Evidence for Stem Cells in Cartilage Regeneration

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Stem cells hold tremendous promise for the treatment of tendonitis and joint repair. There are several therapies currently being used or marketed under the guise of stem cell therapy; therefore, understanding the fundamentals of stem cell biology is important for choosing the appropriate treatment type and application protocol. We will discuss the definitions of stem cells and review current studies and clinical outcomes of the various stem cells therapies being used in tendon and cartilage regenerative efforts. Authors’ addresses: Department of Veterinary Clinical Sciences, VMC C3-181, Cornell University, Ithaca, NY 14853 (Fortier); and Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK (Smith); e-mail: laf4@cornell.edu. © 2007 AAEP.

1. Introduction

What is a stem cell? There are two broad categories of stem cells: embryonic and adult-derived. Embryonic stem cells (ES cells) are by definition derived from embryos, more specifically, from day 8, pre-implantation blastocysts. Adult-derived mesenchymal stem cells (MSCs) can be obtained from bone marrow, fat, muscle, and many other tissues including cartilage, trabecular bone, and tendon. Hematopoietic stem cells (HSCs) are those cells in the bone marrow that are the basis of bone marrow transplantation and are capable of forming all types of blood cells. Arguments can be made regarding the optimal stem cell source for applications in regenerative therapies, and importantly, studies are needed to define the need for stem cells in such endeavors. There are more data available regarding cartilage tissue engineering, and those data support the need for the presence of cells (chondrocytes or stem cells) in a graft composite, but similar data are less abundant for tendon regenerative studies.

The definition/identification of stem cells is constantly evolving. Confounding the issue of stem cell definition is the concept of stem cell plasticity where a lineage committed stem cell might differentiate, or transdifferentiate, into a cell of a completely different tissue lineage; this is commonly termed “plasticity of stem cells.” Therefore, the identification of stem/lineage committed cells can be ambiguous. There is no current consensus on a gold standard assay to isolate or identify stem cells. It is important to remember when evaluating cell surface marker expression that several markers are common to multiple cells types, particularly nucleated white blood cells. Because there is no single marker for MSCs, a panel of markers must be evaluated together. For example, a marker commonly used to assert isolation of stem cells is CD44 (cluster of differentiation 44). However, many cells, including lymphocytes, granulocytes, and thymocytes

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(realistically, nearly everything but platelets) display surface antigens for CD44. Therefore, a mesenchymal or adipose-derived stem cell should be positive for CD44, but should also be negative for CD34, which is only present on HSCs and endothelial cells, or negative for CD45, which is displayed on granulocytes and lymphocytes. Our laboratory is working toward developing a panel of markers for identification of equine adult tissue-derived stem cells. The aim is to use this panel of antibodies to determine the absolute number of stem cells derived from bone marrow aspirate, adipose tissue, and muscle.

In pre-clinical studies, some of the most convincing experiments of stem cell differentiation used cell surface markers to show isolation and in vitro differentiation, and they showed functionality of the stem cells in animal models of disease. However, all of the studies so far have been performed in rodents, the majority using severe, chemical, or irradiation-induced animal models of disease. Many reports simply show that MSCs home or “engraft” to tissues, but do not show any functional consequences of cell transplantation. Similar studies exist for ES cells. There are, however, a few promising studies documenting functional recovery of nerve and hepatocyte function after transplantation of MSCs and recovery of nerve and cardiac function subsequent to ES cell transplantation. The results of these studies hold tremendous promise for the treatment of many clinical maladies such as liver failure, Parkinson’s disease, and spinal cord or peripheral nerve transection. The clinical potential of HSCs, MSCs, and ES cells would seem to be limitless given the emerging reports of in vivo transdifferentiation of HSCs into neuronal cells, neuronal cells into hematopoietic cells, and ES cells into lymphohematopoietic cells. One of the most convincing studies of stem cell application is in a fetal xenograft model; human HSCs were injected into the peritoneum of fetal sheep that had no prior chemical liver injury. The human HSCs localized to the liver and seemed to function as determined by their secretion of human albumin. This study showed transdifferentiation/plasticity of stem cells in the absence of prior tissue injury and function of the newly acquired cell phenotype. To date, equine studies that have investigated the use of “stem cells” contain no information regarding characterization of the cells before implantation or data concerning survival or function of the transplanted/grafted cells.

2. Stem Cells for Tendons: What Is the Evidence?

After tendon injury, the scar tissue that replaces the damaged tendon results in reduced performance and a substantial risk of re-injury (56% risk of re-injury for National Hunt jump horses after superficial digital flexor tendon [SDFT] tendinopathy).7 Therefore, if we are going to be able to avoid these poor functional outcomes, it is necessary to replace the injured tissue with a matrix more like tendon and less like scar tissue. It was hypothesized that the implantation of MSCs, in far greater numbers than is present normally within tenotendyn tissue, would have such potential. This hypothesis was supported by the apparent poorer differentiation capacity of tendon-derived cells,8 and the nature of the lesion that invariably results in an enclosed cavity that can retain implanted MSCs without the need for a scaffold. In support of this hypothesis, equine MSCs cultured in the laboratory are able to synthesize a matrix that is remarkably ordered, and when cultured on acellular sections of equine tendon, they survive, proliferate, and invade the tissue and express genes of tenocytes.9 In addition, this injury provides an environment that potentially provides all the necessary components for tendon tissue engineering by providing a vascularized scaffold (granulation tissue), a rich growth factor milieu and a mechanically appropriate environment. Finally, this hypothesis has been supported by experimental studies in laboratory animals of MSC implantation in surgical lesions that have shown favorable effects on tissue organization, composition, and mechanics of MSCs implanted tendons and ligaments over controls.10–13

3. Stem Cells in Cartilage Regeneration

ES cells have shown promise for cartilage regeneration. Wakitani14 showed that ES cells transplanted into rat joints formed cartilage and not teratomas, which was theoretically possible. Several research groups have optimized culture conditions to enhance the chondrogenic differentiation of ES cells, but because of the remaining barriers of routine transplantation such as immune rejection and the relative difficulty in maintaining ES cells, progress in ES cell transplantation for cartilage repair has been measured. Rather, the focus is being placed on generation of a more readily available and autogenous cell-based product such as MSCs.

When considered together, cartilage studies reveal that three components are needed for cartilage regeneration; cells, scaffold, and growth factor/s. Our laboratory has generated a stem cell concentrate from sternal bone marrow aspirate and we are currently testing it in an equine model of articular cartilage loss. For these studies, bone marrow aspirate is obtained at the time of surgery and centrifuged to concentrate the cellular population and platelets in bone marrow aspirate. Using flow cytometry, our data indicate that the final total nucleated cell population contains ~15% stem cells. The concentrate also contains a large number of platelets, which are the body’s natural reservoir of several growth factors such as insulin-like growth factor (IGF)-I, transforming growth factor (TGF)-β, and fibroblast growth factor (FGF), which are known to enhance cartilage matrix synthesis. The concentrate can be mixed with thrombin to cleave the fibrinogen into a fibrin scaffold to hold the milieu of MSCs and growth factors. This method has the advantages of being a point-of-care technique (no laboratory culture period is necessary) that is completely autogenous, arthroscopically applicable, and delivers all three components believed to be important for car-

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tilage regeneration: cells, growth factors, and a scaffold. We have treated 10 research horses in which 15-mm full-thickness defects were made on the lateral trochlear ridge of the femur and 18 clinical cases. No animal had post-operative synovitis of other detectable adverse reaction. All animals are convalescing and therefore follow-up data is not available.

4. Stem Cells for Tendon Injuries: Practical Aspects
Two techniques are currently available for the treatment of tendon and ligament injuries with MSCs. One uses a mixed cell population derived from adipose tissue and the other relies on a cultured cell population derived from bone marrow. Each technique has its strengths and weaknesses.

Bone Marrow–Derived MSCs
Bone-marrow–derived MSCs (BM-MSCs) were chosen because they seem to perform superiorly to MSCs recovered from other tissues (including tendon) in terms of differentiation into known cell types.8 Furthermore, BM-MSCs have received the most attention scientifically and hence are the best characterized. Bone marrow is collected from the sternum (or the tuber coxae) under standing sedation, followed by isolation and expansion of the nucleated adherent cell population (containing the MSCs) in the laboratory. A 3-wk culture period is needed to expand these selected cells until in excess of $10^6$ cells are available for implantation under standing sedation into the tendon core lesion using ultrasound guidance. The cells are suspended in bone marrow supernatant for implantation so that no “foreign” material is implanted and to gain potential beneficial effects of the rich growth factors present in the supernatant. Research in our laboratory has shown significant anabolic effects of bone marrow supernatant on cell cultures of ligament-derived cells. Thereafter, the horses enter a controlled exercise program for up to 48 wk (see Fig. 1 for an overview of the protocol).

Adipose-Derived MSCs
This technique is based on data that suggested that adipose-derived MSCs exhibited a similar degree of

Fig. 1. The stem cell–based therapeutic approach to the treatment of equine superficial digital flexor tendinopathy. From the left in a clockwise fashion: aspiration of bone marrow from the sternum of the standing horse; recovery and expansion of mesenchymal stem cells (bone marrow stromal cells); re-suspension of $10^6$ stem cells in citrated bone marrow supernatant; sterile implantation of the cells into the central core lesion under ultrasound-guided injection. (Figure courtesy of the British Journal of Sports Medicine.)
multi-potentiality to BM-MSCs, although in many studies they performed less well than BM-MSCs in differentiation.8,15,16 The currently available technique uses a mixture of cells derived from the adipose tissue (taken surgically from the tail head) once the cells containing fat have been removed—there is no culture step. This has the advantage of supplying large numbers of cells (but with only an estimated 2% being MSCs) in a short period of time (48 h). The cells are implanted under ultrasound guidance as outlined above. No references regarding the application of adipose-derived MSCs (A-MSCs) in equine tendonitis are presently available, although the results of a pilot study has been presented.17 In that study of two groups of four horses in which lesions were created in the SDFT with collagenase, there was a significant improvement in histological score in the A-MSC–treated tendons over phosphate-buffered saline–treated control tendons.

5. Does MSC Implantation Work for the Treatment of Tendinopathy?

BM-MSCs
Initially, a phase I trial was performed to ensure safety. This consisted of six horses with large core lesions in their SDFTs; results indicated that the technique did not cause any worsening of the injury. Furthermore, there was no reaction or enlargement of the tendon post-implantation, and no bone (on gamma scintigraphy) or cartilage (on ultrasound) was formed. Core lesions filled in quickly when a hypoechoic lesion was still visible at the time of implantation (Fig. 2). The longitudinal pattern, however, remained inferior to normal tendon but improved with exercise.

Since the initial trial, in excess of 400 horses have been treated with this technique. At the most recent evaluation of clinical outcome (September 2006; see Table 1), 168 racehorses had been treated, and long-term (>1 yr) follow-up was available for 82 horses. For National Hunt racehorses (n = 71), the re-injury rate was 13% (including injuries to untreated contralateral limbs). When only those horses that had entered full training were included, the re-injury rate rose slightly to 18%. This compares favorably with previous analyses for the same category of horse (56% re-injury rate for National Hunt horses),7 although this analysis was over 2 yr rather than 1 yr after a return to full work. Further follow-up of these treated horses after this time period will allow direct comparison. However, in further support for this improvement in outcome, re-injury rates for sports horses (all disciplines combined; n = 24 with >1 yr follow-up) was improved by a similar degree (13% compared with 23–43% reported for different sports horse disciplines by Dyson).7

When comparing those horses that did not re-injure with those that did, there was a significantly longer interval between injury and implantation for the fail-

Table 1. Re-Injury Rates After BM-MSC Treatment

<table>
<thead>
<tr>
<th>Use</th>
<th>No. SDFT-Treated</th>
<th>SDFT With Follow-up for &gt;1 yr</th>
<th>Success Rate (No Re-injury Only Those Horses Returned to Full Training; &gt;1 yr in Full Work; Both Limbs)</th>
<th>Success Rate (No Re-injury Conventional Treatment; &gt;2 yr in Full Work; Both Limbs; Dyson 2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>National hunt</td>
<td>145</td>
<td>71</td>
<td>82%</td>
<td>44%</td>
</tr>
<tr>
<td>Flat</td>
<td>23</td>
<td>11</td>
<td>50%</td>
<td>34%</td>
</tr>
<tr>
<td>Total (all racehorses)</td>
<td>168</td>
<td>82</td>
<td>78%</td>
<td>43%</td>
</tr>
</tbody>
</table>
ures (83 versus 44 days; \(p = 0.0035\)). We hypothesize (and observed ultrasonographically) that the later implanted horses had substantial fibrosis present within the healing tendon before implantation, which may have compromised the efficacy of the treatment. Earlier aspiration of bone marrow and hence implantation in now recommended in an attempt to avoid this. We are aiming therefore to implant after inflammatory phase but before fibrous tissue formation (practically this means aspirating the bone marrow ideally within 1 mo of injury. The time of implantation may be further optimized by pre-injury storage of cells, and we are currently investigating the use of umbilical cord cells recovered at the time of birth and stored for future use.

A more limited number of cases have been treated with injuries to other tendons and ligaments. For lesions present within a tendon sheath, the implantation is done after tenoscopic evaluation to ensure that there are no surface defects through which the cells could leak. Two cases that died through unrelated causes have been analyzed histologically and showed excellent healing with minimal inflammatory cells and crimped organized collagen fibers (Fig. 3). In contrast, a contralateral untreated suspensory ligament injury in one of these horses, which was clinically silent at the time of implantation, showed persistent inflammatory cells and poorly organized collagen fibers (Fig. 4).

A-MSCs

A pilot study into the efficacy of this treatment was performed in a collagenases model of tendon injury in the horse and compared with saline-injected lesions. This showed some beneficial effects of A-MSC implantation with histological evaluation of tissue organization (Fig. 5) and some specific gene expression (e.g., cartilage oligomeric matrix protein [COMP] expression was increased over controls). A large number of horses have been treated in the United States with this technique, although no outcome data has been published to date.

There are thus some encouraging aspects to this technology, although definitive proof of efficacy is still lacking. Furthermore, there have been no direct comparisons between the two techniques. It must be remembered that there are still considerable gaps in our knowledge, although the technology is developing rapidly.

6. What the Future Holds

Current and future stem cell therapy efforts depend somewhat on the type of stem cell (embryonic or mesenchymal) being targeted for clinical applications. In ES studies, efforts are concentrating pri-
Fig. 5. The histological outcome of ADC-treated (bottom two figures) and control (top two figures) collagenase tendons. Hematoxylin and eosin (H&E) staining on the left and polarized light on the right show the collagen crimp pattern. (Figure courtesy of Vet-Stem.)

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