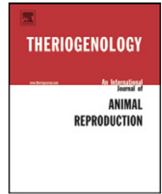
Theriogenology 79 (2013) 744–750

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com



A novel strategy of mesenchymal stem cells delivery in the uterus of mares with endometrosis

L.I. Mambelli [a,b,**], G.H.Z. Winter [c], A. Kerkis [a], E. Malschitzky [d], R.C. Mattos [c], I. Kerkis [a,*]

[a] Laboratório de Genética, Instituto Butantan, São Paulo, São Paulo, Brazil
[b] Programa de Pós-Graduação em Anatomia dos Animais Domésticos e Silvestres da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, São Paulo, Brazil
[c] Reprolab, Faculdade de Medicina Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil
[d] Curso de Medicina Veterinária, ULBRA, Canoas, Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form 27 November 2012
Accepted 28 November 2012

Keywords:
Equine endometrosis
Laminin
Vimentin
Mesenchymal stem cells
Delivery
Homing

ABSTRACT

Mesenchymal stem cells (MSCs), because of their immunomodulation and trophic activities, in addition to their capacity to regenerate damaged tissues, have potential for treatment of many diseases. The success of stem cell therapies depends, in part, on the method of cell delivery, which should provide wide cell distribution and homing in to injured sites. The objective of the present study was to developing a novel strategy for delivery of MSCs into the uterus of mares with endometrosis (degenerative alteration of uterine glands and surrounding stroma). Endometrosis was confirmed in all mares ($N = 6$) used in this study. To trace multipotent equine adipose tissue-derived MSCs (eAT-MSCs) in endometrial tissue, before transplantation, cells were stained with a fluorescent dye. During a synchronized estrus, the eAT-MSCs (2×10^7 diluted in 20 mL of sodium chloride 0.9%) were inoculated into uterus using a simple technique, similar to artificial insemination (AI) in mares. At 7 and 21 days after transplantation, homing of fluorescently labeled eAT-MSCs was observed by confocal microscopy of uterine biopsies collected from the uterine body and in both uterine horns, including glandular and periglandular spaces, in three of four treated mares. Herein, we propose a new method of MSCs delivery in uterus of mares with endometrosis, which was minimally invasive and technically simple.

© 2013 Published by Elsevier Inc.

1. Introduction

The body contains so-called stem cells that are non-specialized and could renew themselves and give rise to specialized cells, e.g., muscles, bone, cartilage, etc. These adult stem cells are “spare parts” of the organism, which are activated in case of injury, disease, or trauma [1]. Discovery of cell isolation and *in vitro* cultivation provided a qualitative leap in stem cell technologies, which became more efficient through employment of mesenchymal stem cells (MSCs) [2,3]. These are multipotent progenitor cells originally identified in bone marrow stroma. They can be expanded *in vitro* and, under appropriate conditions, can give rise to several cell types [2,4]. The *in vitro*-expanded MSCs have remarkable immunoregulatory properties and effects on tissue repair; therefore, their potential use as therapeutic agents *in vivo* has been extensively studied [5–8]. Currently, MSCs can be readily harvested from several sources, including bone marrow, fatty tissue, and dental pulp [2,9,10].

In horses, bone marrow and fatty tissue-derived mononuclear cell therapies have been widely explored in order to recover joint problems, such as osteoarthritis and tendon lesions [11,12]. However, the mononuclear cell population contains only a small proportion of MSCs.

* Corresponding author. Tel.: +55 11 3726 7222 ×2287; fax: +55 11 3726 1505.
** Alternate corresponding author. Tel.: +55 11 3726 7222 ×2287; fax: +55 11 7169 6204.
E-mail addresses: lis_mambelli@yahoo.com.br (L.I. Mambelli), ikerkis@butantan.gov.br (I. Kerkis).

0093-691X/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2012.11.030

Equine endometrosis is an age-associated, degenerative alteration of uterine glands and surrounding stroma, directly related to fertility problems in mares [13]. The MSCs, because of their therapeutic properties, have potential for treatment of this disease. However, the success of stem cell therapies depends at least, in part, on cell delivery, which should ensure wide cell distribution and homing within the injured site [14]. To substitute for damaged cells and aiming at upgrading tissue integrity and function, a cell suspension is simply injected into the damaged tissue or into the blood circulation. Depending on the pathology, treatment strategies can differ considerably [15]. Therefore, favorable application strategies are important for the success of MSCs therapy. Ideally, a cell application method should not be complicated. The aim of the present study was to develop a novel strategy of delivering MSCs in the uterus of mares with endometrosis.

2. Materials and methods

2.1. Mares

All studies were approved by the ethical committee of the School of Veterinary Medicine and Animal Science, University of Sao Paulo, SP, Brazil (Protocol 1804/2009). Six mares of various breeds, 6 to 21 years old, with varying degrees of endometrosis, were used. The mares were systemically healthy and had regular estrous cycles. These mares were part of an experimental herd and were maintained in an open field, supplemented with oats and alfalfa hay, with *ad libitum* access to water.

An endometrial biopsy was taken before the beginning of the experiment and used to classify the mares, of which three were classified as grade IIb (Mares 03, 04, and 05), and three as grade III (Mares 01, 02, and 06), according to Kenney and Doing [16]. One mare from each grade (Mares 02 and 03) was used as control during cell transplantation experiments. Mares were examined for reproductive soundness, including evaluation of perineal conformation, transrectal palpation, and ultrasonography of the genital tract, vaginal examination with speculum, bacteriological cultures, and cytology of the endometrium. Only clinically normal mares with negative cytology and negative cultures were used. Estrus was synchronized in mares with 5 mg prostaglandin F2α im (Lutalyse; Pharmacia Brasil Ltda., São Paulo, São Paulo, Brazil).

2.2. Cells

Equine adipose tissue-derived mesenchymal stem cells (eAT-MSC), previously isolated and characterized by our group, were used. These cells have been extensively expanded *in vitro*, characterized, and stored in liquid nitrogen for at least 2 years, with similar properties before freezing and after thawing [17].

2.3. Fluorescent eAT-MSCs labeling

For cell labeling, Vybrant CFDA SE Cell Tracer Kit (Invitrogen, Carlsbad, CA, USA; V12883) fluorescent-nanocrystal dye (green) was used. The FDA SE 10 mM stock solution was prepared immediately before use by solving the contents of one vial (Component A) in 90 μL of the high-quality DMSO provided in the kit (Component B). Next, stock solution was diluted in phosphate-buffered saline (PBS) until it reached the desired working concentration of 25 μM. The eAT-MSCs were thawed just before staining, after two washes in Dulbecco's Modified Eagle Medium-High Glucose. Cell pellets were obtained by centrifugation ($110 \times g$, 5 minutes) and the supernatant was aspirated. Next, eAT-MSCs were gently resuspended in prewarmed (37 °C) PBS containing the probe and incubated for 15 minutes at 37 °C. Cells were repelleted by centrifugation and resuspended in 20 mL of fresh prewarmed physiologic solution 0.9% for transplantation into mares' uterus.

2.4. Experimental cell transplantation

The procedure of eAT-MSCs application was performed during a synchronized estrus. After cleaning the perineal area, the operator wearing a sterile insemination glove introduced a disposable insemination pipette through the cervix into the uterine body. In order to avoid contamination, the gloved hand was placed over the tip of the pipette during its introduction into the vagina. At this time, the pipette was guided toward the tip of the right horn, assisted by transrectal palpation. The pipette was connected to the syringe containing 2×10^7 cells diluted in 20 mL of sodium chloride 0.9% through a sterile connector rubber. The plunger of the syringe was slowly depressed, introducing 10 mL of cell suspension. Then, the free end was placed on the left uterine horn and the remainder (10 mL of cell suspension) was infused. Immediately thereafter, a second syringe containing 3 mL of sodium chloride 0.9% was coupled to the sterile pipette and infused (to ensure delivery of the entire volume). The pipette was then slowly withdrawn from the vagina.

The two control mares were infused with 20 mL of sodium chloride 0.9% with 10 mL in each horn tip, using the same technique used for cell transplantation.

Biopsies from the uterine body and left and right horns from treated and control mares was collected 7, 21, and 60 days after inoculation (total of 72 biopsies).

2.5. Histology

A total of 72 endometrial uterine biopsies from the six mares were analyzed without knowledge of treatment group (blinded). Uterine biopsies were fixed in 10% buffered formalin, embedded in paraplast, sectioned at 4 to 5 μm, and stained with hematoxylin and eosin. The degree of endometrosis was analyzed as described [18–21]. All specimens had signs of endometrosis varying in quantity, degree (mild to severe), and quality (active or inactive).

2.6. Immunohistochemistry

The peroxidase anti-peroxidase (PAP) method was used for immunohistochemistry. Tissue sections were mounted on superfrost slides (Life Science Int. GmbH, Frankfurt Main, Germany). The paraffin wax sections were rehydrated and endogenous peroxidase activity was inhibited by 3% H_2O_2 in methanol (30 minutes). Primary antibodies were diluted in TRIS-buffered saline with 1% BSA.

Download English Version:

https://daneshyari.com/en/article/10894465

Download Persian Version:

https://daneshyari.com/article/10894465

Daneshyari.com