STEM CELL THERAPY: STUDIES IN ANIMALS
Simon R. Bailey BVMS PhD FHEA DipECVPT MRCVS
Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Victoria

Mesenchymal stem cells (MSCs) are present in all tissues, and isolated MSCs are considered very promising for therapeutic applications due to their potential roles in tissue regeneration and modulation of inflammation. Many aspects of their mechanism of action are still poorly understood, although a large number of studies are being conducted on experimental animals with induced lesions, and our understanding is rapidly increasing. However, despite many anecdotal reports of their beneficial effects in the veterinary field, there are relatively few reports of well controlled studies investigating the efficacy of MSC therapies in naturally occurring diseases in dogs, cats and horses.

Many companies around the world now offer stem cell therapies or the means to obtain and culture or purify these cells. For autologous stem cell use, initially MSCs were cultured from bone marrow, however adipose-derived stem cells are currently the most popular source of MSCs, either as cultivated cells or stromal vascular fraction. Not only is adipose tissue more easily obtained, but MSCs have been shown to be considerably more abundant in adipose tissue than in bone marrow. ASCs tend to exhibit greater proliferative potential and lower chondrogenic and osteogenic potential than bone marrow MSCs.

Although autologous cells were considered safer than allogeneic cells (cells from another individual of the same species), in fact allogeneic MSCs are not recognised by the recipient immune system as ‘foreign’ and do not elicit an immune response, because these cells do not express MHC class II antigens or costimulatory molecules and they suppress T cell proliferation. Therefore by using allogeneic cells, the biggest step forward in ease and availability of MSCs has come recently with the availability of off-the-shelf cell therapy products for dogs and horses, which are now pre-registered in Australia, and are currently being trialled. For canine cells these are typically obtained from fat tissue from donor animals at the time of sterilisation.

Regenerative effects of stem cells
Stem cells are capable of many different functions which support tissue regeneration, and it is important to note that these effects are not just related to their ability to differentiate into other cell types. Other very important mechanisms include trophic support, revascularisation and anti-inflammatory effects.

Regarding differentiation, adipose-derived MSCs show a similarly diverse plasticity to that seen from bone marrow-derived MSCs, including differentiation into adipo-, osteo-, chondro-, myo-, cardiomyo-, endothelial, hepato-, neuro-, epithelial and hematopoietic lineages. These data are supported by in vivo experiments and functional studies that demonstrated the regenerative capacity of adipose-derived MSCs to repair damaged or diseased tissue via transplant engraftment and differentiation (Bruder et al, 1998). However, trophic support within the affected tissue may play an even more important role. Many studies have demonstrated that MSCs secrete active levels of cytokines and growth factors that support angiogenesis, tissue remodeling, differentiation, and anti-apoptotic events.

Revascularisation is another important component of tissue regeneration. Adipose derived cells of the stromal vascular fraction contain endothelial progenitor cells and MSCs that assist in angiogenesis and neovascularization by the secretion of cytokines, such as hepatic growth factor (HGF), vascular endothelial growth factor (VEGF), placental growth factor (PGF), transforming growth factor (TGFβ), fibroblast growth factor (FGF-2), and angiopoietin.

Anti-inflammatory and immunomodulatory effects
Both in vitro and in vivo studies have demonstrated that MSCs limit inflammatory responses and promote anti-inflammatory pathways, by a number of different mechanisms. When present in an inflammatory environment, MSCs may alter the cytokine secretion profile of dendritic cell (DC) subsets and T-cell subsets causing a shift from a pro-inflammatory environment to an anti-inflammatory or tolerant environment. Therefore, there are several inflammatory and immune-mediated conditions which appear to be very amenable to stem cell therapy, including the potentially lethal graft versus host disease in humans receiving transplanted tissue.

Our own studies at the University of Melbourne into the anti-inflammatory effects of MSCs has utilised a mild arthritis in sheep induced by the immune response to collagen in pre-sensitised animals (a large animal model of human rheumatoid arthritis). Sheep treated with mesenchymal progenitor cells (MPCs; a subset of MSCs obtained from bone marrow) demonstrated significantly reduced clinical signs of lameness, joint pain and swelling compared with control sheep. They also showed decreased cartilage erosions, synovial stromal cell activation and angiogenesis. This was accompanied by decreased infiltration of the synovial tissues by CD4+ lymphocytes and CD14+ monocytes/macrophages (Abdalmula et al, 2015). Interestingly, when MPCs were...
administered intra-articularly, no beneficial effects were observed – the effects were only seen when the cells were administered intravenously. Because these cells are acting to modulate the behaviour of T and B lymphocytes early in the inflammatory pathway, it appears that much of this cross-talk may be occurring in sites such as the spleen, rather than the locally inflamed tissues. This is reinforced by the effect of MPCs on systemic inflammatory markers and vascular endothelial dysfunction that accompanies these immune-mediated conditions (Dooley et al., 2014).

Therefore route of administration is an important consideration for stem cell therapy. Homing (chemotaxis) is an event by which a cell migrates from one area of the body to a distant site where it may be needed for a given physiological event. Homing is an important function of MSCs and other progenitor cells and one mechanism by which intravenous or parenteral administration of MSCs permits an auto-transplanted therapeutic cell to effectively target a specific area of pathology. For example, labelled cells of bone lineage injected intravenously into mice can engraft, form bone, and give rise to osteocytes and bone lining cells detectable on the mouse femur. Peripheral intravenous experiments using a cerebral arterial occlusion model of stroke has demonstrated that labelled MSCs administered 24 hours and 7 days post-injury can migrate to the area of injury as well as causing a dramatic reduction in stroke infarct size.

**Studies in domestic animals**

Several previous studies have described the results of stem cell therapy for artificially induced conditions in dogs, cats, and horses. The use of animals as models for diseases, with surgically or chemically induced lesions, can provide valuable information on specific aspects of available therapies. However, while this information is important, induced lesions cannot adequately reproduce the conditions present in spontaneously affected patients.

Many of the reports of therapeutic efficacy of stem cell therapy in naturally occurring conditions are descriptive or anecdotal, because of the ethical and clinical problems associated with obtaining sham-treated controls and blinded objective measurements. The scarcity of strong scientific evidence is a major problem with the progression of stem cell therapies in domestic animals. However, a number of important clinical trials are now being instigated at institutions such as Colorado State University, University of Pennsylvania and UC Davis.

Seven studies in dogs describing adipose-derived stem cell therapy in naturally occurring cases have been published to date, mainly for orthopaedic conditions. Quantitative data was only obtained in two of these studies, using a force platform to measure lameness due to osteoarthritis (Vilar et al., 2013; 2014). In one study, atopic dermatitis was treated with no effect (Hall et al., 2010). In some studies, cells were combined with platelet rich plasma, which also contains growth factors which may complicate observations. Several other limitations are observed in these reports, and only one of them included a control group (Black et al., 2007). This was a double blinded controlled trial in dogs with chronic arthritis of coxofemoral joints, where significant clinical improvement on a subjective rating scale was observed using 18 dogs (9 treated and 9 control).

Two studies have been published in cats, where adipose-derived stem cell therapy was used in spontaneously affected animals treated for chronic kidney disease. No control groups were included, and although a modest improvement in GFR was observed in a small pilot study, no clinical improvement was found in the subsequent larger study. The probably suggests that only certain specific disease processes may suitable for stem cell therapy, and there needs to be a clear rationale (based on sound evidence) for their clinical use.

A number of reports of studies using MSC therapy in horses have been published, most of them addressing cases of tendonitis. Clinical and ultrasonographic analyses have shown regeneration of injured tendons and functional recovery, but the absence of a control group and use of cells combined with PRP often limits the significance of the results in terms of the efficacy of MSCs. Osteoarthritis in the horse appears to be a more difficult indication for stem cell therapy, and some trials have shown no appreciable effect while others have been more encouraging. Most studies using MSCs in an attempt to repair damaged cartilage in animals have involved injecting a suspension of MSCs into a joint space; at best this serves to retard the progression of osteoarthritis, most likely through paracrine anti-inflammatory effects, but does not generate any long-term physical repair of the cartilage, almost certainly because the MSCs fail to engraft. In contrast, studies in which the MSCs are spatially supported by a matrix show more promising results, especially when they are combined with biomaterials, such as hyaluronic acid, that support chondrogenesis (Whitworth and Banks, 2014).

**Conclusions**

It appears that many of the characteristics and actions of canine and equine MSCs may be similar to the characteristics described for human cells. However, more trials and robust evidence is required in veterinary species in order to maximise the enormous potential of cell therapies in veterinary medicine.
References


