Survival of mesenchymal stem cells in collagenase induced tendonitis in an ovine model

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Abstract

The purpose of this study was to evaluate the efficacy and the survival of local injection of allogenic MSC marked with Red Fluorescent Protein (RFP) (Lentigen-Italy) in collagenase induced tendonitis in the ovine Achilles' tendon. The study was performed after the approval by the National Animal Care and Use Committee. Four sheep (2 years old, female, 45 bwt) have been enrolled in the study. After some days for the acclimatation, the sheep have been investigated to exclude any previous Achilles' tendon lesion. Three weeks before starting of the study one sheep was randomly selected for Bone Marrow harvesting for MSCs cultivation. The MSCs obtained has been transfected with a lentivirus for integration of a gene for expression of Red Fluorescent Protein (RFP). After a week the other 3 sheep was injected in both Achilles' tendon with 400 U.I. of Collagenase IA of Cl. hystoliticum (Sigma-Aldrich-Italy). After two weeks the left Achilles' tendon of each sheep was injected with a solution of 6x10⁶ RFP-MSCs (MSCRFP) in 1 ml of fibrine glue (TISSUCOL, Baxter). The remaining tendons were used as negative control and received the same volume of saline solution as placebo. At 3-4-6 weeks from the treatment the tendons were harvested and evaluated for morphology, collagen I and III expression, and visualized at fluorescence microscope to assess RFP expression of the grafted MSCRFP. The results of these investigations evidenced the presence of MSCRFP in the treated tendons respect to the control ones at 3, 4 and 6 weeks after the treatment. Moreover, the RFP positive tissue showed high expression of collagen I and low collagen III with good morphology in comparison to the lesions treated with placebo. The presence of high expression of collagen I and low collagen III with good morphology, in term of restored tendon architecture, can be related to the MSCRFP injected into tendon lesions, as a large number of cells can survive in the site of injection. These results showed that intralocal administration of MSCs into the tendon lesion can lead to a good effect on injured tendon. The local infusion delivery entails injecting MSCs directly into the tissue of interest and guarantees a higher number of engrafted cells and optimal therapeutic effect. Besides the survival of high numbers of positive RFP-MSCs in treated samples have been demonstrated at 3, 4 (Fig.1) and 6 weeks from the treatment. We have evaluated also that quality of tendon healing in MSCRFP treated tendons has been based on a better architecture of collagen fibers and high expression of collagen I respect to collagen III (Fig.2), related to the control tendons. The data obtained in this study confirm that MSCs allograft have a positive effect on tendon healing, its lack of significant immunogenicity permitting allogenic transplantation without immunosuppressive drugs and that the local injection in the tendon allows the survival of MSCs with good engraftment efficiency.