

**ASSESSING THE USE OF MESENCHYMAL STEM CELL
THERAPY FOR FELINE CHRONIC KIDNEY DISEASE**
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Regenerative medicine refers to the process of using living function tissues to repair or replace organs that are functionally damaged. Stem cell therapy, in particular, is an innovative new field of scientific investigation and clinical application that holds promise for a variety of diseases in veterinary medicine as well as human medicine. Recent years have brought increased interest in the potential for adult stem cells to help in the treatment of many diseases through both their regenerative properties as well as their apparent ability to alter the environment in injured and diseased tissues. In particular, adult stem cells called mesenchymal stem cells can migrate to affected areas and may be able to support the growth of other stem cells as well as moderate the response of the immune system.

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. Stem cells are generally divided into two groups, embryonic stem cells and adult stem cells. Adult stem cells can be obtained from many differentiated tissues including, but not limited to, bone marrow, bone, fat, and muscle. Obtaining adult stem cells also does not raise ethical concerns. For most studies, the adult stem cell in question is actually a mesenchymal stem cell or mesenchymal stromal cell. Mesenchymal stem cells are multipotent but not pluripotent, which means they can differentiate into some, or “multiple,” but not all tissue types (Reinders et al. 2010).

Sources

Mesenchymal stem cells can be isolated from virtually every tissue in the body. In cats, sources of mesenchymal stromal cells (MSCs) that have been utilized for expansion and clinical therapy include bone marrow, adipose, testicular and ovarian tissue salvaged from routine sterilization procedures, and fetal membrane tissues discarded from pregnant ovariohysterectomy. A recent study in cats compared the proliferative capacities of MSCs from different sources (Webb et al. 2012). In addition to a relatively easier collection procedure, adipose-derived MSCs were found to be superior in proliferative potential than bone marrow-derived MSCs and were considered therefore to be the preferred source for MSC therapy in cats (Webb et al. 2012). Although most MSC therapy in acute kidney injury (AKI) and chronic kidney disease (CKD) rodent models utilize bone-marrow derived MSCs, more recent studies indicate similar efficacy with adipose-derived MSCs (Furuichi et al. 2012; Kim et al. 2012).

Two different types of MSC products are currently being investigated as a novel therapy for CKD in cats: aMSC expanded in culture and stromal vascular fraction (SVF) cells (also known as non-expanded aMSC). SVF is the initial product of adipose tissue enzymatic digestion and is the type of cellular product produced from point of care tissue processors and by several private stem cell companies. In contrast to aMSC cultures, which contain a relatively homogeneous population of activated and proliferating MSCs, the SVF product is a mixture of multiple cell types, primarily cell types such as adipocytes and endothelial cells that do not give rise to MSCs. These are thought to include mesenchymal stem cells as well as a mixture of B and T lymphocytes, endothelial cells, fibroblasts, macrophages, pericytes, and pre-adipocytes (Gimble et al. 2012). Currently, not enough information is known about SVF to determine if such a product with a mixed cellular composition is a therapeutic advantage or disadvantage for the intended applications. Culture-expanded MSCs (both bmMSCs and aMSCs) are the type predominantly used in the rodent model literature; however, more recent rodent studies have started to explore the therapeutic potential of the SVF cellular product with promising results (Riordan et al. 2009; Yasuda et al. 2012).

Stem cells that are harvested from the patient with the intention of administering them back to that patient are termed autologous MSCs. Stem cells that are harvested from healthy donors for administration to a different, genetically unrelated patient are termed allogeneic MSCs. The relative efficacy of autologous versus allogeneic cells is an area of controversy. Although allogeneic MSCs traditionally are thought to be immune-privileged and are not expected to incite an immune response, more recent evidence suggests that the terminology “immune-evasive” may be more appropriate as antibody formation against and rejection of allogeneic donor MSCs has been documented (Ankrum et al. 2014). As a result, it is argued that autologous MSCs may survive longer in the body in comparison to allogeneic cells, resulting in improved efficacy over the latter (Togel et al. 2009). However, allogeneic MSCs have been widely used in experimental stem cell transfer investigations in rodents, as well as clinical trials in humans, with positive results (McTaggart and Atkinson 2007).

The advantages of using allogeneic MSCs include sparing the patient from undergoing the harvest procedure as well as the use of MSCs from young, healthy donor animals. Recent studies in humans and rodents support the view that MSCs obtained from young, healthy individuals have greater proliferation potential and have greater therapeutic potential than those collected from elderly, diseased individuals (Kretlow et al. 2008; Lei et al. 2007; Wang et al. 2013). Another concern for autologous MSC administration in animals with kidney disease is the growing body of literature supporting the theory that MSCs are adversely affected by uremia. Recent studies have documented that MSCs obtained from uremic rats have reduced proliferation in culture, loss of regenerative potential, premature senescence, decreased capacity to induce angiogenesis, and an altered secretome (Idziak et al. 2014; Klinkhammer et al. 2014; Noh et al. 2012; van Koppen et al. 2012). Uremic effects also have been documented in vitro as a reduced capacity of MSCs from uremic individuals to ameliorate renal damage in experimentally-induced CKD in comparison to MSCs from healthy rats. This information does imply, however, that uremic patients are not the best MSC source, a concern for autologous MSC therapy. Little data has been gathered on whether MSCs transplanted into a uremic recipient environment will become compromised. The success of MSCs in palliation of AKI and CKD in rodent models argues against this being an issue.

Characterization

Mesenchymal stem cells or mesenchymal stromal cells (MSCs) 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. Additionally cell surface marker characterization via flow cytometry differentiates them from hematopoietic cells, though no truly unique MSC molecule has been identified. In part, the lack of definitive markers probably reflects the diverse lineage of MSCs and the fact that each MSC population reflects to some degree the characteristics of tissues from which they were derived. Most importantly, stem cells possess the ability to differentiate into cell types of multiple lineages including adipocytes, chondrocytes, and osteocytes (Reinders et al. 2010).

Immunologic Properties

Mesenchymal stem cells clearly modulate immune responses, as demonstrated by both in vitro and in vivo studies. Among their other immunological properties, MSCs inhibit lymphocyte proliferation and cytokine production, suppress dendritic cell function and alter DC cytokine production, and decrease IFN- γ production by NK cells (Reinders et al. 2010). MSCs also have the ability to be home to injured tissues and can produce growth factors, cytokines and chemokines, release bioagents such as microvesicles, and work through cell to cell contact, all of which could help maintain or improve renal repair and function (de Almeida et al. 2013).

Several mechanisms of action have been proposed to explain the apparent renoprotective effects of MSCs, as evaluated in vitro and in rodent models. These include anti-inflammatory, pro-angiogenic, anti-apoptotic, anti-fibrotic, and anti-oxidant qualities as well as stimulation of production of endogenous progenitor cells (de Almeida et al. 2013). These processes are well documented in AKI models, but the extent to which they occur in naturally-developing feline CKD is less well understood. However, mesenchymal stem cell therapy would seem promising for treatment of CKD in cats, since feline CKD is histologically characterized by tubulointerstitial inflammation, tubular cell death, formation of fibrosis, and rarefaction of vasculature, and oxidative stress has been demonstrated in CKD cats (Chakrabarti et al. 2013; Keegan and Webb 2010; McLeland et al. 2014).

Stem Cells and Kidney Disease

There are numerous studies of MSC therapy in rodent models of renal failure, though most studies have focused on models of short-term protection from acute renal disease (Little and Rae 2009). The majority of these studies provide evidence that systemic administration of bone marrow-derived or adipose-tissue derived MSCs can help preserve renal function in the face of acute insults and can also help reduce tubular injury and fibrosis. Several studies have also demonstrated incorporation of small numbers of MSCs into the renal parenchyma (Morigi et al. 2004). It has been proposed that some of these MSCs may actually differentiate into functional renal tubular epithelial cells, though this theory remains controversial. Most investigators believe that paracrine effects from the injected MSCs are more important than the effects of direct cellular incorporation into the kidney (Togel et al. 2007; Togel et al. 2008).

Fewer studies have investigated the effects of MSC therapy in rodent CKD models. Rodent models of CKD are most commonly created by performing a 5/6 nephrectomy. A limitation of these models is that, frequently, MSC therapy

is administered a relatively short time after nephrectomy (days to weeks). In the majority of CKD rodent model studies that have been performed, administration of both bmMSCs and aMSCs has demonstrated significant renoprotective effects including reduction of intrarenal inflammatory infiltrate, decreased fibrosis, and glomerulosclerosis (Lee et al. 2010; Semedo et al. 2009; Villanueva et al. 2011). Parameters of renal function and clinical health, including weight, creatinine, BUN, proteinuria blood pressure, and hematocrit have also been demonstrated to improve as a result of MSC therapy. Several routes of administration—intrapararenchymal, subcapsular, intravenous—have been explored and all seem to be effective. Multiple, repeated injections of MSCs appear to be even more effective than single injections (Lee et al. 2010; Semedo et al. 2009).

Current Clinical Trials for Feline Chronic Kidney Disease

At present, there is little published work regarding the use of MSCs for treatment of CKD. At the Center for Immune and Regenerative Medicine at Colorado State University, we are currently conducting research into the immunological properties of feline MSCs, as well as the potential use of MSCs for treatment of CKD in cats. Over the past six years, the feline stem cell program at Colorado State has investigated basic feline stem cell biology and therapeutic applications. Other feline diseases under investigation for MSC therapy include feline asthma and inflammatory bowel disease. We initially completed a pilot study investigating the safety and potential effectiveness of unilateral intra-renal autologous MSC injections for cats with CKD (Quimby et al. 2011). In that study, we found that the MSC injections were well-tolerated and may have improved renal function in some animals. However, the number of sedations required for the procedure limited its clinical applicability. In a recent series of pilot studies, we investigated the effectiveness of repeated, intravenously-delivered MSCs for the treatment of feline CKD, using allogeneic MSCs derived from healthy, young donor animals (Quimby et al. 2013). The effect of MSC administration on kidney function in this clinical trial was variable. We continue to develop clinical trials to explore and optimize this potentially powerful treatment modality.

Conclusion

The fields of stem cell therapy and regenerative medicine are expanding rapidly. Veterinary medicine is poised to take a leading role in these fields, as there are a number of diseases in companion animals that would be amenable to stem cell therapy. Among the challenges facing these emerging fields are standardization of treatment protocols and adherence to strict principles of evidence-based medicine in reporting study results and conclusions. Nonetheless, it is likely that stem cell therapy will make significant progress in changing treatment paradigms for a number of important diseases of dogs and cats in the relatively near future.

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