically, 9 of 11 treated horses recovered from SDF injury, had an excellent ultrasound image of tendons after a period ranging 3–6 months, and returned to racing with good or even optimal results in 9–12 months without any re-injuring event. Of the control horses, all had re-injured by 12 months. These results are encouraging for the use of bone marrow derived stem cells in the treatment of soft tissue lesions. In the future, comparative studies assessing various different therapeutics will help delineate where and when the use of bone derived stem cells are indicated.

In conclusion, the field of stem cell research is an ever evolving science and much work is still needed in the clinical application of this treatment modality. It is important for clinicians to continue to communicate openly on the success and failures with the emerging modality, under evidence-based (EBM) conditions.

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Manufacturers' addresses

[1]Vet-Stem, Poway, California, USA.
[2]VetCell Bioscience Ltd., Cambridge, UK.
[3]Advanced Regenerative Therapies, Fort Collins, Colorado, USA.

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