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Original Research

Characterization of Nucleated Cells From Equine Adipose Tissue and Bone Marrow Aspirate Processed for Point-of-Care Use

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ABSTRACT

The objective of this study was to compare nucleated cell fractions and mesenchymal stromal cells (MSCs) from adipose tissue to bone marrow processed by a point-of-care device that are available for immediate implantation. A paired comparison using adipose and bone marrow from five horses was done. The number of nucleated cells, viability, total adherent cells on day 6 of culture and colony-forming unit fibroblasts (CFU-Fs) were determined. Gene expression for markers of stemness, adipogenic, chondrogenic, osteogenic lineage, and collagen formation was measured in total RNA isolated from adherent adipose and bone marrow cells. Day 6 adherent adipose-derived MSC was frozen briefly, whereas day 6 adherent bone marrow–derived MSC was passaged two additional times to obtain adequate cell numbers for chondrogenic, osteogenic, and adipogenic cell differentiation assays. The total cell count per gram was significantly greater for bone marrow, whereas total adherent cells per gram and the CFU-F per million nucleated cells on day 6 were significantly greater for the adipose. In undifferentiated adherent cells, relative gene expression for CD34, adipogenic, and chondrogenic markers and collagen II was significantly lower in the adipose-derived cells. Conversely, expression of collagen I was significantly higher in the undifferentiated adipose-derived cells. Cell density and total RNA were higher in differentiated adipogenic and osteogenic cultures of adipose cells and in chondrogenic cultures of bone marrow cells. This cell preparation method provides a stromal vascular fraction with a large proportion of multipotent MSCs. There are differences in the cells obtained from the two sources. This method can provide an adequate number of multipotent cells from adipose tissue for immediate implantation.

1. Introduction

Autologous cells from bone marrow and adipose tissue, characterized as mesenchymal stromal cells (MSCs), have been used as cell-based regenerative therapy in humans and animals [1,2]. There is increasing evidence from multiple species that MSCs can be used for the treatment of tendon, ligament, and joint diseases [3–7]. Equine practitioners have embraced this therapy for the treatment of musculoskeletal disease. Mesenchymal stromal cells may enhance both the rate and quality tissue healing [4,8,9].

In the horse, both adipose-derived MSC (Ad-MSC) and bone marrow–derived MSC (BM-MSC) have been used with positive and negative results. Sport horses with tendon injuries treated with BM-MSCs had only a 13% reinjury rate [10] compared with 23% or greater in similarly managed horses without MSCs [11]. Furthermore, the tendon

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reinjury rate of National Hunt Horses after BM-MSC treatment was only 27.5%, which was significantly below similarly managed horses without MSC therapy [8]. In Thoroughbred racehorses with BM-MSC–treated superficial digital flexor tendon injuries, there was only an 18% reinjury rate after returning to full work [4]. In a collagenase tendon injury model, there was an increase in stiffness of the tendons treated with BM-MSCs compared with controls [12]. When Ad-MSCs were injected into tendons with collagenase induced tendinitis, the tendons had significantly improved histologic scores and fiber alignment [13]. Adipose-derived MSCs are considered an easily accessible source of MSCs for use in the horse [14,15].

Even with the increased frequency of the use of MSCs, there are still questions as to optimal source, cell type, and timing of treatment. Isolation of equine MSC and growth of an adequate population from bone marrow require 2–4 weeks, and there is a variation in cell numbers, growth rates, and viability [16,17]. The advantage of culturing the cells is that culturing can result in a large number of homogeneous cells. In fresh bone marrow aspirate, actual MSCs are a small percentage of the total population of nucleated cells, being estimated to be one MSC in 50,000–100,000 cells [18].

Cells from other sources including adipose, muscle, and tendon have shown potential for therapeutic application [19–21]. In cell culture on a tendon matrix, cells from equine tendon and muscle yielded greater cell numbers than cells from bone marrow in a shorter time [19]. Interest in using adipose has increased because of the potential to harvest fairly large volumes yielding a high number of MSCs [22]. The adipose-derived nucleated cell fraction can be cultured to obtain a homogeneous population or used as a fresh nucleated cell population [22,23]. The stromal vascular fraction has many cells in addition to MSCs that may be clinically important to tissue healing compared with bone marrow [13,22,23]. Material obtained for direct application is more appropriately referred to as the stromal vascular fraction, which contains a mixture of Ad-MSCs, fibroblasts, endothelial cells, leukocytes, and very small embryonic-like (VSEL) cells [24,25].

There is no consensus antigen profile for MSC's in the horse. Mesenchymal stromal cells from different sources may have different characteristics [26–30]. Culture-expanded equine Ad-MSCs and BM-MSCs have been characterized [31]. There is limited information as to the composition of the stromal vascular fraction from equine adipose available for immediate implantation. The development of equipment that allows for isolation of the stromal vascular fraction at the point-of-care indicates the need for this information. The objective of this study was to compare the nucleated cell fractions from donor-matched samples of adipose to bone marrow available for immediate implantation.

2. Materials and Methods

2.1. Preoperative and Postoperative Care

Five mature healthy female horses were used. There were 3-, 5-, 8-, and 10-year-old Quarter Horses and a 15-year-old Belgian horse. The study was approved by the Institutional Animal Care and Use Committee. The collection procedures were done under sedation with detomidine (20 μg/kg IV). Local anesthesia with mepivacaine HCl was placed subcutaneously for the stab incisions at the collection sites for the bone marrow and adipose. Furthermore, lidocaine HCl was in the tumescent fluid used for the liposuction. Both harvest sites were evaluated weekly for 4 weeks and any complications noted.

2.2. Collection of Bone Marrow

The technique for marrow aspiration has been well described [32]. Briefly, ultrasound was used to identify approximately the body of the fifth sternebrae. Local anesthesia was placed subcutaneously, and a small stab incision was made with a number 15 scalpel blade and an 8-ga Jamshide needle (T-Lok bone marrow biopsy needle; Angiotech, Vancouver, BC, Canada) was placed in the incision and guided into the body of the sternebrae. A 12-mL syringe preloaded with 1 mL of 1,000 units of sodium heparin per 1 mL of sterile-buffered physiological saline solution was used to aspirate a 10-mL sample. The samples were placed into a 50-mL centrifuge tube (Centrifuge Tube 50 mL; Millipore, Billerica, MA) and put in a cooler with cold packs until processed within 6 hours.

2.3. Collection of Adipose

Ultrasound was used to identify the area with the greatest amount of adipose at approximately the sacrococcygeal level, 3–5 cm from midline. The area was clipped and aseptically prepared. A number 15 scalpel blade was used to make a stab incision and to provide analgesia, tighten the tissue, and minimize bleeding, 60 mL of tumescent anesthesia fluid (2 mg epinephrine; 1,000 mg/L lidocaine in normal saline) was infiltrated subcutaneously with an 18-ga 6-in spinal needle in the region to be liposuctioned approximately 10 minutes before the syringe liposuction procedure [33]. A liposuction cannula (3-CD-4H-3 × 15 disposable luer lock liposuction cannula; Shippert Medical Technologies Corp, Centennial, CO) was introduced through the incision and a 30-mL locking aspiration syringe (VacLok Syringe; Merit Medical, South Jordan, UT) was used to obtain up to 30 mL of adipose with tumescent fluid. The lipoaspirate was placed in a 50-mL centrifuge tube and placed in a cooler with cold packs until processed within 6 hours. Each sample, from both sources, was labeled with a randomized number matching the horse and type of sample. Blinding was maintained until data acquisition was completed for statistical analysis.

2.4. Cell Isolation and Counts

Equine lipoaspirates were weighed and processed in aliquots of approximately 10 g using a commercially available system to isolate stromal vascular fraction from adipose (ARC; InGeneron Inc, Houston, TX). Each lipoaspirate was incubated at 37°C and agitated in the processing unit with a proprietary blend of enzymes (Matrase; InGeneron Inc) for 30 minutes to dissociate the tissue and