MESENCHYMAL STEM CELLS FOR EQUINE TENDON REPAIR

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INTRODUCTION

Cell therapy based on use of Mesenchymal Stem Cells (MSCs) is of high interest in human and veterinary medicine, due to their ability to repair damaged tissues. Autologous and allogenic MSCs can be used in regenerative medicine thanks to their immunomodulatory properties.
In veterinary field the equine species represents an important target for cell therapy, because horses are often subjected to lesions that can occur during competitions. Accordingly the purpose of this study was to evaluate the ability of MSCs to repair tendon lesions.

MATERIALS & METHODS

MSCs isolation - In this study, 23 horses with tendon injuries were submitted to autologous MSCs implantation. Bone marrow was aspirated from sternum and it was collected in tubes containing 6000 IU of sodium heparin. MSCs were isolated by a density gradient centrifugation and seeded in flasks at the final concentration of $1x10^6$ cells/cm^2 in NH Expansion Medium (Miltenyi Biotech®) and incubated at 37°C in 5% of CO_2.
Amplification - At confluence, cells were amplified for a maximum of 5 serial passages using another medium (40% D-MEM low glucose, 40% Ham's F12, 20% Foetal Bovine Serum, 1500 µg/ml Streptomycin and 3000 IU/ml Penicillin).
Characterization - To verify the ability of MSCs to days colonies were stained with 2% Crystal Violet.
MSCs were differentiated into three cell culture lineages (chondrocytes form colonies, $2x10^5$ cells were seeded in 6-well plates with cultural medium (NH CFU-F Medium, Miltenyi Biotec®) and incubated at 37°C and 5% CO_2. After 15-21, adipocytes and osteocytes) to test their pluripotency. MSCs were cultivated with three specific differentiation media (NH ChondroDiff Medium - NH AdipoDiff Medium - NH OsteoDiff Medium, Miltenyi Biotec®).
Safety - MSCs were tested for virus, eubacteria and mycoplasma contaminations.
Final cell preparation, inoculation and follw up - Platelet Rich Plasma (PRP) was obtained from autologous venous blood and MSCs were suspended at the final concentration of $5x10^6$ cells/ml. The suspension was inoculated into tendon lesion by ultrasonic guide 1 month after the lesion event. After MSCs treatment, horses were subjected to a 12-month rehabilitative period and an ecographic control every 30 days.

RESULTS

MSCs isolation and amplification – MSCs, after their isolation and cultivation, reached the subconfluence in 7-10 days. 48 hours after seeding, fibroblastic-like cells were observed (Fig. n°1).
The mean number of MSCs obtained after serial passages was about $20x10^6$ cells.
Characterization – One CFU was obtained every 10^4 seeded cells (Fig. n°2). MSCs demonstrated the ability to differentiate into adipogenic (Fig. n°3), osteogenic (Fig. n°4) and chondrogenic lineages (Fig. n°5).
Safety – Only cells free from any contamination were used for *in vivo* application.

Fig. n°1: MSCs, 200x.

Fig. n°2: presence of MSCs colonies, 40x .

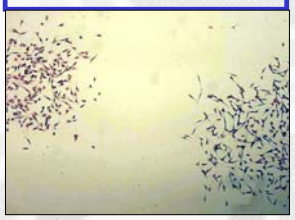


Fig. n°3: presence of lipidic accumulation (Oil red O staining), 400x.

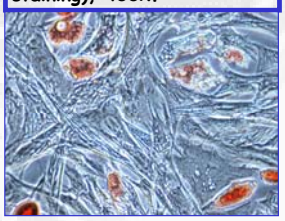


Fig. n°4: calcification after 10 days (Von Kossa staining), 100x.

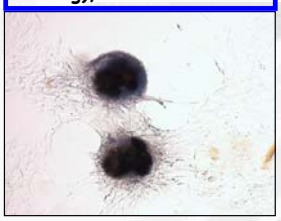


Fig. n°5: formation of chondrocitic nodule after 21 days.

Sixty days after the MSCs inoculation, no difference was detected in 85% of the injured tendons in comparison with the healthy controlateral tendon; concerning ecogenicity, fiber alignment and traversal section area of the tendon (Fig. n°6-7-8).

Fig. n°6: ecographic image of the lesion before MSCs inoculation.

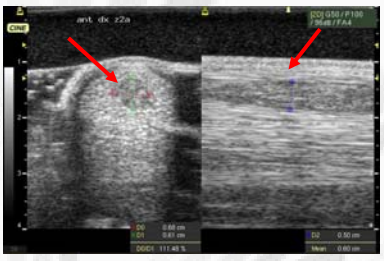


Fig. n°7: ecographic image of the lesion 40 days after MSCs therapy.

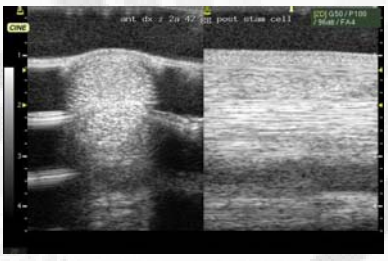
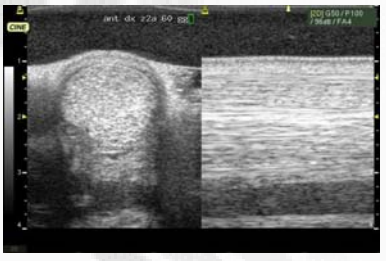


Fig. n°8: ecographic image of the lesion 60 days after MSCs therapy.



DISCUSSION

MSCs demonstrated the ability to grow and to differentiate into three lineages, attesting their pluripotency. They did not cause any immunologic response and no one of the treated animals showed adverse features after MSCs injection, thanks to their immunomodulatory features. Ecographic examinations showed a good regeneration of the tendon. To demonstrate the advantages of this therapy in comparison with traditional approaches and to evaluate the rate of recidivism, it is necessary to wait for the return of treated horses to competitions.

REFERENCES
Krampera M et. Curr. Opin. Pharmacol. 2006.
Richardson LE et al. Trends in Biotechnlogy 2007.
Koch TG et al. Can. Vet. J. 2008.
Zuk P et al. Mol. Biol. Cell. 2002

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