Homing of Stem Cells

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Cell-based therapy with mesenchymal stem cells (MSCs) has become an accepted practice for equine tendon and ligament injuries. [1, 2] MSCs are most commonly injected intralesionally (IL). Less invasive administration of MSCs through intraarterial (IA) or intravenous (IV) regional limb perfusion (RP) has recently been reported. In that report the use of MSCs labelled with Tc\textsuperscript{99m} proved to be a sensitive and quantifiable measurement of MSCs distribution in the normal equine distal limb. [3]

Several reports suggests that MSCs are capable of mobilizing from bone marrow or other sources to home to injured tissues through mediators and growth factor signals, which attract MSCs and facilitate their migration. [4, 5] This characteristic of MSCs could improve cell-based therapies, with the hope that vascular MSCs administration would lead to homing of the cells to acute lesions or lesions difficult to treat intralesionally.

The survival of IL implanted MSCs has been investigated using labelled cells and subsequent evaluation of the tissue up to three to four months after implantation has demonstrated persistence of implanted cells but only in relatively small numbers [6, 7]. However, it is not clear when this loss occurs and how many survive the initial implantation process.

Presently, it is a common practice to use autologous MSCs to treat tendon and ligament injuries. This technique includes harvesting the source of MSCs and culturing them. These steps could be eliminated using allogenic MSCs. The difference in lesion homing, persistence after administration and distribution between autologous and allogenic MSCs has not been reported.
In the present study, the distribution of autologous and allogenic MSCs after administration IV, by RP and IL using Tc$^{99m}$ to label MSCs is assessed. The measurement of the radioactivity would determine the persistence of injected MSCs over a 24-48 hour period. We hypothesised that MSCs would be retained within the tendon after IL injection and that MSCs can “home” to the sites of injury in digital flexor tendons when injected remotely either by intravenous injection into the jugular vein or via the digital veins while a proximal tourniquet is in place. Furthermore, autologous and allogenic MSCs may display a similar distribution, persistence and homing effect.

**Materials and Methods:**

**Cases:**

18 horses were utilized in this study. These horses were all presented with a non-traumatic over-strain injury to one of the palmar soft tissue supporting structures of the metacarpal region of the forelimb, 13 presented injury of the superficial digital flexor tendon (SDFT) and 5 had lesion of the accessory ligament of the deep digital flexor tendon (ALDDFT). Both the affected and contralateral limbs were palpated and examined ultrasonographically to confirm the diagnosis. For inclusion in the study, lesions had to have occurred less than 21 days prior to presentation. Ethical permission for this study was granted by the Animal Ethics and Welfare Committee of the Colegio de Veterinarios de Malaga – Spain.

**Recovery of MSCs:**

Bone marrow was harvested from the sternum in the standing horse under sedation. In 13 horses 10 million cell aliquots were suspended in autologous citrated bone marrow supernatant. In the other 5 horses allogenic MCSc were used with identical protocol.

**Labelling and implantation of MSCs for clinical study:**

Ten million autologous (13 horses) or allogenic (5 horses) MSCs labelled with Tc$^{99m}$-HMPAO were administered to each horse by each of three randomized routes every 7 days as follows:

1) Intraleisonally under ultrasonographic guidance using a 1.5 inch 20 gauge needle at one site at the maximum injury zone. (Intraleosonal – IL; n=18).
2) Intravenously by first diluting the MSCs in 19ml PBS and administering it via the lateral palmar digital vein in the pastern region while a rubber tourniquet was
applied in the proximal metacarpal region (regional perfusion – RP; n=17) The tourniquet was left in place for 30 minutes after injection before release.

3) Intravenously via a preplaced catheter in the jugular vein (intravenous – IV; n=18).

The activity of Tc\textsuperscript{99m} used for labelling for each of the administration routes used were 1000 MBq, 2000 MBq and 7000 MBq for intralesional, regional perfusion and intravenous respectively.

**Imaging protocol:**

Planar gamma scintigrams using a gamma camera\textsuperscript{1} were obtained within 5 minutes of injection (IL and IV) or tourniquet release (RP) (time 0) and then at 1, 3, 6, 12, 24, 36 and 48 hours after administration of Tc\textsuperscript{99m}-HMPAO labelled MSCs. Images were obtained from the lesion area, the equivalent area of the contralateral limb, the left lung field and the left thyroid. Images were displayed and analysed using software\textsuperscript{2}. Radioactivity levels at the lesion site were measured by analysing identically-sized regions of interest for each animal at the different time-points, which were then expressed as a percentage of the ROI at time 0, having corrected for the predicted decay of the technetium, to give the percentage of cells remaining.

**Results**

**Distribution after implantation:**

*Intralesional administration:* Diffuse uptake of radiopharmaceutical was imaged immediately after implantation throughout the lung field in 2/18 horses, in a single focal area within the lung field in 3 other horses and in multiple focal areas in 2 horses. In the remaining 11/18 horses no uptake of radiopharmaceutical was evident within the lungs. No uptake of Tc\textsuperscript{99m} was evident in the thyroid (as free pertechnetate) or within the contralateral limb. Focal uptake was evident at the site of the injection in all 18 horses, with similar results between autologous and allogenic. No proximodistal spread was evident after masking the injection site in 9/18 horses. In two horses the spread was diffuse proximally. In 4/18 horses had diffuse distal spread, 2/13 had proximodistal spread and 1/13 had a focal proximal spread separated from the injection site.

*Regional perfusion:* Uptake throughout all lung fields was identified after removing the tourniquet. Radiopharmaceutical uptake in the thyroid region was mild to marked in all

\begin{footnotesize}
\begin{enumerate}
\item GE-400A, GE Healthcare. Little Chalfont, UK
\item MicasX plus, Bartec Medical Imaging Solutions, Surrey, UK
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horses. In the contralateral limb no uptake of Tc\(^{99m}\)-HMPAO was evident in any horse. Radiopharmaceutical uptake was identified at the site of the lesion in 11/17 horses (64%, 75% in the autologous and 60% in the allogenic group) (Figure 1). In 5 horses with no uptake at the lesion site had one or two intense focal areas of uptake in other regions of the limb and in the lungs.

*Intravenous administration:* Marked diffuse distribution throughout all lung fields was evident at five minutes in 13/18 (72%) of horses; a milder diffuse distribution was seen in the remaining 5 horses. In all but one horse, the gamma camera could not detect Tc\(^{99m}\) within the lung fields after 24 hours. In the contralateral limb no uptake of Tc\(^{99m}\) was evident in any horse. Only one horse (horse 3, autologous group) showed an area of focal uptake in the region of its SDFT lesion which was evident within 5 minutes of administration of the labelled cells and persisted for 24 hours.

*Quantification of cell persistence after intralesional and regional perfusion administration*

The absolute counts from regions of interest over the lesion with regional perfusion was 2.6 (+/-1.9) % of the counts after intralesional injection at time 0. When taking into account the normal decay of Tc\(^{99m}\), there was still a rapid drop off in the percentage of cells remaining at the lesion site after both intralesional and regional perfusion (figure 2), with an average of 32% and 24% retained at 12 and 24 hours respectively after intralesional autologous administration compared to 9% retained after 12 hours with autologous regional perfusion. At 24 hours an 18% of the allogenic MSCs were present after intralesional administration compared to 11% retained 12 hours after RP administration.

**Discussion**

Based on these findings, a homing effect is present after intravenous administration of MSCs with 30 minutes of regional perfusion. Regional perfusion appeared to deliver significantly lower cell numbers than after intralesional administration. This technique seems a promising modality for treating diffuse lesions or lesions in areas difficult to reach by intralesional administration.

The distribution of cells to the lungs after intravenous route was not surprising given that that has been observed after white blood cell labeling. There was occasional focal uptake in the lungs after regional perfusion which is most likely to reflect clumping of cells within the venous system which are then released after the tourniquet is removed and are ‘filtered out’ by the lung microvasculature. However, the presence of
cells in the lungs was not associated with any adverse clinical signs and all horses were successfully rehabilitated back to performance, an important finding with regards to the safety of these administration routes for humans.

Around a 20% of the cells were still present after 24 hours after intra-lesional implantation. This figure may represent a low but close estimate of cell persistency since it is possible that label dissociated from the cells when in vivo. Relatively low levels of label dissociation occurred since deposit of free Tc$^{99m}$ in the thyroid was rare. This level of cell retention, however, is consistent with other labeling studies which estimated less than 5% of the implanted cells still present 10 days after implantation. Although a large percentage of cells appear to be lost soon after implantation, only smaller numbers may be necessary to exert paracrine influences on the resident cells.

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**References:**


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Figure 1: Gamma scintigraphs from a lesion on the distal superficial digital flexor tendon 22 cm distal to the accessory carpal bone (representative drawing and ultrasonographs shown on top). They were performed at 5 minutes, 1 hour, 3 hours and 6 hours after administration by intravenous regional perfusion (via the palmar digital vein in the pastern region). Note the uptake over the distal superficial digital flexor tendon.

Figure 2 – Percentage of cell remaining, as estimated from radioactivity levels, after intralesional injection and regional perfusion. A: Autologous. B: Allogenic