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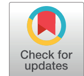
Review

Practical considerations for clinical use of mesenchymal stem cells: From the laboratory to the horse



L. Barrachina[a,b], A. Romero[a,b], P. Zaragoza[a,c], C. Rodellar[a,c], F.J. Vázquez[a,b,*]

[a] Laboratorio de Genética Bioquímica LAGENBIO, Instituto de Investigación Sanitaria de Aragón (IIS), Universidad de Zaragoza, Zaragoza, Spain
[b] Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, 50013 Zaragoza, Spain
[c] Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza-Centro de Investigación y Tecnología de Aragón (CITA), Zaragoza, Spain

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ABSTRACT

Since the clinical use of mesenchymal stem cells (MSCs) for treating musculoskeletal injuries is gaining popularity, practitioners should be aware of the factors that may affect MSCs from tissue harvesting for MSC isolation to cell delivery into the injury site. This review provides equine practitioners with up-to-date, practical knowledge for the treatment of equine patients using MSCs. A brief overview of laboratory procedures affecting MSCs is provided, but the main focus is on shipping conditions, routes of administration, injection methods, and which commonly used products can be combined with MSCs and which products should be avoided as they have deleterious effects on cells. There are still several knowledge gaps regarding MSC-based therapies in horses. Therefore, it is important to properly manage the factors which are currently known to affect MSCs, to further strengthen the evidence basis of this treatment.

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Introduction

Mesenchymal stem cells (MSCs) are gaining popularity in equine practice for regenerative purposes, not only because of their potential for differentiation but also because of their trophic, anti-inflammatory and immunomodulatory abilities (da Silva Meirelles et al., 2009). In the horse, their most common application is the treatment of musculoskeletal injuries, which will constitute the main focus of this review. However, MSC-based therapies have also been explored for respiratory (Zucca et al., 2016), reproductive (Falomo et al., 2015) or ophthalmologic diseases (Sherman et al., 2017).

Notwithstanding reported beneficial effects and increasing clinical use of MSCs, their actual therapeutic efficacy is not yet entirely clear. In general, results of MSC treatment of equine tendinopathies have been more consistent than of joint pathologies (Colbath et al., 2017). Ultrasonographic and/or histopathologic improvements have been reported after MSC treatment of tendinopathies in experimental models (Caniglia et al., 2012; Romero et al., 2017) as well as in naturally occurring disease, with 77 to 98% of racehorses returning to racing with reinjury rates lower than 30% (Godwin et al., 2012; Van Loon et al., 2014). In equine osteoarthritis (OA) models, MSCs have shown different results ranging from only a slight improvement (Frisbie et al., 2009) to a significantly improved outcome (Mokbel et al., 2011). In one retrospective study, 78% of horses with naturally occurring OA returned to work after MSC treatment (Broeckx et al., 2014a). Similarly, 76% of horses with different stifle injuries receiving MSCs after surgery returned to work, with the percentage of horses with meniscal injury returning to work being significantly higher than in previous studies using only arthroscopy (Ferris et al., 2014).

The wide variety of study designs, including different natural or experimental models as well as different treatment setups (e.g. time for treatment, MSC source or MSC number, etc.), precludes drawing definitive conclusions about actual MSC effectiveness. Furthermore, MSCs are often combined with other products or with surgical procedures, complicating the formulation of a conclusion on the role of MSCs. Although MSCs may be a promising treatment for equine musculoskeletal injuries, it is important to highlight that their actual therapeutic potential still remains unclear and that there are still several gaps in the knowledge to be investigated. Current knowledge of MSC therapies has been covered in other reviews (Colbath et al., 2017; Durgam and Stewart, 2017). The current review does not aim to report the efficacy of MSCs and associated challenges, but offers practical guidelines to manage factors affecting the clinical use of MSCs.

Considerations ranging from laboratory procedures to shipping conditions and MSC administration may affect the clinical use of

* Corresponding author.
E-mail address: pvazquez@unizar.es (F.J. Vázquez).

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MSCs. Therefore, clinicians should be aware that MSCs are not a 'traditional drug', but a biological compound that must be handled carefully to ensure optimal administration. The aim of this review is to summarise the current knowledge about appropriate MSC management in clinical practice, focusing on practical considerations to optimise the conditions in which MSCs are delivered to the equine patient.

Tissue harvesting for MSC isolation

In the horse, MSCs have been isolated from bone marrow (BM), adipose tissue (AT) (Ranera et al., 2011), peripheral blood (PB) (Dhar et al., 2012), synovial membrane and synovial fluid (Prado et al., 2015), amniotic membrane and fluid or umbilical cord (UC) blood and tissue (Iacono et al., 2012; Iacono et al., 2017), amongst others. Despite the wide variety of sources, not all of them are equally suitable for clinical purposes. Bone marrow and AT currently are the most extensively investigated sources for MSC isolation for clinical purposes (Colbath et al., 2017). Nevertheless, peripheral blood and perinatal sources are of raising interest because tissue harvesting is not invasive (Broeckx et al., 2014a,b; Tessier et al., 2015). Additionally, MSCs derived from perinatal sources present lower expression of immunogenic markers, potentially making them more suitable for allogeneic application (Tessier et al., 2015).

Equine BM and AT-MSCs properties have been compared quite extensively in vitro, with BM-MSCs showing higher chondrogenic (Vidal et al., 2008) and osteogenic (Toupadakis et al., 2010) potential. However, although MSCs from both sources display immunomodulatory properties, AT-MSCs seem slightly superior (Remacha et al., 2015) and elicit their regulatory effects through different mechanisms (Carrade Holt et al., 2014). Different properties shown in vitro by equine BM and AT-MSCs may be relevant for their clinical application, depending on the injury. However, only few studies have compared BM-MSCs and AT-MSCs in vivo. Iacono et al. (2015) reported beneficial effects of both BM-MSCs and AT-MSCs without significant differences between them in naturally occurring tendinopathies. Similarly, both treatments showed similar efficacy when surgically created meniscal defects were treated with scaffolds loaded with either BM-MSCs or AT-MSCs (Gonzalez-Fernandez et al., 2016). Our group has compared equine BM-MSCs and AT-MSCs for treating experimentally induced tendon injuries. Although differences between treatments were relatively small, BM-MSCs resulted in a better outcome than AT-MSCs (Romero et al., 2017). In general, BM-MSCs are considered superior to AT-MSC for musculoskeletal therapy, which may be influenced by a larger number of scientific studies on BM-MSC (Schnabel et al., 2013).

Adipose tissue is usually harvested from the supra-gluteal subcutaneous area and BM can be collected from sternum or ilium with a Jamshidi needle. As aggregates formation may diminish cells recovery (Bastos et al., 2017), BM must be collected with an anticoagulant and the syringe gently agitated to ensure proper mixing. The preferred anticoagulant for BM collection is sodium heparin at 250–500 IU/mL BM (Kasashima et al., 2011; Delling et al., 2012). No differences have been observed between BM-MSCs from sternum or ilium regarding proliferation, phenotype or differentiation (Adams et al., 2013; Lombana et al., 2015). The number of nucleated cells obtained from each location is similar in horses younger than 5 years (Adams et al., 2013) but lower in BM obtained from the ilium in older horses, in which iliac BM aspiration may be harder than sternal BM aspiration (Delling et al., 2012). Therefore, in animals older than 5 years, it is recommended to sample the sternum, whereas in animals younger than 5 years, the choice between sternum and ilium relies on individual preferences. The highest concentration of nucleated cells is contained in the first 5 mL of BM aspirate for both sampling locations (Adams et al., 2013).

Harvested tissue is sent to the laboratory for MSC isolation in containers at 4 °C. Our laboratory has observed significantly lower numbers of colony forming units when BM is processed after 24 h of transport compared with isolation immediately after aspiration, which could result in longer time required for expansion. Therefore, processing BM immediately after harvesting is preferred whenever possible (Ranera, 2012).

What happens in the laboratory?

Even though this review does not aim to describe laboratory techniques, it is important to understand the laboratory procedures performed once the sample for MSC isolation is received. Particular focus will be given to aspects potentially influencing MSC application.

MSC isolation and culture

When MSCs are isolated from 'liquid' sources (BM, PB, UC-blood), the fraction of mononuclear cells is usually isolated by gradient centrifugation, whereas 'solid' sources (AT, UC-tissue) require enzymatic digestion. Subsequently, the mononuclear cell fraction is plated in tissue culture-treated plates to allow cell attachment while non-adherent cells will be removed with media replacements (Ranera et al., 2011; Tessier et al., 2015). Liquid harvests can also be directly plated mixed with culture media (Sharma et al., 2014) and MSCs can be isolated from solid tissues using explants techniques, although this results in lower MSC yields which can delay therapy (Gittel et al., 2013). After isolation, colonies of adherent cells appear within a few days and will progressively cover the plate bottom until they reach confluence. Cells are then enzymatically detached and reseeded in lower density to allow continuing expansion. This step is repeated each time cells become confluent and is known as a 'passage'. To complete a passage takes about 1 week, depending on technical and individual factors. MSCs are commonly applied at low passage to maintain stemness, i.e. passage 2–4, to obtain both an appropriate cell number and a homogeneous cell population (Colbath et al., 2017). For clinical application, the use of MSCs beyond passage 6–7 should be avoided, as these cells become senescent, showing decreased proliferation and morphological abnormalities (Vidal et al., 2012). In conclusion, it takes about 2–3 weeks to obtain autologous cells ready for therapy. However, the required expansion time also depends on other points such as MSC proliferation potential, which may be lower in elderly patients (Choudhery et al., 2014), or the isolation protocol used (Bourzac et al., 2010; Gittel et al., 2013), etc. The process to obtain MSCs has been briefly outlined in Fig. 1.

The use of fetal bovine serum in the culture media

To provide the cells with nutrients and growth factors, fetal bovine serum (FBS) is commonly used to supplement MSC culture medium. This is a pivotal aspect for MSC clinical application as FBS is a xenogeneic compound that may generate an immune reaction (Sundin et al., 2007). Even if MSCs are rinsed exhaustively, cells may internalise some FBS-compounds. Mild inflammatory reaction may occur after intra-articular (IA) administration of both autologous and allogeneic MSCs in an equine healthy joint (Carrade et al., 2011; Pigott et al., 2013b; Ardanaz et al., 2016). This issue could be associated with xeno-contamination from the FBS, as the immune system may react against xeno-proteins internalised by MSCs (Sundin et al., 2007). Moreover, the animal might have been previously immunised because some xeno-proteins can be used for

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