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Review Article

The role of mesenchymal stem cells in veterinary therapeutics – a review

RA Webster*§, SP Blaber*, BR Herbert*, MR Wilkins† and G Vesey‡

Abstract

Adult mammalian tissue contains a population of cells known as mesenchymal stem cells (MSC), that possess the capability to secrete regenerative cytokines and to differentiate into specialised cell types. When transplanted to a site of injury MSC embed in damaged tissue and repair and regenerate the tissue by secreting cytokines. The immuno-privileged and immuno-regulatory capabilities of MSC enhance their therapeutic potential not only in autologous but also allogeneic recipients. Studies have demonstrated the beneficial effects of MSC in the treatment of a variety of clinical conditions including osteoarthritis, tendon injuries, and atopic dermatitis in domestic animals. Studies using animal models have shown promising results following MSC or MSC secretion therapy for induced injury in musculoskeletal and nervous systems and some organ diseases.

This review describes the stem cell types relevant to regenerative medicine and the procedures used for isolation, identification, expansion, enrichment and differentiation of these cells. We also review the use of MSC in animal models of disease as well as diseases in the clinical veterinary setting.

KEY WORDS: *Adult stem cells, mesenchymal cells, hematopoietic cell, adipose tissue, bone marrow*

Introduction

Stem cells are part of the tissue repair mechanism found in all mammalian tissues. They are unspecialised cells capable of renewing themselves through division, sometimes after lengthy periods of inactivity. Under certain physiologic or experimental conditions, stem cells can differentiate into tissue- or organ-specific cells with specialised functions (e.g. muscle, blood or neural cells). It is these characteristics that distinguish stem cells from other cell types.

There are two types of stem cells: embryonic and adult stem cells. Embryonic stem cells (ESC) are derived from the inner cell mass of the blastocyst. They are considered to be of vast potential in regenerative medicine because they are capable of becoming any differentiated cell in the body; this ability is known as pluripotency. There is, however, some risk associated with therapeutic use of ESC due to immunological rejection, mutation and the formation of tumours (Amariglio *et al.* 2009). Furthermore, the use of ESC is fraught with controversy due to the ethical and legislative issues surrounding embryo-derived material. Adult stem cells are multipotent meaning they can give rise to a defined number of cell types. These cells, which are capable of self-renewal, carry none of the ethical issues associated with ESC because they are obtained from fully developed adult tissues. Some adult stem cells are considered to be more valuable than others, due to their accessibility, abundance, self-renewal properties, plasticity and ability to proliferate and differentiate. Of most therapeutic relevance are mesenchymal stem cells (MSC) and hematopoietic stem cells (HSC). MSC can differentiate into many cell types including the chondrogenic, adipogenic, osteogenic and myogenic lineages (Zuk *et al.* 2001). MSC are immuno-compatible and have an immuno-regulatory effect, both of which are advantageous when making therapeutic use of them (Augello *et al.* 2007). HSC are found in bone marrow in high numbers and also in blood in low numbers in a mobilised cytokine-induced state. They give rise to platelets, red and white blood cells and can form any cellular components of the blood.

The purpose of this article is to provide a brief background on stem cells, review the use of stem cell therapy in animals and provide a guide for the therapeutic use of stem cells in domestic animals. This review focuses on MSC and their therapeutic use. Although HSC are relevant in the human clinical setting, currently they are not utilised in veterinary medicine.

Sources of stem cells

Stem cells can be obtained from many sites. For veterinary applications the common sites are bone marrow, adipose tissue and umbilical cord blood. The number of stem cells isolated from these tissues can vary, however, it should be noted that a known therapeutic dose of stem cells has not been yet defined for any disease in any species.

Bone marrow

Bone marrow contains both MSC and HSC, with, MSC being present at lower numbers than HSC (Baksh *et al.* 2004). Bone marrow is harvested using an aspiration needle such as a jamshidi. In dogs and cats, marrow is collected from the femur, tibia or

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ESC Embryonic stem cell(s)
HSC Hematopoietic stem cell(s)
MSC Mesenchymal stem cell(s)
SVF Stromal vascular fraction

humeral head with the humeral head being reported as the least traumatic (Frimberger *et al.* 2006). In horses, due to ease of access, marrow is collected from the sternum (Smith and Webbon 2005).

The volume of bone marrow that can be easily harvested from a cat or dog tibia or femur is approximately 1 mL per 1 kg of body weight. From 10 mL of bone marrow approximately 6×10^7 nucleated cells can be harvested, of which 600 to 6000 are stem cells (Pittenger *et al.* 1999; Varma *et al.* 2005). To separate the nucleated cells from red blood cells, a density gradient centrifugation procedure such as Ficoll-paque is performed. After washing by gentle centrifugation in saline, cells can be administered to the animal immediately, or can be further purified or enriched.

Adipose tissue

Mesenchymal stem cells are present in fat at higher levels than bone marrow (Varma *et al.* 2005) and are associated with the vast network of dense capillary beds lining adipose tissue (Crisan *et al.* 2008). Suitable tissue can be obtained using surgical excision of inguinal, abdominal and thoracic wall fat in companion animals. In horses following sedation adipose tissue can be obtained from the superficial gluteal fascia (Vidal *et al.* 2007).

The isolation of stem cells from adipose tissue involves mincing the tissue, digesting with collagenase and centrifugation. Adipocytes will float to the top whereas stem cells and other non-adipocyte cells form a stromal vascular pellet (Zuk *et al.* 2001).

The stromal vascular fraction (SVF) contains MSC, endothelial progenitor cells known to be involved in angiogenesis, monocytes and macrophages known for the high expression of anti-inflammatory cytokine interleukin-10, as well as pericytes, mast cells, preadipocytes, fibroblasts and smooth muscle cells (Varma *et al.* 2005; Riordan *et al.* 2009; Tallone *et al.* 2011). The therapeutic use of the entire SVF offers its own benefits, in that a mixed cell population allows for immediate, same day treatment. Furthermore, it is likely that the non-MSC cell types in the SVF possess therapeutic properties, and may act in a concerted manner with each other, the MSC and the local cells to produce cumulative therapeutic effects.

Umbilical cord blood

Umbilical cord blood should be collected immediately after foaling, using venipuncture with a 16 g needle attached to a blood collection bag containing an anticoagulant (Koch *et al.* 2007). Cells can then be cryopreserved either before or after *in vitro* expansion.

It appears that fresh umbilical cord cells and cryopreserved thawed cells have similar cell numbers and cell viability (Koch *et al.* 2007). Umbilical cord blood has lower numbers of MSC compared with bone marrow, but there is evidence to suggest this source has greater plasticity (Koch *et al.* 2007), with differentiation of these cells into cartilage, bone, hepatocytes and endodermal-derived cells described (Reed and Johnson 2008).

Processing prior to use

There are a number of common techniques that can be used to confirm the presence of MSC, and/or enrich or expand numbers of MSC prior to therapeutic use. Three criteria are used for the definition of multipotent MSC, according to the International Society for Cellular Therapy (Dominici *et al.* 2006), including the expression of cell markers CD73, CD90 and CD105 and lack of expression of the hematopoietic cell markers CD34 and CD45. Confirmation of MSC identity can be achieved using antibodies that recognise surface markers using techniques such as flow cytometry or fluorescence microscopy. In addition, the MSC must be capable of differentiating into cells of various lineages. This is generally achieved by growing the cells in a chemically defined medium. Finally, the MSC must display plastic adherence. This feature of MSC can be exploited to obtain a relatively pure population of MSC that can be expanded to achieve the desired number of cells for therapeutic use. However, there are concerns regarding the effect of long-term culture on these cells. Cultured cells may display genetic instability (Ross *et al.* 2011) and may not only lose functional characteristics but also differentiation potential (Mitchell *et al.* 2006). A further issue is that some cells have shown malignant transformation when cultured for long periods (Gimble *et al.* 2007). There are numerous on-going trials in humans assessing the long-term safety and efficacy of these cells.

Enrichment of MSC from mixed cell populations can be achieved through the use of density gradients, immuno-magnetic techniques, and fluorescent activated cell sorting. Furthermore, MSC can be seeded or cultured onto materials that facilitate adherence and integration of new connective tissues. (Bruder *et al.* 1998; Butler *et al.* 2008; Del Bue *et al.* 2008). These resorbable materials are designed to act as a scaffold until the introduced and recruited cells can commence tissue healing.

These techniques are not widely available in the veterinary setting, but some form of analysis of cells is useful for quality control and to help understand which cells are contributing to clinical effects (Horie *et al.* 2009). Moreover, this quality control approach may help to answer some of the unresolved questions such as the number of stem cells required to achieve a therapeutic effect.

Therapeutic delivery

Once stem cells are isolated, and if required expanded, the method used for the reintroduction of the cells depends on the condition that is being treated. Delivery mechanisms that direct the maximum number of cells to the diseased area are essential.

For the treatment of osteoarthritic joints, an intra-articular injection is used resulting in therapeutic cells remaining within the affected joint (Horie *et al.* 2009). For damaged tendons, ultrasound guidance permits direct implantation. Other localised conditions such as corneal eye ulceration can be treated with a local, topical application (Oh *et al.* 2008).

For the administration of cells to an organ, direct injection would be the most targeted approach, however, this may not be achievable in veterinary practice. Interestingly, it has been shown that MSC can localise to damaged organs (Fang *et al.* 2007). This localisation suggests that I/V injections of cells can be used to treat certain conditions. For example, I/V injections of stem cells into rats improved renal function following ischemic kidney damage (Fang *et al.* 2007; Semedo *et al.* 2007).

If *in vitro* expansion of stem cells is not required, the delivery of the cellular preparation should take place rapidly, preferably on

the same day. Cryopreservation of cells is possible and it has been reported that long-term cryopreservation of canine MSC did not result in a loss of stem-like features (Martinello *et al.* 2011). However, whether the cryopreserved MSC retain the same functionality as fresh cells is not fully understood. Also there are challenges associated with maintaining viability and preventing biological contamination in the storage and shipping of cryopreserved cells.

Autologous, allogeneic and xenogeneic therapy

There are several different approaches to the use of stem cells in regenerative therapy. An autologous approach uses an individual's own cells for treatment. Cells are harvested from the animal, the stem cells purified and then re-introduced into the same animal. Allogeneic transplants use cells from a genetically non-identical member of the same species, whereas xenotransplantation is the use of tissue or cells from a different donor species. Autologous procedures are considered minimal risk and ethically uncomplicated. In contrast, allogeneic stem cells could be used to provide a valuable off-the-shelf product, given that MSC are immune privileged (Fang *et al.* 2007). However, to date, limited data has been generated on the long-term safety of these cell therapies in animals. Xenotransplantation has been used more as a research tool to examine the immuno-privileged status of MSC and their mechanism of action *in vivo*.

Proposed mechanisms of action of MSC

The mechanisms by which stem cells repair and regenerate diseased sites are not yet fully understood. However, there is evidence for multiple mechanisms being involved. These are summarised in Table 1. Newly introduced stem cells have the capability to differentiate into specific cell types that can replace damaged or missing cells. Alternatively the introduced cells may mix or embed with existing cells thereby enhancing the existing cells function and ability to replicate (Kraus and Kirker-Head 2006). In the last decade, research has revealed additional mechanisms related to the secretion profiles of MSC and the beneficial properties of these secretions.

Table 1. The regeneration mechanisms of stem cells.

Replacement of damaged or lost cells. Introduced stem cells differentiate into specific cell types and replace the function of damaged or lost cells. For example, stem cells introduced into a joint could differentiate into chondrocytes and form new cartilage (Wakitani *et al.* 1994).
Cell embedding. Introduced cells embed with host cells and enhance cellular function (Rehman *et al.* 2004).
Recruitment of cells. Introduced cells elicit the recruitment of cells from other parts of the body (Gimble *et al.* 2007).
Anti-inflammatory effects. Secretions including interleukin-10 and hepatocyte growth factor from introduced cells have an immediate anti-inflammatory effect. The introduced cells may embed in tissue and continually secrete anti-inflammatory cytokines (Rehman *et al.* 2004).
Immune modulation. Introduced cells suppress the local immune environment, reducing inflammation and blocking autoimmune responses (Kang *et al.* 2008).
Growth factors. Introduced cells secrete growth hormones such as transforming growth factor beta (Kraus and Kirker-Head 2006).

Mesenchymal stem cells have potent anti-inflammatory and immuno-suppressive functions and secretions from these cells have similar capabilities (Baltzer *et al.* 2009; Table 1). The secretions have an immediate effect following introduction of the cells into the diseased site owing to the ability of the stem cells to respond to the local environment. It is recognised that the production of pro-inflammatory cytokines such as tumour necrosis factor-α and interleukin-1 α and $-\beta$ from immune cells decreases following the introduction of MSC (Semedo *et al.* 2007), and a number of the growth factors and cytokines secreted from MSC can have potent anti-inflammatory effects, such as interleukin-10 (Augello *et al.* 2007) Introduced stem cells have been shown to embed in tissues and it is likely that these cells continue to secrete regenerative substances for a significant length of time (Rehman *et al.* 2004). It is now evident that the secretions from MSC have potent angiogenic, anti-apoptotic and anti-scarring properties (Rehman *et al.* 2004) which, along with their ease of administration, make them a powerful potential therapy for veterinary use.

Treatment of arthritis, atopic dermatitis and tendon injury with MSC

A number of studies have reported the results of stem cell therapy in the treatment of domestic animals, these have focused on the treatment of arthritis, atopic dermatitis and tendon injury, and are summarised in Table 2.

Arthritis

There have been two reports on the use of adipose-derived MSC for the treatment of canine osteoarthritis. Both studies involved intra-articular injection with a mixed population of cells. The first study was a double-blinded trial of 21 dogs with osteoarthritis of the coxofemoral joint. The dogs treated with cells had a significant improvement in lameness and functional ability compared with the dogs that received a saline injection (Black *et al.* 2007). In the second study treatment of dogs with arthritic humeroradial joints resulted in improvements in lameness that remained evident at 180 days post-treatment (Black *et al.* 2008).

Atopic dermatitis

One open, non-controlled, non-blinded pilot clinical trial of the treatment of canine atopic dermatitis with MSC found no significant improvement in clinical signs in the five cases treated (Hall *et al.* 2010).

Tendon injury

Three approaches have been used to treat equine tendon injury. The first is the intra-lesion injection of autologous adipose-derived cells, containing a mixed population of non-cultured cells, but no clinical results have been published for this method. The second approach uses cultured bone marrow-MSC. and in over 100 horses with superficial digital flexor tendinopathy, was reported to produce rapid in-filling of the tendon lesion with no negative side effects (Smith and Webbon 2005). Follow up of the horses that entered full training (>1 year) found a re-injury rate of 18%. An alternative approach using cultured adipose-derived -MSC in conjunction with platelet-rich plasma was reported to result in 14/16 horses returning to their full pre-injury function (Del Bue *et al.* 2008).

Table 2. Results of treatment of dogs and horses with arthritis, atopic dermatitis and tendon injury using mesenchymal stem cells (MSC).

Condition	Type of stem cells	Number of cells	Reintroduction site	Results	Reference
Canine coxofemoral joint OA	Autologous AD-MSC, not cultured	4.2–5x10^6	Intra-articular	Statistically significant improvement in lameness	Black *et al*. 2007
Canine humeroradial joint OA	Autologous AD-MSC, not cultured	3–5x10^6	Intra-articular	Statistically significant improvement in lameness, joint stiffness and functional disability	Black *et al*. 2008
Canine atopic dermatitis	Autologous cultured AD-MSC	1.3x10^6 per kg	Intravenous	No significant improvement in the clinical signs of atopic dermatitis	Hall *et al*. 2010
Strain injury to superficial flexor tendon in a polo pony	Autologous cultured BM-MSC	6.4x10^5	Tendon under ultrasound guidance	Safe to use. No clinical results reported	Smith *et al*. 2003
Equine tendon injury	Autologous cultured BM-MSC	>4x10^6	Tendon under ultrasound guidance	Rapid in-filling of the tendon lesion with no negative side effects in over 100 horses	Smith and Webbon 2005
Equine tendon injury	Allogeneic cultured BM-MSC	Undefined	Tendon under ultrasound guidance	14 of 16 horses returned to their full pre-injury function	Del Bue *et al*. 2008

AD = adipose-derived; BM = bone marrow; OA = osteoarthritis

Treatment of induced injury using MSC

A large number of trials have investigated the use of stem cells in animal models, where injury has been induced in the musculoskeletal and nervous systems. A summary of the findings of these trials is presented in Table 3. It should be noted that induced injuries do not necessarily reflect their natural counterparts. Care should therefore be taken in the interpretation of these results in the veterinary clinical setting.

Musculoskeletal system

The repair of damaged tendons, ligaments, menisci, cartilage and bones have been demonstrated using cultured autologous and allogeneic bone marrow- and adipose-derived -MSC delivered into the site of injury (Table 3). New skeletal tissue was observed as early as 2 weeks post-treatment in rabbits, with hyaline cartilage forming with a compliance rating similar to natural hyaline cartilage (Wakitani *et al.* 1994).

Treatment of induced cartilage defects in the horse with bone marrow-derived cells has shown limited effects when compared with controls (Wilke *et al.* 2007), and treatment of induced osteoarthritis with expanded bone marrow-MSC influenced concentrations of prostagladin E_2 but had no other effects when compared with controls (Frisbie *et al.* 2009).

Collagenase-induced tendonitis is a common model to demonstrate efficacy using MSC in equine tendon repair. Results of these trials consistently demonstrate improved tendon fibre alignment, an improved collagen type I to III ratio as well as increased expression of cartilage oligomeric matrix protein, when compared with controls (Nixon *et al.* 2008; Crovace *et al.* 2010). In collagenase-induced injury in mice, where there was development of a rheumatoid condition as well as tissue damage, the introduction of stem cells appeared to both repair the damaged tissue and modulate the immune response attenuating the rheumatoid condition (Augello *et al.* 2007).

Bone regeneration using MSC have been demonstrated in dogs. The use of different types of scaffolds has been shown to be successful in keeping MSC localised to the surgically induced bone defects. Allogeneic MSC treatments produced no major immune response and healing was equivalent to that of dogs treated with autologous cells (Bruder *et al.* 1998; Arinzeh *et al.* 2003).

Nervous system

In rats with an induced spinal lesion, remyelination has been reported following I/V injection of bone marrow stromal cells and direct implantation of cultured bone marrow stromal cells into the site of injury (Akiyama *et al.* 2002ab). In contrast, no remyelination was reported in another study, but the introduced MSC migrated from the lesion site and deposited collagen in areas of healthy myelination (Hunt *et al.* 2008). The differences in outcomes in these studies are likely due to the culturing methods. In the former study, media contained neural progenitor cells, epidermal growth factor, basic fibroblast growth factor and neural survival factors (Akiyama *et al.* 2002b); whereas in the later study cells were cultured in low glucose Dulbecco's modified eagle medium and 10% foetal calf serum (Hunt *et al.* 2008).

Other organ systems

A number of trials have investigated the use of stem cells for the treatment of other damaged organs. The results of trials in rats for ischemia-induced kidney injury, urinary incontinence and corneal injury, and in pigs with induced diabetes are presented in Table 4.

Heart

The stem cell treatment of five dogs with dilated cardiomyopathy resulted in three dogs dying within 5 days of the procedure. This was likely due to the use of direct injection into the epicardial surface. The two surviving patients responded well, suggesting some value in this therapy (Borenstein *et al.* 2002). The safety of treating dilated cardiomyopathy in dogs was assessed by introducing varying quantities of cultured myoblasts via a

Table 3. Results of treatment of induced injury to the musculoskeletal and nervous systems using mesenchymal stem cells (MSC).

Condition (n)	Type of stem cells	Number of cells	Reintroduction site	Results	Reference
Surgically excised medial meniscus and resected cruciate ligament in goats (24)	Autologous, cultured BM-MSC	$1x10^7$	Intra-articular	Regeneration of meniscal tissue. No detectable repair of ligament	Murphy *et al*. 2003
Surgically induced full thickness defects of articular cartilage in New Zealand white rabbits (68)	Autologous, cultured BM or periosteum MSC	$5x10^6$	Medial femoral condoyle with collagen gel	Hyaline like cartilage formed in defects. No significant difference between cells from BM or periosteum	Wakitani *et al*. 1994
Arthroscopically induced cartilage defects in the femoropatellar articulations of horses (6)	Autologous, cultured BM	$12x10^6$	Defect with autologous fibrin or in contralateral defect autologous fibrin alone	Accelerated healing in BM-MSC group in the short-term. No long-term differences between the control and BM-MSC groups	Wilke *et al*. 2007
Arthroscopically induced OA in the middle carpal joint of horses (24)	Autologous uncultured AD-MSC and autologous, cultured BM	$16.3x10^6$ and $10.5x10^6$	OA-affected joint 14 days after arthroscopy	No significant treatment effects seen in either treatment group compared with control group	Frisbie *et al*. 2009
Collagenase induced tendon injury in horses (8)	Autologous AD-MSC, not cultured	$4x10^7$	Tendon under ultrasound guidance	Improved tendon structure. No clinical results reported	Nixon *et al*. 2008
Collagenase induced tendon injury in horses (6)	Autologous, cultured BM-MSC and uncultured autologous BM cells	$5.5x10^6$ and $1.2x10^8$	Tendon under ultrasound guidance	High expression of COMP and improved collagen type I/III ratio and fibre alignment in both treatment groups compared with placebo	Crovace *et al*. 2010
Collagenase induced tendon injury in horses (8)	Autologous cultured AD-MSC	$10x10^6$	Tendon under ultrasound guidance	No clinical or ultrasonographic changes. Significant improvement seen in histopathology fibre alignment and collagen type 1:3 ratio in treated group compared with control group	de Mattos Carvalho *et al*. 2011
Collagenase induced auto immune arthritic condition in mice	Allogeneic, cultured BM-MSC	$5x10^6$	Intra-peritoneal injection	Reduced bone and cartilage damage and T lymphocyte response	Augello *et al*. 2007
Surgically induced full thickness cranial bone defect in dogs (8)	Autologous, cultured AD-MSC with scaffold implantation	$3x10^5$	Inserted into bone defect	Greater bone growth in cell and coral treated group than control coral group	Cui *et al*. 2007
Surgically induced segmental bone defect in dogs (15)	Autologous, cultured BM-MSC. With scaffold implantation	$3.7x10^7$	Inserted into bone defect	Substantially greater bone growth in cellular implant than in control groups. Large osseous callus formed	Bruder *et al*. 1998
Surgically induced segmental bone defect in dogs (12)	Allogeneic, cultured BM-MSC with scaffold implantation	$3.7x10^7$	Inserted into bone defect	Substantial bone growth, no immune response noted	Arinzeh *et al*. 2003
Induced demyelinated spinal lesions in rats (30)	Allogeneic BM cells, not cultured	$1x10^7$	Intravenous peripheral introduction (femoral vein)	Extensive remyelination in 8 out of 15 subjects	Akiyama *et al*. 2002a
Induced demyelinated spinal lesions in rats (21)	Allogeneic, cultured BM-MSC	$5x10^3$	Directly into spinal lesion site	Remyelination of demyelinated spinal cord axons	Akiyama *et al*. 2002b
Induced demyelinated spinal lesions in rats (8)	Allogeneic cultured BM-MSC	$5x10^3$	Directly into spinal lesion site	No remyelination reported	Hunt *et al*. 2008

AD = adipose-derived; BM = bone marrow; COMP = cartilage oligomeric matrix protein; OA = osteoarthritis

Table 4. Results of treatment of induced injury to the kidney and cornea, and induced diabetes using stem cells.

Condition (n)	Type of stem cells	Number of cells	Reintroduction site	Results	Reference
Induced renal ischemia and reperfusion injury in rats	Allogeneic, cultured BM-MSC	$2x10^5$	Intravenous injection	Improved renal function. Reduced serum urea and creatinine	Semedo *et al*. 2007
Induced urinary incontinence in rats (30)	Allogeneic, cultured muscle derived stem cells	$1x10^5$, $1x10^6$, or $1x10^7$	Two injections one on each side of the urethra	Increased muscle twitch contractibility	Kwon *et al*. 2006
Chemical burns of rat corneas (18)	Allogeneic, cultured BM-MSC	Cells $2x10^6$ and secretions	Applied to cornea	Rapid corneal repair	Oh *et al*. 2008
Induced diabetes in pigs (10)	Allogeneic, cultured BM-MSC	$5x10^7$	Injected into pancreas	Reduction in blood glucose levels and newly formed islets produced insulin	Chang *et al*. 2008

BM = bone marrow; MSC = mesenchymal stem cells

fluoroscopically guided catheter in the femoral artery with differing effects. Use of 5×10^6 cells caused severe arrhythmia and cardiac infarction whereas a transplant of 3×10^6 cells proved to be well tolerated. The different response seen with varying cell numbers needs to be carefully considered in the clinical setting (Oyama 2005).

Urethra

Injection of autologous adult stem cells into the middle urethra has been shown to improve or restore contractile response (Jack *et al.* 2005). Under ultrasound guidance, it is possible to introduce adult stem cells to dogs through a percutaneous injection into the urethra.

Liver

No clinical studies in animals have described the treatment of liver disease with MSC. However, some preliminary studies have been performed in humans with promising outcomes and similar results may be expected in the veterinary setting. Improvement in liver function were reported following treatment with bone marrow derived cells in human patients with alcoholic liver cirrhosis (Pai *et al.* 2008). The process of isolating, expanding and re-introducing autologous stem cells into the hepatic artery appears relatively safe and effective in the treatment of human liver disease (Khan *et al.* 2008).

Conclusions

Autologous transplants of stem cells have proven efficacious in a number of applications. Stem cells can be derived from adipose tissue or bone marrow with procedures that are easily performed in the veterinary clinic, are low risk and are reasonably non-invasive. The use of adult stem cells in a number of situations has been shown to be predominantly safe. The best-documented examples are the treatment of tendon and ligament injury, osteoarthritis and inflammatory conditions. Results of the treatment of induced renal failure and diabetes are also promising.

Future stem cell therapies may also include allogeneic transplants. At this time there are no data on the use of such therapies in animals for naturally occurring diseases. There are, however, studies in dogs that demonstrate a surprising lack of immunological reaction to transplant. This suggests that the immuno-privileged and immuno-modulatory effects of MSC may render them suitable for allogeneic therapies.

A further alternative to the use of stem cells is the use of allogeneic or autologous stem cell secretions. It is increasingly evident that they have important immuno-modulatory and regenerative properties. Future research into the safety of this type of approach could result in an off-the-shelf stem cell secretion product. Such a product could be beneficial for conditions for which there are no current therapies or the therapies available are not very effective, or for patients which are unable to be anaesthetised for harvesting bone marrow or fat. Suitable clinical applications may include the topical treatment of corneal ulceration, skin and ear inflammation.

In assessing the clinical and experimental research, it is clear that stem cell-based therapies have potential as a powerful treatment for regeneration and repair of disease and injury. In the future, this may help reduce the use of invasive procedures and surgery in animals.

Declaration of interest

All authors are involved with Regeneus Pty Ltd in varying capacities. Rebecca Webster and Graham Vesey are employees of Regeneus Pty Ltd, Ben Herbert and Sinead Blaber are consultants of Regeneus Pty Ltd, Graham Vesey, Ben Herbert and Marc Wilkins are founding members of Regeneus Pty Ltd and are members of the board of directors of Regeneus Pty Ltd. All authors are shareholders of Regeneus Pty Ltd. Regeneus Pty Ltd have various patents filed in the adipose derived stem cell area.

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