

Results of treatment of bone marrow derived mesenchymal stem cell therapy.

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Introduction

Regenerative medicine offers the prospect of restoring normal, or as close to normal, structure and function to an injured organ and thereby resulting in a successful restoration of activity without the risk of re-injury. Over-strain and traumatic tendon and ligament injuries are common in the horse and, for the most part, heal (repair) naturally by the formation of scar tissue. However the scar tissue formed in this repair is functionally deficient compared to normal tendon, which has important consequences for the animal in terms of reduced performance and a substantial risk of re-injury, in spite of a multitude of treatments that have been proposed. As pain is not usually a feature of these conditions in the long-term, the primary need is to restore functionality and so this has encouraged the development of regenerative strategies.

Mesenchymal progenitor cells (MPCs) have been considered an ideal source of cells for regenerative medicine because it can be demonstrated, in horses as in other species, that they are capable of differentiating into different cell lines and synthesise new matrix (usually chondrogenesis, adipogenesis and osteogenesis). These cells are thought to be present in small numbers in most tissues but we have chosen to harness the action of MPCs recovered from bone marrow because of ease of recovery, minimal donor site morbidity, and, as these stem cells can be recovered from adult tissue, the possibility of autologous re-implantation which carries fewer regulatory and safety issues. Furthermore in comparative experiments assessing multipotency, bone marrow-derived MPCs tend to out-perform MPCs from other sources.

Equine digital flexor tendon strain injuries provide many of the elements required for tendon tissue engineering – the lesion manifests within the central core of the tissue thus providing a natural enclosure for implantation and, by the time of stem cell implantation, is filled with granulation tissue which acts in the role of a scaffold. It has the added advantage of being highly vascularised and therefore capable of nutritional support of the implanted progenitor cells. The cytokine and mechanical environment, which are potentially important drives for differentiation, is provided by the intra-tendinous location of the cells and the suspension of MPCs in bone marrow supernatant which we have shown to have significant anabolic effects *in vitro*[1].

Post injury, tendon does not exhibit a problem with cellular infiltration but those cells actually involved in the synthesis of new tissue are mostly locally derived cells [2]. Most tissues have a small population of precursor cells (tissue-specific progenitor cells) that are used to replenish cells due to natural turnover and aid in repair post-injury. Evidence of multipotency has been shown for cells derived from young tendon, however, in adult tendon, it has been difficult in our laboratories to demonstrate the presence of a cell sub-population capable of differentiating into multiple cell lines, other than possibly their own, with similar ability to bone marrow derived cells, which may explain why this component of the repair process is limited and hence natural repair inferior to normal tendon.

We have therefore hypothesised that the implantation of autologous MPCs, in far greater numbers than are present normally within tendon tissue, would have the potential of improving the repair of the tendon both structurally (by optimising mechanical properties, organisation and composition) and functionally (by reduced re-injury rates).

Materials and Methods

Clinical data: Bone marrow was recovered from the sternum under standing sedation, generally within 1 month of injury, and transferred to a laboratory for culture and expansion of MPCs. After approximately 3 weeks, the cultured cells were transferred back to the veterinarian ($10-50 \times 10^6$ cells, depending on the extent of the lesion) and implanted into the damaged tendon of the same horse under ultrasound guidance. After implantation, the limb was bandaged and the horses underwent a week of box rest followed by a controlled exercise programme for up to 48 weeks.

Experimental study: 10 horses with naturally occurring SDFT injury were randomly allocated to treatment groups - 1×10^7 autologous bone marrow derived MPCs, obtained as described above) were implanted into the damaged SDFT of the treated group. Saline was injected into the control group. Horses received controlled exercise and were euthanased after 6 months. Non-destructive mechanical testing assessed structural stiffness of the SDFT and morphological and compositional analysis was performed on the tendon tissue.

Results

Clinical data: To date in excess of 1500 horses have been treated worldwide with this technique. Ultrasonographic appraisal of treated cases showed a rapid filling-in of the hypoechoic lesions although a reduced longitudinal striated pattern usually persists. Occasional hypoechoic needle tracts can be identified in some horses for up to 3 months after implantation. Analysis of clinical outcome in 113 treated racehorses gave a re-injury rate of 27% for those horses which had returned to full training and had been followed up for 3 years after treatment. This re-injury rate was significantly better than for racehorses treated conventionally and analysed in the same way (57%[3]; $p < 0.05$). A more limited number of injuries to other tendons and ligaments cases have also been treated so that firm conclusions on efficacy for these injuries can not be made. For lesions present within a tendon sheath, the implantation was performed after tenoscopic evaluation to ensure that there are no surface defects through which the cells could leak.

Histopathological examination has been carried out on 17 tendons from post mortem samples obtained from 12 horses which have undergone MPC implantation. These have shown both good quality healing with minimal inflammatory cells, and crimped organised collagen fibers. Furthermore, there was no evidence of any abnormal tissue or neoplastic transformation. In addition, labeled MPCs were detected in enclosed lesions for up to 4 months, similar to that described previously [4].

Experimental study: MPC-treated tendons exhibited normalisation of their mechanical, morphological and compositional parameters towards that of uninjured tendons. This was significantly different ($p < 0.05$) from saline treated tendons for cross-sectional area, cellularity, crimp pattern, and DNA content.

Conclusions

Treatment with MPCs appears to reduce re-injury rates in superficial digital flexor tendon injuries in racehorses. This is supported by improvement in mechanical, morphological and compositional parameters in a controlled experimental study using natural disease.

References

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Acknowledgements and Declarations

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