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Efficacy of allogeneic mesenchymal stem cell administration in a model of acute ischemic kidney injury in cats

Desiree D. Rosselli [a], Jennifer L. Mumaw [a], Vanna Dickerson [a], Cathy A. Brown [b], Scott A. Brown [c], Chad W. Schmiedt [a,*]

[a] Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, 2200 College Station Road, Athens, GA 30602, USA
[b] Georgia Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, 501 DW Brooks Drive, Athens, GA 30602, USA
[c] Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, 501 DW Brooks Drive, Athens, GA 30602, USA

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ABSTRACT

Objective: To evaluate the effects of allogeneic mesenchymal stem cells (MSCs) in a model of ischemic acute kidney injury (AKI).
Study design: Randomized controlled trial.
Animals: Adult, purpose-bred research cats (n = 15) and a historical reference group (n = 3).
Methods: Cats underwent unilateral, in vivo, warm renal ischemia, then intravenous administration of 4 million adipose-derived MSCs, bone marrow-derived MSCs, or fibroblasts (n = 5/treatment) 1 h after reperfusion. Serum creatinine and blood urea nitrogen concentrations were measured at baseline and days 1 and 6. Urine specific gravity, urine protein to urine creatinine ratio, and glomerular filtration rate were measured at baseline and day 6. Both kidneys were harvested on day 6; histopathology was described and scored and smooth muscle actin was quantified with histomorphometry. A 2-way ANOVA was used to compare time and treatment. Chi square analysis was used to determine the % of cats with at least International Renal Interest Society (IRIS) Grade 1 AKI.
Results: Time, but not treatment, had a significant effect on renal function. No difference was noted in % of cats with IRIS AKI. Significantly fewer mitotic figures were observed in ischemic kidneys that received bone-marrow derived MSCs vs. fibroblasts. No differences in smooth muscle actin staining were noted.
Conclusions: This study did not support the use of allogeneic MSCs in AKI in the regimen described here. Type of renal injury, MSC dose, allogenicity, duration, and route or timing of administration could influence the efficacy MSCs.

1. Introduction

Acute kidney injury (AKI) is an important clinical disease in cats, with mortality over 50% despite therapy (Segev et al., 2013; Lee et al., 2012). AKI results from a variety of insults including: toxins, obstruction, infection, and ischemia (Worwag and Langston, 2008). Ischemic damage is a common cause of AKI in human patients (Schrier et al., 2004). Ischemic causes of AKI in cats include hypovolemia, hypotension, and thromboembolic disease (Monaghan et al., 2012; Worwag and Langston, 2008). Renal ischemia followed by damage from reperfusion causes inflammation, acute reduction in renal function, and tubular epithelial cell damage (Bonventre and Yang, 2011). Ischemic AKI is also inherent in the renal transplantation process during the time between harvest and restoration of blood flow. This ischemic injury is known to contribute to chronic allograft dysfunction (Bernsteen et al., 1999). More generally, AKI of any type is linked to chronic kidney disease in people and other animals (Chawla, 2011). The pathological consequences of ischemia-induced AKI mimic those observed in chronic kidney disease in cats (Schmiedt et al., 2016).

Mesenchymal stem cells (MSC), also known as multipotent stromal cells, are cells harvested from adult tissues that have the capacity for both self-renewal and differentiation into mesenchymal cell types (Pittenger et al., 1999). The role of systemically administered MSCs in treatment of disease increasingly has been focused on their ability to modulate inflammation rather than replace damaged tissues. Autologous stem cells are harvested from the recipient and require weeks to isolate and expand. Allogeneic stem cells have the advantage of increased availability for acute insults, such as AKI, and reduced anesthetic events for the recipient. There is evidence supporting a protective role of MSC in AKI, particularly through anti-inflammatory and immune modulatory pathways (Erpicum et al., 2014; Lindoso et al., 2011; Tögel et al., 2005 and 2007). In rodent models of ischemic AKI, both autologous and allogeneic MSC had positive effects following AKI, including

* Corresponding author.
E-mail addresses: ddross@uga.edu (D.D. Rosselli), jmumaw@uga.edu (J.L. Mumaw), vannadvm@uga.edu (V. Dickerson), cathybro@uga.edu (C.A. Brown), sbrown01@uga.edu (S.A. Brown), cws@uga.edu (C.W. Schmiedt).

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preservation of kidney architecture, improvement of renal function, and anti-inflammatory and anti-oxidative effects (Chen et al., 2011; Feng et al., 2010; Semedo et al., 2009). Likewise, allogeneic MSCs have proven efficacious in renal allograft transplantation models in rats for reducing interstitial fibrosis and tubular atrophy, reducing inflammation, and improving allograft function (Franquesa et al., 2012). Finally, in human renal transplantation, patients who received autologous stem cells had lower incidence of graft rejection and improved renal function 1 year after transplant (Tan et al., 2012). This effect may be due to a reduction of peri-transplantation AKI or by chronic modulation of graft-associated inflammation.

Characterization of feline MSC was first described in 2002 (Martin et al., 2002). Adipose derived MSC and bone marrow derived MSC have been described as phenotypically similar in cats (Webb et al., 2012). Intravenous administration of allogeneic MSC for the treatment of chronic kidney disease in 16 cats showed little clinically relevant improvement in renal function parameters (Quimby et al., 2013).

Therefore, while feline MSCs have been evaluated in the context of chronic kidney disease, studies documenting the effect of MSC in cats with AKI are lacking. Logistically, allogeneic MSCs may be best suited for this application because of their potential rapid availability at the time of diagnosis of AKI. Our hypothesis was that administration of MSCs to cats immediately following unilateral ischemia-induced AKI would lessen renal damage, as indicated by improved renal function and reduced histopathologic damage, compared to control cats that were administered nothing or fibroblasts.

2. Materials and methods

2.1. Animals and treatment groups

15 intact, adult male and female purpose-bred domestic shorthair cats were used in the study. Cats were housed individually, fed standard laboratory diets, and given water ad libitum. Approximately 10 months prior to entrance into the study, all cats were inoculated with *Brugia malayi* as part of a separate project designed to induce microfilariasis (Hawking, 1962). All cats included in the present study failed to become microfilemic and were scheduled for euthanasia because exposure to a human pathogen precluded adoption. Prior to entrance into the study, all cats were evaluated for health and normal renal function based on physical examination, complete blood count (CBC), serum chemistry profiles, urinalysis, and urine protein to urine creatinine ratio (UP/C). All procedures were approved by the Institutional Animal Care and Use Committee.

All cats underwent in vivo unilateral warm renal ischemia for 60 min using a described model of unilateral renal ischemia (Schmiedt et al., 2016). In this model, significant loss of renal function occurs; GFR is reduced to 57% of baseline with the most significant reduction occurring around day 6. Because the contralateral kidney is preserved, there is no clinical evidence of uremia, nor does serum creatinine concentration change dramatically. BUN concentration increases significantly at days 1 and 3; histologically, at day 6, there is substantial tubular necrosis, inflammation, and early evidence of tubular regeneration.

For the study reported here, cats were divided into 3 groups: group 1 (AMSC) cats ($n = 5$) received 4×10^6 adipose-derived MSCs; group 2 (BMSC) cats ($n = 5$) received 4×10^6 bone marrow-derived MSCs; group 3 (FIBRO) cats ($n = 5$) received 4×10^6 cultured fibroblasts. Data from these cats were compared to a reference group of cats that were treated identically, ($n = 3$), received no cell administration, and were previously reported in the original description of this model (Schmiedt et al., 2016). These 3 untreated cats were not studied contemporaneously with groups 1–3. In cats that received cell therapy, cells were administered intravenously 1 h after kidney reperfusion via jugular catheter in 10 mL of PBS at a rate of 1 mL/min. After administration, the catheters were flushed with 2 mL of heparinized saline. All cats survived 6 days after renal ischemia.

2.2. Anesthesia, surgery, and renal ischemia-reperfusion

Two days prior to surgery, all cats were briefly anesthetized for placement of an indwelling jugular catheter. Cats were sedated and anesthesia was induced with the regimen described below. A 20-gauge, 12-cm length catheter was placed in the right jugular vein using a modified Seldinger technique. Jugular catheters were flushed three times daily with heparinized saline. All cats underwent 1 h of unilateral renal ischemia as part of a model described previously (Schmiedt et al., 2016). Cats were fasted for 12 h prior to surgery and sedated with intravenous ketamine (7 mg/kg), buprenorphine (0.04 mg/kg), and acepromazine (0.01 mg/kg). Anesthesia was induced with isoflurane delivered by facemask. Cats were endotracheally intubated and anesthesia was maintained via isoflurane delivered in 100% oxygen. Cats received intravenous lactated Ringers solution IV at 10 mL/kg/h. Body temperature was supported with a hot water blanket and a hot air patient warming system (Bair Hugger Animal Health, 3M, St. Paul, MN). Temperature, pulse, respiration, systemic arterial blood pressure (811B, Parks Medical Electronics, Inc., Las Vegas, NV), and oxygen saturation via pulse oximetry were recorded every 5 min during anesthesia. No perioperative antibiotics were administered. As previously described, the left kidney was exposed through a ventral midline incision, and the renal artery and vein were exposed and skeletonized at their origin. A nontraumatic vascular clamp was placed across the renal artery and vein for 60 min (Schmiedt et al., 2016).

Post-operatively, a transdermal fentanyl patch (25 mcg/h, Fentanyl Transdermal System, Myaln Pharmaceuticals Inc., Morgantown, WV) was placed on the lateral abdomen in all cats. Based on a discomfort score (Schmiedt et al., 2010) assigned during monitoring, post-operative analgesia was augmented via transmucosal buprenorphine (0.03 mg/kg) in the 24 h following fentanyl patch application. After that, SC hydromorphone (0.1 mg/kg) was provided as needed for analgesia. In all cats, lactated Ringer's solution (60 mL/kg/day) was administered SC 2 days and was continued until the cat started eating and drinking. To ensure the well-being of the cats, each animal was monitored at least twice daily for signs of pain (discomfort score assigned) and to assess appetite, thirst, urination, and defecation.

2.3. Cell isolation, expansion, and characterization

BMSCs, AMSCs, and fibroblasts were harvested from a single, 2 year old, male, purpose-bred research cat. The cat was euthanized for reasons unrelated to this project. Tissue was immediately and sterilely collected from the medullary cavity of both humeri, the falciform fat, and the skin on the flank. The resulting MSCs and fibroblasts have been previously characterized (Mumaw et al., 2015). In that previous study, the cells demonstrated trilineage differentiation, characteristic CD marker morphology, and a dose dependent reduction in neutrophil oxidative burst following stimulation with 10^{-7} M phorbol myristate acetate (Mumaw et al., 2015). In the study reported here AMSCs and BMSCs were used at passage 3 and the fibroblasts were used at passage 4.

2.4. Analysis of renal function

Renal function was assessed using serum creatinine and blood urea nitrogen (BUN) concentrations, urine specific gravity, UP/C, and glomerular filtration rate (GFR; plasma clearance of iohexol). Analysis was performed by a commercial clinical pathology laboratory (Clinical Pathology Laboratory, College of Veterinary Medicine, University of Georgia). Plasma clearance of iohexol used to estimate GFR, determined with a 2-point decay curve, (Goodman et al., 2010; Heiene et al., 2009), was evaluated the day prior to surgery and on post-operative day 6. In non-sedated cats, iohexol (300 mg iodine/kg IV) was administered via the medial saphenous vein. Blood samples were drawn from the indwelling jugular catheter or a cephalic vein prior to iohexol administration and at 2 and 3 h after administration. Plasma was separated within