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Cell Therapy for Tendon Repair in Horses: An Experimental Study

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Abbreviations: BMMNCs, Bone Marrow Mononucleated Cells; cBMSC, cultured Bone Marrow Mesenchymal Stem Cells

INTRODUCTION

Tendonitis and desmitis are high morbility diseases in the horse, (Sharma and Maffulli, 2005; Smith and Webbon, 2005) and their clinical approach is a challenge for clinicians. (Smith *et al*., 2003; Sharma and Maffulli, 2005; Smith and Webbon, 2005). In 2003, Smith *et al*. suggested the use of cultured bone marrow mesenchymal stem cells (cBMSC) as treatment for spontaneous tendinitis in the horse (Smith *et al*., 2003; Smith, 2004; Smith and Webbon, 2005). The MSCs regenerated tendon tissue either in experimental lesions or spontaneous ones, respectively, in lab animals and equine species, but this procedure is epensive. Bone Marrow Mononuncleated Cells (BMMNCs) could represent an alternative cell source for tissue engineering (Blatt *et al*., 2005; Cho *et al*., 2005). Concentration of MSCs contained in bone marrow and their grafting directly into lesions allows their differentiation in specialized cells. The aim of this study was to compare cBMSCand BMMNCs vs. placebo for treatment of collagenase induced tendonitis in the horse.

MATERIALS AND METHODS

Three male Standardbred horses, 4 years old, were used in this investigation. Any previous lesion was assessed by clinical and ultrasound examination. Horses were rested in a box 4 weeks prior to beginning of the study. 4000 IU of *Cl. histolyticum* Type 1A collagenase was injected in zone 2B of each superficial digital flexor tendon (SDFT) of three limbs (left front, right front, and right hind) under ultrasonographic guidance. The left hindlimb was not injected and used as control. At this time point a first bone

marrow harvesting has been performed for cBMSC culturing. After 3 weeks the lesions were investigated by clinical ultrasound examination. A second bone marrow harvesting was collected from tuber coxae with the horse sedated and standing. The cBMSCs cultured for 3 weeks, and the BMMNCs harvested at this time point were obtained using a prevoiusly described procedure. (Crovace *et al*., 2004) Cell grafting was performed under ultrasound guidance directly into the lesion. Ultrasound examination was performed using a 7.5 MHz linear probe at 0, 1, 3, 6, 8, 12, 16, 18, 21 weeks from treatment. The type lesion score (TLS), fiber pattern score (FPS) and percentage of cross sectional area of the lesion (% of CSA-l) was recorded at the Maximum Injury Zone (MIZ). Quantitative data were valuated by ANOVA. The level of significance was considered as $p<0.05$.

RESULTS

After 3 weeks from collagenase injection we obtained lesions with a mean length of 36.5 mm, with % CSA-l of 30.5%, the TLS and the FPS at T0 was 2.83. We collected approximately 35 ml of bone marrow for BMSC culture, while 45 ml was collected to obtain BMMNCs. The number of Colony Forming Units, (CFU-f), obtained precentrifugation (on entire bone marrow) was 428 CFU-f$\times$ml^{-1}, while the post centrifugation (post-Ficoll) CFU-f was 1696. Mean 122×10^6 were injected. Suspended in a mean of 3.13 ml of fibrinogen. 5.5×10^6 BMSCs were obtained at the first passage, after 3 weeks of cultivation. At T 8w the % of CSA-l did not differ between cBMSC and BMMNC while both cell treatments were statistically different from placebo ($p< 0.05$). At this time point the FPS and TLS were not normal, but better than at T0w. At T16w until T21w, % CSA-l, FPS and TLS were statistically different. Histological and immunohistochemisty stained with H&E and Herovici, collagen type I and III revealed mature type I collagen with normal architecture in tendons treated with cBMSC and BMMNC, while random collagen type III organization was observed in the tendons treated with placebo.

DISCUSSION

Our results provide evidence that cBMSC and BMMNCs cause tendon regeneration in an equine collagenase-induced tendonitis model while placebo repairs lesions with scar tissue.

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