Equine mesenchymal stem cells from umbilical cord matrix: isolation, culture and phenotypic characterization

Danielle J. Barberini[a], Marjorie Golim[b], Fernanda da C. Landim-Alvarenga[c], Rogério M. Amorim[a]

[a] Departament of Veterinary Clinics, College of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, SP – Brazil
[b] Hemocenter Division of Botucatu Medical School, São Paulo State University (UNESP), Botucatu, SP – Brazil
[c] Department of Animal Reproduction and Veterinary Radiology, College of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, SP – Brazil

Abstract

In equine species, bone marrow (BM) is one of the most studied and used sources to obtain mesenchymal stem cells (MSCs). However, adipose tissue (AT) is also an abundant and accessible source of MSCs that can provide a large number of cells required for use in cell therapy. Additionally, cells from the amniotic membrane and umbilical cord (UC) are a promising source of MSCs because they are less immunogenic and their collection is noninvasive. In this study we aimed to evaluate cell culture, immunophenotypic characteristics and the differentiation potential into mesenchymal lineages of MSCs from umbilical cord tissue (UC-MSCs). To obtain the UC (n = 6), two samples were collected from two births and four samples from the slaughter of horses. Approximately 10 cm of umbilical cord from the fetal portion was collected and stored in a sterile 50 mL conical tube containing HBSS plus 2% penicillin/streptomycin. Then, UC samples were washed three times in HBSS/penicillin and submitted to mechanical dissection to separate vein and arteries, which were discarded. The UC tissue was fragmented using a scalpel and anatomical forceps to approximately 0,2-0,3 cm size and then placed in a 0.04% solution of type 1 collagenase at 37°C for 60 minutes. After this period, the solution was filtered through a 70-micrometer filter, DMEM high glucose (1:1) was added, and the solution was centrifuged twice at 340 × g for 15 minutes. Cells were cultured at a density of approximately $10x10^3$ cells/cm2 in 25-cm2 flasks containing a culture medium consisting of 80% DMEM high glucose/F12 (1:1), 20% fetal bovine serum, 1% penicillin/streptomycin and 1.2% amphotericin B at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. MSCs were cultured until third passage (P3) and evaluated in vitro for their osteogenic, adipogenic and chondrogenic differentiation potential, immunophenotypic characterization with CD44, CD90, CD105, CD34 and MHC-II markers by flow cytometry, and MHC-II was also assessed by immunocytochemistry. UC-MSCs were able to differentiate into osteogenic (after 15 days), adipogenic (after 15 days) and chondrogenic (after 21 days) lineages, confirmed by Alizarin Red S, Oil Red O and Alcian Blue and toluidine blue staining, respectively. Immunophenotypic analysis revealed UC- MSCs

with high expression of CD105 (94.2±2.1), CD44 (95.7±1.5) and CD90 (67.7±6.5) markers, negative expression of CD34 (0.20±0.3) and low expression of MHC-II (5.9±1.8) markers. The MHC-II was not detected by immunocytochemistry techniques. Equine UC is a viable source for obtaining MSCs, confirmed by the immunophenotypic and multipotentiality characteristics of these cells. Due to the low expression of MHC-II by UC-MSCs, this source could be used in clinical trials involving allogeneic therapy in horses.

Support: FAPESP, CNPq.