Efficacy of ultrasound to identify mesenchymal stem cell transplantation in equine muscle

Denis J. Svicero^[a], Danielle J. Barberini^[a], Gustavo F. Viana^[b], Vânia M. de V. Machado^[b], Fernanda da C. Landim-Alvarenga^[b], Marjorie Golim^[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
- Department of Animal Reproduction and Veterinary Radiology, College of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, SP – Brazil
- [c] Hemocenter Division of Botucatu Medical School, São Paulo State University (UNESP), Botucatu, SP Brazil

Abstract

Prospects of the studies with mesenchymal stem cells (MSCs) and tissue engineering have shown that MSCs may benefit the health of both humans and domestic animals. However, in horses, many of its biological characteristics remain unknown and scarce, like the residence time at the site of transplantation. In this context, the use of cell markers associated with methods of getting images *in vivo* are essential for tracking these cells. This study aimed to compare images obtained through ultrasound (US) of MSCs marked and unmarked with Nanocrystal Qtracker 655® transplanted into Brachiocephalicus muscle of horses, with the aim of the potential traceability of the method employed.

Bone marrow (BM) was obtained from one healthy equine and sent to the laboratory (LANÇA) for isolation and cultivation to create a cell bank for allogeneic transplantation. Whan a confluence of approximately 80% was reached, MSCs were labeled with the nanocrystal and visualized with a fluorescence microscope. The amount of 1x106 labeled BM-MSCs were transplanted guided by ultrasound into the Brachiocephalicus muscle on the right side of the neck, and the same amount of unlabeled cells were transplanted on the left side of the neck of 12 healthy horses. The transplantation of BM-MSCs was performed at an average depth of 1 cm. Monitoring with ultrasound was performed at 0, 24, 48, 72 and 96 hours to evaluate dispersion of labeled MSCs into adjacent muscle tissue and echogenic differences.

Muscle with labeled MSCs showed regular, defined and hyperechoic images surrounded by a slight reverberation forming area at 0, 24 and 48 hours. A higher dispersion and an increase in heterogeneous echotexture along the muscular region were visualized at 72 and 96 hours. The muscle with unlabeled MSCs showed scattered and hyperechoic images, forming reverberation effect and moderate acoustic shadowing at 0 hour. A mildly heterogeneous muscle echotexture, suggestive of a healing process after inoculation, was seen at 96 hours. No changes in the muscular topography were visualized at all other times. Ultrasound monitoring of transplanted labeled MSCs

proved to be very useful to clarify the behavior of dispersion of MSCs in muscle tissue *in vivo*, also being able to contribute and encourage research on traceability of MSCs.

Financial support: FAPESP, CAPES.

Ethics Committee: CEUA (Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia

da UNESP – Campus Botucatu – SP). Protocolo 209/2012.