

## Stem Cell Therapy for CKD: What do we know? (2018)

**Jessica M. Quimby, Columbus, Ohio, USA**

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### **Introduction**

Regenerative medicine is an innovative new area of research, and clinical applications hold promise for a variety of diseases in veterinary medicine. Stem cells have regenerative properties as well as an apparent ability to alter the environment in injured and diseased tissues. This type of therapy may be useful in chronic kidney disease (CKD), however research into this application of stem cells is still in preliminary stages and continued investigation is necessary.

### **What are stem cells?**

A stem cell is a generic term referring to any unspecialized cell that is capable of long-term self-renewal through cell division but that can be induced to differentiate into a specialized, functional cell. Adult stem cells can be obtained from many differentiated tissues and are not associated with ethical concerns like embryonic stem cells. Mesenchymal stem cells (MSC) have been one of the most commonly used types of adult stem cells and are multipotent but not pluripotent, which means they can differentiate into some, or “multiple,” but not all tissue types.

### **What tissues can stem cells be obtained from?**

Mesenchymal stem cells can be isolated from virtually every tissue in the body. The tissue source with the highest potential for proliferation of MSC appears to vary from species to species.<sup>1,2</sup> For most studies in veterinary medicine, MSC are most commonly obtained from bone marrow (bmMSC) or adipose (aMSC) sources. Various types of MSC products have been investigated as novel therapies, including MSC expanded in culture and stromal vascular fraction (SVF). SVF is the initial product of adipose tissue processing and is the type of cellular product produced from point of care processors and several private companies. While isolation and expansion in culture allows the expanded aMSC product to have a purer population of MSC, SVF contains multiple cell types. These are thought to include MSC as well as a mixture of B and T lymphocytes, endothelial cells, fibroblasts, macrophages, pericytes, and preadipocytes.<sup>3</sup> Currently not enough information is known about SVF to determine if a cellular product with a mixed cellular type is a therapeutic advantage or disadvantage.

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### **What donors can stem cells be obtained from?**

Stem cells that are harvested from the patient with the intention of administering them back to that patient are termed autologous. Stem cells that are harvested from healthy donors for administration to the clinical patient are termed allogeneic. The relative efficacy of autologous vs. allogeneic cells is an area of controversy. Although allogeneic MSC are immune-privileged and are not expected to incite an immune response, according to some authors they may not be as effective as autologous cells.<sup>4</sup> It is argued that autologous MSC may survive longer in the body in comparison to allogeneic cells, which could reduce efficacy of the latter. The advantages of using allogeneic MSC include sparing the patient from undergoing the harvest procedure as well as the ability to use MSC from young, healthy donor animals. Recent studies in human beings and rodents support the view that MSC obtained from young, healthy individuals have greater proliferation potential and have greater therapeutic potential than those collected from elderly diseased individuals.<sup>5,6</sup> This is of particular concern for application to kidney disease as it has been demonstrated that MSC obtained from uremic rats have reduced proliferation in culture, premature senescence, and decreased capacity to induce angiogenesis.<sup>7-9</sup> This concern was also noted in a pilot study assessing the safety and feasibility of autologous intrarenal MSC therapy in cats in which investigators observed that producing sufficient numbers of autologous MSC in culture from elderly diseased patients was challenging due to poor proliferation.<sup>10</sup>

### **What characterizes a stem cell?**

MSC are plastic-adherent and assume a fibroblast-like morphology during culture. They proliferate easily in culture and can be cryopreserved without loss of phenotype or differentiation potential. However, whether cryopreservation affects their immunomodulatory capabilities has not been fully investigated. The characterization of cell surface markers via flow cytometry differentiates them from hematopoietic cells, but no truly unique MSC marker has been identified.<sup>11</sup> For the most part, MSC have been reported to be CD 44 positive, CD 90 positive, CD 105 positive, CD 45 negative, HLA-DR negative and these markers are similar in both bmMSC and aMSC.<sup>2,11</sup> Most importantly stem cells from both adipose and bone marrow sources possess the ability to differentiate into cell types of multiple lineages including adipocytes, chondrocytes, and osteocytes demonstrating their multipotent potential.<sup>11</sup>

### **How could MSC be applied to CKD?**

Several studies have investigated the effects of MSC therapy in rodent models of CKD.<sup>12-15</sup> In the majority of these model studies, administration of both bmMSC and aMSC has demonstrated significant renoprotective effects including reduction of intrarenal inflammatory infiltrate, fibrosis and glomerulosclerosis.<sup>12,13,15</sup> Parameters of renal function

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function and clinical health, including weight, creatinine, BUN, proteinuria, blood pressure and hematocrit have also improved as a result of MSC therapy.<sup>12,13,15</sup> The mechanism of action for the effects seen in CKD is thought to be paracrine in nature, coming both from anti-inflammatory capabilities as well as protection of vascular integrity as mediated by VEGF.<sup>12-15</sup> Pro-fibrotic molecules and cytokines and pro-inflammatory cytokines, specifically TGF- $\beta$ , MCP-1 and IL-6, are found to be decreased in MSC treated rodents.<sup>12,13</sup> Anti-inflammatory cytokines such as IL-10 and vasculoprotective factor VEGF have been shown to increase as a result of MSC therapy.<sup>12,14,15</sup> Several routes of administration – intraparenchymal, subcapsular, intravenous - have been explored and all seem to be effective. Multiple repeated injections of MSC appear to be even more effective than single injections.<sup>12,13</sup> Although this body of literature demonstrates the immense potential of MSC therapy for kidney disease, it remains to be seen if these results in rodent models translate to veterinary or human patients. Rodent models of CKD are most commonly created by performing a 5/6 nephrectomy and a limitation of these models is that frequently MSC therapy is administered a relatively short time after nephrectomy (days to weeks), a time frame which may not predict success in the clinic where stem cell therapy would usually be applied much later in the disease process.

### **What studies have been performed in veterinary patients with CKD?**

To date no studies assessing MSC in dogs with CKD have been performed, but pilot studies assessing the safety and efficacy of MSC in cats with CKD have been conducted.<sup>10,16,17</sup> The first MSC study in cats with CKD was a pilot study assessing the safety and feasibility of autologous intrarenal MSC therapy.<sup>10</sup> Six cats (two healthy, four with CKD) received a single unilateral intrarenal injection of autologous bm-MSC or aMSC via ultrasound guidance. Two IRIS Stage 3 CKD cats that received aMSC experienced modest improvement in GFR and a mild decrease in serum creatinine concentration. Intrarenal injection of MSC did not induce immediate or longer-term adverse effects but it was concluded that the number of sedations and interventions required to implement this approach made it unattractive for clinical application. A more recent study in which one healthy cat received an intrarenal injection of amniotic-derived allogeneic MSC documented hematuria and significant stress as a result of the procedure and this study also concluded the technique was not clinically feasible.<sup>18</sup>

The feasibility of IV administration of allogeneic aMSC to cats with CKD has been investigated.<sup>16</sup> Cats with stable CKD with no concurrent illness were enrolled in a series of pilot studies and received an IV infusion of allogeneic aMSC collected and cryopreserved from healthy young specific pathogen free research cats every 2 weeks. In the first pilot study, cats had few adverse effects from infusions of cryopreserved aMSC and there was a statistically significant decrease in serum creatinine concentrations during the study period. However, the magnitude of the decrease in serum creatinine concentrations was considered unlikely to be clinically significant. In a second pilot study cats received a higher dose of cryopreserved aMSC. Adverse effects of aMSC infusion were observed in the majority of cats. Vomiting occurred in 2/5 cats during infusion and increased respiratory rate and effort was noted in 4/5 cats. In contrast, cats in a third pilot study that received aMSC cultured from cryopreserved

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adipose did not experience any adverse effects. Serum creatinine concentrations, urinary cytokines and GFR did not change significantly in cats in either of these latter studies. It was suspected that the use of higher doses of aMSC taken directly from cryopreservation was the source of the treatment-related adverse effects, and that this finding may represent an instant blood mediated inflammatory reaction (IBMIR) where cells clump as they contact the blood and result in micro pulmonary thromboembolism.<sup>19</sup>

A randomized, placebo-controlled, blinded one-way crossover clinical study assessing the efficacy of allogeneic MSC expanded from cryopreserved adipose has also been performed in CKD cats.<sup>17</sup> Seven cats were randomized to receive aMSC or saline placebo IV at two, four, and six weeks. While administration of aMSC was not associated with adverse effects, significant improvement in renal function (as determined by serum creatinine and GFR by nuclear scintigraphy) was not observed in the 8 weeks following administration.

The IV administration of allogeneic MSC derived from amniotic membrane has been assessed in nine cats with CKD who received two injections of MSC 21 days apart.<sup>18</sup> One cat experience vomiting during the first administration, but otherwise MSC were well tolerated. A statistically significant but small decrease in serum creatinine was seen with stable body weight over the course of the study. Numerical reduction in proteinuria and increase in urine specific gravity were also seen. However, studies with a control group are necessary to determine if changes are attributable to MSC therapy or normal variation in values.

### **Conclusion**

Although MSC have great potential applicability to kidney disease, and some veterinary studies have documented small improvements in renal parameters in some patients, results have not recapitulated those from rodent studies. Additionally there are still many questions to be answered regarding the logistics of MSC therapy (route of administration, dose and frequency of dosing, source of cells etc.). At this time MSC therapy for CKD should still be considered an experimental and unproven therapy. Additional studies are needed to determine the clinical applicability of this potential therapy.

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