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THE RESULTS OF USING STEM CELLS TO TREAT FLEXOR TENDONITIS.

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Rationale behind the use of exogenous stem cells to treat tendon over-strain injuries

Tendon naturally heals (repairs) well but 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. We have hypothesised that the implantation of autologous mesenchymal stem cells, in far greater numbers than are present normally within tendon tissue, would have the potential of regenerating or improving the repair of the tendon.

The equine digital flexor tendon strain injury provides 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 stem 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 MSCs in bone marrow supernatant which has been shown to have significant anabolic effects on cultures of equine ligament-derived cells.

Technique

BM-MSCs are still the most investigated and characterised post-natally derived stem cell as they appear to perform superiorly to MSCs recovered from other tissues in terms of differentiation into known cell types. Bone marrow is recovered from the sternum (or tuber coxae) under standing sedation and transferred to a laboratory in specially designed containers for culture and expansion of MSCs. Separation is largely dependent on the MSC's property of adhesion to culture plastic which produces an enriched rather than pure cell preparation. As with other species, the presence of stem cells within equine MSC preparations has been shown by differentiating the cells along multiple cell lines using defined media (e.g. usually osteogenic, adipogenic and chondrogenic). After approximately 3 weeks, the cultured cells are transferred back to the veterinarian ($10\text{-}50 \times 10^6$ cells, depending on the extent of the lesion) and implanted into the damaged tendon of the same horse under ultrasound guidance. The cells are suspended in bone marrow supernatant for implantation so that no 'foreign' material is implanted and to gain potential beneficial effects of the rich mix of growth factors present in the supernatant. After implantation, the limb is bandaged and the horses undergo a week of box rest to allow the cells to 'take up residence in the tissue'. Thereafter the horses enter a controlled exercise programme for up to 48 weeks. This procedure has now become routine in equine clinical practice in many countries world-wide. Training courses have been run to educate veterinarians on the technology, criteria for treatment and the practicalities of the technique to minimise inappropriate use.

Current outcome data

Initially, a Phase I trial was performed to ensure safety. This consisted of 6 horses with large core lesions in their SDFTs. Results indicated that the technique did not cause any worsening of the injury. Furthermore, there was no reaction or enlargement of the tendon post-implantation, and no bone or cartilage was formed based on gamma scintigraphy and ultrasonography. Core lesions filled in quickly when a hypoechoic lesion was still visible at the time of implantation. The longitudinal pattern, however, remained inferior to normal tendon but improved with exercise.

Since the initial trial, in excess of 600 horses have been treated with this technique. At the most recent evaluation of clinical outcome (September 2006), 168 racehorses had been treated and long term (>1 year) follow-up was available for 82 horses. For National Hunt

racehorses (n=71), the re-injury rate was 13% (including injuries to untreated contralateral limbs). When only those horses which had entered full training were included, the re-injury rate rose slightly to 18%. This compares favourably with previous analyses for the same category of horse (56% re-injury rate for National Hunt horses, Dyson (2004)) although this latter analysis was over 2 years after a return to full work. Further follow-up of these treated horses after this time period will allow direct comparison. Re-injury rates for sports horses (all disciplines combined; n=24 with more than 1 year follow-up) was improved by a similar degree (13% compared to 23-43% but with longer follow-up reported by Dyson (2004)).

We proposed that the optimum time to implant the cells is after the initial inflammatory phase but before fibrous tissue formation. It was hypothesised that the presence of mature fibrous tissue within the tendon would (a) make implantation more difficult and (b) reduce the benefits of the stem cell therapy due to its persistence both of which have been supported by clinical experience of delayed implantation of bone marrow-derived MSCs and outcome - successes had an average interval between injury and implantation of 44 days while, horses suffering re-injury, this was 83 days (p=0.0035). Current recommendations are that bone marrow is aspirated within 1 month of injury and, for the same reason, known recurrent injuries are not considered ideal cases because significant fibrosis would already be present. The time of implantation may be further optimised by pre-injury storage of cells.

Two cases which died through unrelated causes have been analysed histologically and showed excellent healing with minimal inflammatory cells, and crimped organised collagen fibres. In contrast, a contralateral untreated suspensory ligament injury in one of these horses, which was clinically silent at the time of implantation, showed persistent inflammatory cells and poorly organised collagen fibres.

An increasing number of injuries to other tendons and ligaments have also been treated. For lesions present within a tendon sheath, the implantation is done after tenoscopic evaluation to ensure that there are no surface defects through which the cells could leak.

Conclusions

There are thus some encouraging aspects to this technology although definitive proof of efficacy is still lacking which is essential before full confidence in the technology can be achieved. Furthermore there have been no direct comparisons between the two techniques currently available for use commercially. It must be remembered that there are still considerable gaps in our knowledge although the technology is developing rapidly. Although cell-based therapies are likely to be another instrument for tackling orthopaedic disease in the future, it is also likely that we will need to be selective in choosing the right clinical cases. It is also hoped that experience gained from treating clinical cases in horses will provide sufficient supportive data to encourage the translation of this technology into the human field where largely randomised control trials will lead to better evidence-based medicine.

Reference

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