Published in final edited form as:

Am J Vet Res. 2021 June ; 82(6): 487–493. doi:10.2460/ajvr.82.6.487.

Intravenous administration of allogeneic Wharton jelly–derived mesenchymal stem cells for treatment of dogs with congestive heart failure secondary to myxomatous mitral valve disease

Vicky K. Yang, DVM, PhD, Dawn M. Meola, BS, Airlie Davis, BS, Bruce Barton, PhD, Andrew M. Hoffman, DVM, DVSc

Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA 01536 (Yang, Meola, Davis, Hoffman); and Department of Population and Quantitative Health Sciences, University of Massachusetts Medical School, Worcester, MA 01655 (Barton). Dr. Davis' present address is the Astellas Institute for Regenerative Medicine, Marlborough MA 01752. Dr. Hoffman's present address is the Office of the Dean, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Abstract

OBJECTIVE—To evaluate whether mesenchymal stem cells (MSCs) can be safely administered IV to dogs with congestive heart failure (CHF) secondary to myxomatous mitral valve disease (MMVD) to improve cardiac function and prolong survival time.

ANIMALS—10 client-owned dogs with CHF secondary to MMVD.

PROCEDURES—Dogs with an initial episode of CHF secondary to MMVD were enrolled in a double-blind, placebo-controlled clinical trial. Five dogs in the MSC group received allogeneic Wharton jelly–derived MSCs (2×10^6 cells/kg, IV), and 5 dogs in the placebo group received a 1% solution of autologous serum (IV) for 3 injections 3 weeks apart. Cell-release criteria included trilineage differentiation, expression of CD44 and CD90 and not CD34 and major histocompatibility complex class II, normal karyotype, and absence of contamination by pathogenic microorganisms. Patients were followed for 6 months or until death or euthanasia. Echocardiographic data, ECG findings, serum cardiac biomarker concentrations, CBC, and serum biochemical analysis results were obtained prior to and 4 hours after the first injection and every 3 months after the final injection.

RESULTS—Lymphocyte and eosinophil counts decreased significantly 4 hours after injection, and monocytes decreased significantly only in dogs that received an MSC injection. No significant differences were seen in the echocardiographic variables, ECG results, serum cardiac biomarker concentrations, survival time, and time to first diuretic drug dosage escalation between the 2 groups.

CONCLUSIONS AND CLINICAL RELEVANCE—This study showed that MSCs can be easily collected from canine Wharton jelly as an allogeneic source of MSCs and can be safely delivered IV to dogs with CHF secondary to MMVD.

Address correspondence to Dr. Yang (vicky.yang@tufts.edu).

The authors declare that there were no conflicts of interest.

Myxomatous mitral valvular disease is the most common acquired cardiac disease and the most common cause of CHF in dogs, constituting two-thirds of all cases of heart failure in dogs.¹ The incidence of the disease increases with age, and the prevalence of the resulting valvular changes approaches 100% in older small breed dogs on the basis of necropsy findings.² Myxomatous degeneration of the mitral valve leads to valvular insufficiency, cardiac enlargement, and sometimes contractile dysfunction in the later stages of MMVD with fibrous tissue deposition and an increase in inflammatory cytokines in the myocardium.^{2,3} As MMVD progresses, worsening valvular regurgitation results in progressive volume overload and finally CHF. Once a dog is in CHF, the median survival time is between 1 and 9 months.² Currently, the only medical intervention that has been shown to delay the onset of CHF is pimobendan administration, which extends overall survival time for approximately 5 months.⁴ Surgical repair of the mitral valve and chordae tendineae has shown potential in reversing some cardiac changes and prolonging survival time,⁵ but to date, this remains a high-risk surgery that few veterinarians are capable of performing. The cost of the procedure is also financially prohibitive to most dog owners.

Mesenchymal stem cell treatment has garnered attention for heart disease treatment because of the cells' immunomodulatory and antifibrotic effects and their regenerative potential, with numerous trials underway for the treatment of heart failure in humans.^{6–11} These cells can be easily harvested from a variety of autologous or allogeneic sources (ie, bone marrow, umbilical cord, placenta, muscle, and adipose tissue) without the ethical dilemma or potential safety issues encountered with the transplantation of pluripotent embryonic stem cells.^{10,12,13} Under in vitro conditions, MSCs can differentiate into cells of mesodermal lineages, including adipocytes, chondrocytes, and osteocytes.¹³ Treatment with MSCs in myocardial infarction and heart failure models has shown improvement in fibrosis and scar size, cardiomyocyte apoptosis, and cardiac function, including improved ejection fraction and end systolic volume.^{14–16} Furthermore, MSC treatment has shown a wide safety margin with no observed acute infusion toxicoses, organ or systemic complications, infection, death, or malignancy.¹⁷ One study¹⁸ published in the veterinary medical literature evaluated the use of stem cells for the treatment of MMVD and CHF; deciduous teeth stem cells were administered IV. Results showed increased left ventricular ejection fraction 30 days after treatment, but the effects diminished 60 days after treatment.¹⁸ In a separate study¹⁹ of intracoronary injection of bone marrow MSCs to deliver stromal-derived factor-1 to Doberman Pinschers with dilated cardiomyopathy, no improvement in survival time was observed. Nevertheless, dogs have been used as experimental models for myocardial infarction, in which treatment with intracardiac injected bone marrow MSCs showed promise in reducing fibrosis and increasing vascular density.¹⁹

Wharton jelly, or the umbilical tissue, is a particularly attractive source of MSCs. Wharton jelly–derived MSCs have a homing capability toward damaged tissues after they are administered systemically,^{13,17} are easy to harvest and expand, and exhibit superior angiogenic and paracrine effects, compared with bone marrow–derived MSCs.⁶ Moreover, because these MSCs are from neonatal donors, they exhibit fewer features of cellular aging.⁶

The purpose of the preliminary study presented here was to evaluate whether MSCs can be safely administered IV to dogs with CHF from MMVD to improve cardiac function and prolong survival time. We hypothesized that treatment with Wharton jelly–derived MSCs is safe when administered IV to dogs in CHF from MMVD and that MSC treatment will result in improved cardiac function as assessed by echocardiography and measurement of cardiac biomarkers and will prolong survival time.

Materials and Methods

Animals

Ten client-owned dogs with a first episode of CHF secondary to MMVD admitted to the Tufts Foster Hospital for Small Animals were enrolled in this double-blind, placebo-controlled clinical trial. Congestive heart failure was confirmed on the basis of findings from thoracic radiographs by the attending cardiologist and reviewed by the radiologist, and the diagnosis of MMVD was made on the basis of echocardiographic findings. Dogs with chronic kidney disease, hepatic disease, uncontrolled hypothyroidism, neoplasia, hypertension, active infection, metabolic disorders, and autoimmune disease were excluded from the study. Standard of care treatment for CHF was initiated after enrollment, and treatment with MSCs was not initiated until 1 week after discharge from the hospital with clinical signs of CHF well controlled as determined by the attending cardiologist. The study was approved by the Tufts University Institutional Animal Care and Use Committee and Clinical Studies Review Committee. Informed consent was obtained from owners of privately owned dogs.

Of the 10 dogs recruited, 5 received MSC injections (MSC group dogs) and 5 received placebo (vehicle) injections (placebo group dogs). To that end, treatment group (MSC vs placebo) was assigned on a random basis, and the attending cardiologist and the pet owners were both unaware of group assignments. Three injections were administered as follows: the first dose was given at the first recheck examination 7 to 10 days after pharmacological stabilization from active CHF; the second dose was given at the 3-week recheck examination; and the third dose was given at the 6-week recheck examination. The injection schedule was modified from a previous study²⁰ of cats with chronic kidney disease. For dogs in the MSC group, a dose of 2×10^6 MSCs/kg in a 1% solution of autologous serum was administered IV each time. Dogs in the placebo arm of the study received IV injections of vehicle (1% autologous serum solution) at the same interval. In addition, standard-of-care treatment for CHF was provided to all dogs enrolled in the study, including the administration of diuretic drugs, an angiotensin-converting enzyme inhibitor, pimobendan, or other antiarrhythmics or cardiac medications deemed necessary by the attending cardiologist. The duration of the study was 6 months after the day of enrollment. However, patients were followed past the 6-month time point if they were still alive until they were euthanized or lost to follow-up.

Isolation and characterization of Wharton jelly–derived MSCs

Donors of Wharton jelly–derived MSCs included healthy whelping dogs admitted to a Clinical Studies Review Committee–approved veterinary clinic for elective cesarean section.

Donors were required to be current on immunizations for canine parvovirus, distemper, adenovirus I, parainfluenza, and rabies and to have negative test results for *Borrelia burgdorferi*, *Anaplasma phagocytophila*, *Ehrlichia canis*, *Dirofilaria immitis*, and *Brucella canis* infections.

The MSCs were isolated within 24 hours of cesarean section from donor umbilical tissues as previously described.²¹ Briefly, explanted tissue fragments were incubated in collagenase (3 mg/mL) at 37°C for 1 hour. The tissue digest was then plated in α -modified Eagle minimum essential medium supplemented with a 15% solution of fetal bovine serum, penicillin-streptomycin (100 U/mL), and L-glutamine (2mM). Media was refreshed every 48 to 72 hours. Three cell lines at passage 1 were combined and plated on plastic to form a mixed Wharton jelly-derived MSC line and subsequently passaged to passage 3 for characterization and clinical use. The cell lines were harvested from litters of Great Danes, Labrador Retrievers, and American Staffordshire Terriers.

The mixed Wharton jelly-derived MSCs were immunophenotypically characterized for CD44 and CD90, CD34, and MHCII expression by use of flow cytometry.^a The primary antibodies used were the following conjugates: anti-CD34-PE,^b CD44-allophycocyanin,^c MHCII-fluorescein isothiocyanate,^d and CD90-allophycocyanin.^e 7-aminoactinomycin D was applied to exclude dead cells during analysis. Phenotype was determined by comparison with the corresponding isotype control.

Additional MSC release criteria tests included normal karyotype^f and absence of contamination by pathogenic microorganisms (on the basis of aerobic, fungal, mycoplasma, and *Ureaplasma* spp culture results and mycoplasma PCR assay results). Further, absence of canine adenovirus, canine herpesvirus-1, canine distemper virus, and canine parvovirus was confirmed on the basis of PCR assay results.^g Endotoxin concentrations were evaluated by use of the *Limulus* ameocyte lysate test^h with a limit of detection of 0.01 ng/mL.

Mesenchymal stem cell trilineage differentiation potential was achieved with chondrogenic, adipogenic, and osteogenic differentiation growth conditions. Chondrogenic culture media consisted of DMEM with a high concentration of glucose (4.5 g/L), sodium pyruvate (1 μ M), dexamethasone (100 μ M), L-ascorbic acid 2-phosphate (50 μ M), proline (40 μ g/mL), insulin-transferrin-selenium (1X concentration),ⁱ bone morphogenetic protein 2 (50 ng/L), and transforming growth factor- β 1 (10 ng/mL). Adipogenic culture media consisted of DMEM with a low concentration of glucose (1 g/L), rabbit serum (10% solution), dexamethasone (1 μ M), insulin (10 μ M),^j and indomethacin (200 μ M). Osteogenic culture media consisted of DMEM with a low concentration of glucose (1 g/L), fetal bovine serum (10% solution),

^a.Accuri C6, BD Biosciences, Indianapolis, Ind.

^b.Mouse anti-dog clone 1H6, AbD Serotec, Hercules, Calif.

^c.Rat anti-dog clone YKIX337.8.7, AbD Serotec, Hercules, Calif.

^d.Rat anti-dog clone YKIX334.2, AbD Serotec, Hercules, Calif.

^e.Rat anti-dog clone YKIX337.217, eBioscience, San Diego, Calif.

^f.Breen Laboratory, North Carolina State University, Raleigh, NC.

^g.Animal Health Diagnostic Center, Cornell University, Ithaca, NY.

^h.LAL Chromogenic Endotoxin Quantitation Kit, Pierce Bio-technology Inc, Rockford, Ill.

ⁱ.Lonza Walkersville Inc, Walkersville, Md.

^j.Humulin-N, Lilly USA LLC, Indianapolis, Ind.

dexamethasone (100nM), and β -glycerophosphate (10 μ M). Verification of chondrogenic, adipogenic, or osteogenic differentiation was confirmed with Alcian blue stain, oil red O stain, or Alizarin red stain, respectively.

Clinical data collection

Echocardiographic^k examinations were performed at each appointment by either a cardiology resident under the supervision of a board-certified cardiologist or a board-certified cardiologist, and echocardiographic measurements were obtained by a single observer (VKY). Echocardiography was performed on unsedated dogs positioned in lateral recumbency. Measurements included fractional shortening, left atrium-to-aorta ratio, left ventricular end-diastolic and end-systolic internal diameters, and left ventricular ejection fraction with the Simpson method of discs. Measurements were made on 3 consecutive cardiac cycles, and the mean value was determined. Data on blood pressure, ECG, CBC, and serum biochemical analysis results were collected as well. Four hours prior to the first injection, patients were fitted with a Holter ECG monitor, and baseline serum NT-proBNP concentrations,^l serum renal values, and serum hs-cTnI concentrations^m were analyzed. Patients remained in the hospital and were monitored for 4 hours after receiving injections, and ECG results and respiratory rate and effort were continually monitored. Measurements of serum NT-proBNP and hs-cTnI concentrations and blood pressure were repeated 4 hours after administering the injection.

On the right lateral radiographic images of the thorax, VHS was calculated by measuring the cardiac silhouette from the carina to the apex of the heart (long axis), in addition to the widest part of the cardiac silhouette perpendicular to the carina-to-apex axis (short axis). Vertebral heart score was obtained by adding the corresponding number of vertebrae starting from the fourth thoracic vertebra for both the long and short axes.

At 3- and 6-week recheck examinations, echocardiography; measurements of serum NT-proBNP concentration, blood pressure, and serum hs-cTnI concentration; and ECG examination were repeated. The patients returned every 3 months until at least the 6-month recheck examination or until euthanasia or death, and echocardiography; measurement of blood pressure, serum NT-proBNP concentration, and serum hs-cTnI concentration; thoracic radiography; ECG analysis; CBC; and serum biochemical analyses were repeated at each visit. These patients continued to be managed by the cardiology service at the Cummings School of Veterinary Medicine, Tufts University following completion of the study until the time of death.

Statistical analysis

Statistical analysis was performed with the linear mixed model for repeated measurements to compare multiple measurements for the same patient over time with commercially available software.^{22, n} The distribution of normality was determined with the Shapiro-Wilk test, and

^k.GE Vivid 6, GE Healthcare, Chicago, Ill.

^l.Cardiopet proBNP test, Idexx Laboratories Inc, Westbrook, Me.

^m.Gastrointestinal Laboratory, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Tex.

ⁿ.SPSS Statistics, version 25.0, IBM Corp, Armonk, NY.

comparison between age and weight between groups was performed with the independent sample *t* test. A value of $P < 0.05$ was used as the cutoff for significance. Kaplan-Meier curves were generated for analysis of the survival time and time to the first escalation of diuretic drug dosage and compared between groups by use of log-rank tests. Two-sided log-rank tests for sample size calculation in a larger study²³ were performed on the basis of the survival time data obtained from this preliminary study.^o Unless otherwise stated, quantitative data are expressed as mean $\pm$ SD.

Results

MSC characteristics

The mixed Wharton jelly–derived MSCs exhibited characteristics typical of MSCs. First, they had expected morphological traits (ie, spindle-shaped and elongated) and were adherent to plastic. These cells were also capable of trilineage differentiation into chondrocytes, adipocytes, and osteocytes in vitro (Figure 1). In addition, they were positive for MSC markers (ie, CD44 and CD90) and negative for non-MSC markers (ie, CD34 and MHCII; Figure 2).

Animals

For MSC group dogs ($n = 5$), body weight was 5.7 ± 0.9 kg, age was 11.2 ± 2.2 years, and 3 were castrated males and 2 were spayed females. For placebo group dogs ($n = 5$), body weight was 7.8 ± 4.3 kg, age was 12.2 ± 0.8 years, and 3 were castrated males and 2 were spayed females. No significant differences were found in age and body weight between the 2 groups. Dogs in the MSC group included Papillon ($n = 1$), Pomeranian (1), Cavalier King Charles Spaniel (2), and mixed-breed dog (1). Dogs in the placebo group included Papillon (1), Boston Terrier (2), Havanese (1), and mixed-breed dog (1).

MSC injection safety profile

For the MSC and placebo group dogs, no significant differences were found in neutrophil counts; serum BUN, creatinine, and total bilirubin concentrations; and serum alkaline phosphatase, γ -glutamyl transferase, alanine aminotransferase, and aspartate aminotransferase activities between values before the first injection and values 4 hours after injection. In MSC and placebo group dogs, respectively, eosinophil counts were 670 ± 420 cells/ μ L and 340 ± 190 cells/ μ L before injection and 280 ± 260 cells/ μ L and 170 ± 160 cells/ μ L after injection. Overall, eosinophil counts decreased significantly ($P = 0.007$) after injection with placebo and MSCs, but changes were not significantly different between the 2 injection types (MSC vs placebo). In MSC and placebo group dogs, respectively, lymphocyte counts were $1,720 \pm 900$ cells/ μ L and $1,660 \pm 970$ cells/ μ L before injection and 870 ± 330 cells/ μ L and $1,520 \pm 970$ cells/ μ L after injection. Overall, lymphocyte counts decreased significantly ($P = 0.011$) after injection with the placebo and MSCs, and changes were also significantly ($P = 0.042$) different between the 2 injection types (MSC vs placebo). Lastly, in MSC and placebo group dogs, respectively, monocyte counts were 510 ± 200 cells/ μ L and 390 ± 150 cells/ μ L before injection and 340 ± 340 cells/ μ L and 670 ± 90 cells/

^o.SAS PROC GLM, version 9.4, SAS Institute Inc, Cary, NC.

μL after injection. Overall, monocyte counts did not change significantly after injection with the placebo and MSCs, but the changes were significantly ($P = 0.049$) different between the injection types (MSC vs placebo), with an increase in the monocyte count in placebo group dogs after injection and a decrease in the monocyte count in MSC group dogs after injection. When examining changes in values over the entire follow-up period, no significant changes were found in any of the CBC and serum biochemical analysis variables for either treatment group.

In the comparison between serum NT-proBNP and hs-cTnI concentrations immediately before and 4 hours after the first injection of MSCs and placebo, no significant changes were observed. Prior to injection, the serum NT-proBNP concentration was $1,399.8 \pm 1,628.6$ pmol/L for placebo group dogs and $2,398.0 \pm 1,900.8$ pmol/L for MSC group dogs, and after the injection, it was $1,019.0 \pm 948.3$ pmol/L for placebo group dogs and $2,195.2 \pm 1,495.7$ pmol/L for MSC group dogs. Similarly, no significant change was observed in the serum hs-cTnI concentration. Prior to injection, the serum hs-cTnI concentration was 0.084 ± 0.042 ng/mL for placebo group dogs and 0.11 ± 0.043 ng/mL for MSC group dogs, and after the injection, it was 0.170 ± 0.083 ng/mL for placebo group dogs and 0.403 ± 0.439 ng/mL for MSC group dogs. The reference limit for hs-cTnI was < 0.06 ng/mL. No significant differences were found in baseline serum NT-proBNP and hs-cTnI concentrations between the 2 groups.

Prior to injection, a Holter ECG monitor was placed on each patient and recorded ECG data prior to injection until 4 hours after injection to monitor for any immediate effects on heart rhythm from the injection. No significant changes in heart rate and rhythm were detected. In addition, no hypersensitivity reaction or change in body temperature was observed after each injection.

Long-term effects of MSCs on echocardiographic and radiographic findings and serum cardiac biomarkers

No significant changes from baseline values were found in serum NT-proBNP and hs-cTnI concentrations and echocardiographic variables for either placebo or MSC group dogs. The only significant ($P = 0.029$) change observed was an increase in VHSs for both groups. For placebo group dogs, the baseline VHS was 12.3 ± 0.9 and VHS at the end of the study was 12.6 ± 1.5 . For MSC group dogs, the baseline VHS was 12.6 ± 1.0 and VHS at the end of the study was 13.2 ± 1.6 .

Effects of MSC injection on survival time and escalation of diuretic drug dosage

No significant differences were found in survival time between placebo and MSC group dogs (Figure 3). The overall median survival time for all dogs was 39 (95% CI, 8 to 70) weeks. For placebo group dogs, the median survival time was 52 (95% CI, 24.1 to 80.0) weeks, compared with 39 (95% CI, 10.0 to 68.0) weeks for MSC group dogs. Overall mean survival time for all dogs was 60 (95% CI, 35.6 to 84.6) weeks. Mean survival time was 57 (95% CI, 25.9 to 88.1) weeks for placebo group dogs and 63.2 (95% CI, 20.9 to 105.5) weeks for MSC group dogs. All (5/5) dogs in the placebo group died naturally or were euthanized because of complications secondary to heart disease. For the MSC group, 1 dog

was lost to follow-up beyond week 113, and 1 dog was euthanized at week 113 because of seizures. All other dogs (3/5) were euthanized because of complications secondary to heart disease.

The time from treatment to the first adjustment (increase) of diuretic drug dosage, as deemed necessary by the attending cardiologist, was also analyzed. No significant differences were found between the 2 groups for the time to first adjustment of diuretic drug dosage (Figure 4). Overall median time to the first adjustment of diuretic drug dosage was 12 (95% CI, 0 to 29.0) weeks. For placebo group dogs, median time to the first adjustment of diuretic drug dosage was 26 (95% CI, 0 to 57.2) weeks, compared with 12 (95% CI, 5.6 to 18.4) weeks for MSC group dogs. Overall mean time to the first adjustment of diuretic drug dosage was 32.1 (95% CI, 7.5 to 56.6) weeks. Mean time to the first adjustment of diuretic drug dosage was 21.3 (95% CI, 6.0 to 36.6) weeks for placebo group dogs and 47.6 (CI, 7.2 to 88.0) weeks for MSC group dogs. One of 5 dogs in the placebo group died before any adjustment of diuretic drug dosage was made. For the MSC group, 1 of 5 dogs died before any adjustment was made, and 1 dog did not have any adjustment made before it was lost to follow-up.

Discussion

Results of the present preliminary study showed that IV administration of MSCs was safe for dogs with MMVD and CHF; however, we did not see any significant difference in the effects of treatment on survival time, time to the first adjustment of diuretic drug dosage, echocardiographic variables, and serum cardiac biomarker concentrations between placebo and MSC group dogs. This finding is different from that observed in the study by Petchdee et al¹⁸ that showed an increase in the left ventricular ejection fraction in dogs that received IV injection of deciduous teeth–derived MSCs 30 days after treatment. However, this positive effect was absent when the dogs were reexamined 60 days after treatment.¹⁸ That study¹⁸ did not include data on survival time, and results from that study could not be compared with our results. Furthermore, authors of that study¹⁸ did not perform cellular characterization to confirm the identity of the deciduous teeth–derived MSCs.

Despite the lack of significant effects on survival time in the present preliminary study, findings nevertheless provided important information for MSC treatment in dogs with MMVD and for the design of future trials. By analyzing the results of the present preliminary study using a 2-sided log-rank test to achieve a power of 80% with a value of $P < 0.05$, we calculated that a larger study with 29 dogs (14 in the placebo group and 15 in the MSC group) may allow the detection of a hazard ratio (MSC group-to-placebo group hazard ratio) of 0.3059, assuming that 5% of patients in the placebo group remain alive 2 years following treatment.

In many human MSC trials,^{24,25} treatment benefits are often observed in the short term but diminish in the longer term. In the present study, we observed decreases in blood lymphocyte, monocyte, and eosinophil counts immediately after MSC injection. These changes may represent an anti-inflammatory effect of MSCs. Although decreases in eosinophil and lymphocyte numbers were also observed in patients that received the placebo

treatment, their monocyte counts increased after treatment. This combination of changes seen in patients in the placebo group may represent a response to stress. Conversely, in the MSC group, all 3 cell types decreased in number, although only immediately after the MSCs were administered. Similar to results from some of the clinical trials in people with heart disease, observed potential short-term effects did not translate into long-term benefits.^{13,17} The common finding in these human trials was the extremely low MSC survival rate and low rates of cell engraftment and retention.^{13,17,25} The efficacy and potency of the treatments were also likely a function of the cell source, delivery method, and dose.^{13,17} For our study, we elected to deliver the cells IV because of the ease of administration without the need for high-risk delivery procedures (intracoronary or intramyocardial delivery) under anesthesia for patients with advanced heart disease. This route of administration may greatly reduce the engraftment rate into the myocardium, although we did not confirm the engraftment rate of the MSCs delivered to our patients.

Despite low engraftment rates, benefits may still be obtained from MSC treatment through its so-called paracrine effects. It is now known that MSCs produce extracellular vesicles as part of this paracrine signal to transfer nucleic acids, proteins, lipids, and growth factors to affect the behavior of existing adult cells such as cardiomyocytes or existing progenitor cells.^{13,25,26} These extracellular vesicles can modulate the inflammatory process or elicit cardioprotective effects.^{21,25} However, our study was not designed to evaluate the effects of MSC extracellular vesicles; therefore, we cannot determine whether these extracellular vesicles played any role in our study outcome.

The limitations of the present study included the small sample size, as would be expected for a preliminary study intended to determine the safety profile of MSC treatment in dogs with MMVD and to help determine the appropriate sample size of a large study. We were also unable to determine the structural effects of the MSC treatment (ie, effect on myocardial fibrosis, angiogenesis, and inflammation) and MSC engraftment rate into the heart without the collection of postmortem cardiac samples. Nevertheless, the results of the present study showed that MSCs can be easily collected from canine Wharton jelly as an allogeneic source of MSCs and can be safely delivered IV into dogs with MMVD and CHF.

Acknowledgments

Supported by a grant from the Shipley Foundation.

The authors thank Diane Welsh for assistance in the clinical trials and Dr. Sarah Crain for assistance with canine MSC collection.

ABBREVIATIONS

CHF	Congestive heart failure
DMEM	Dulbecco modified Eagle medium
hs-cTnI	High-sensitivity cardiac troponin I
MHCII	Major histocompatibility complex class II

MMVD	Myxomatous mitral valve disease
MSC	Mesenchymal stem cell
NT-proBNP	N-terminal pro-brain natriuretic peptide
PE	Phycoerythrin
VHS	Vertebral heart score

References

1. Parker HG, Kilroy-Glynn P. Myxomatous mitral valve disease in dogs: does size matter? *J Vet Cardiol* 2012;14:19–29. [PubMed: 22356836]
2. Borgarelli M, Buchanan JW. Historical review, epidemiology and natural history of degenerative mitral valve disease. *J Vet Cardiol* 2012;14:93–101. [PubMed: 22386588]
3. Dillon AR, Dell'Italia LJ, Tillson M, et al. Left ventricular remodeling in preclinical experimental mitral regurgitation of dogs. *J Vet Cardiol* 2012;14:73–92. [PubMed: 22386719]
4. Boswood A, Haggstrom J, Gordon SG, et al. Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiomegaly: the EPIC study-a randomized clinical trial. *J Vet Intern Med* 2016;30:1765–1779. [PubMed: 27678080]
5. Uechi M. Mitral valve repair in dogs. *J Vet Cardiol* 2012;14:185–192. [PubMed: 22366571]
6. Bartolucci J, Verdugo FJ, Gonzalez PL, et al. Safety and efficacy of the intravenous infusion of umbilical cord mesenchymal stem cells in patients with heart failure: a phase 1/2 randomized controlled trial (RIMECARD trial [randomized clinical trial of intravenous infusion umbilical cord mesenchymal stem cells on cardiopathy]). *Circ Res* 2017;121:1192–1204. [PubMed: 28974553]
7. Chugh AR, Beache GM, Loughran JH, et al. Administration of cardiac stem cells in patients with ischemic cardiomyopathy: the SCIPIO trial: surgical aspects and interim analysis of myocardial function and viability by magnetic resonance. *Circulation* 2012;126:S54–S64. [PubMed: 22965994]
8. Fan M, Huang Y, Chen Z, et al. Efficacy of mesenchymal stem cell therapy in systolic heart failure: a systematic review and meta-analysis. *Stem Cell Res Ther* 2019;10:150. [PubMed: 31151406]
9. Perin EC, Willerson JT, Pepine CJ, et al. Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. *JAMA* 2012;307:1717–1726. [PubMed: 22447880]
10. Sanina C, Hare JM. Mesenchymal stem cells as a biological drug for heart disease: where are we with cardiac cell-based therapy? *Circ Res* 2015;117:229–233. [PubMed: 26185208]
11. Schulman IH, Hare JM. Key developments in stem cell therapy in cardiology. *Regen Med* 2012;7:17–24.
12. Ksiazek K. A comprehensive review on mesenchymal stem cell growth and senescence. *Rejuvenation Res* 2009;12:105–116. [PubMed: 19405814]
13. Yu H, Lu K, Zhu J, et al. Stem cell therapy for ischemic heart diseases. *Br Med Bull* 2017;121:135–154. [PubMed: 28164211]
14. Mathiasen AB, Haack-Sorensen M, Jorgensen E, et al. Auto-transplantation of mesenchymal stromal cells from bone-marrow to heart in patients with severe stable coronary artery disease and refractory angina—final 3-year follow-up. *Int J Cardiol* 2013;170:246–251. [PubMed: 24211066]
15. Mathiasen AB, Jørgensen E, Qayyum AA, et al. Rationale and design of the first randomized, double-blind, placebo-controlled trial of intramyocardial injection of autologous bone-marrow derived Mesenchymal Stromal Cells in chronic ischemic Heart Failure (MSC-HF Trial). *Am Heart J* 2012;164:285–291. [PubMed: 22980293]
16. Williams AR, Suncion VY, McCall F, et al. Durable scar size reduction due to allogeneic mesenchymal stem cell therapy regulates whole-chamber remodeling. *J Am Heart Assoc* 2013;2:e000140. [PubMed: 23686370]
17. Fu H, Chen Q. Mesenchymal stem cell therapy for heart failure: a meta-analysis. *Herz* 2020;45:557–563. [PubMed: 30341444]

18. Petchdee S, Sompeewong S. Intravenous administration of puppy deciduous teeth stem cells in degenerative valve disease. *Vet World* 2016;9:1429–1434. [PubMed: 28096616]
19. Silva GV, Litovsky S, Assad JA, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation* 2005;111:150–156. [PubMed: 15642764]
20. Quimby JM, Webb TL, Habenicht LM, et al. Safety and efficacy of intravenous infusion of allogeneic cryopreserved mesenchymal stem cells for treatment of chronic kidney disease in cats: results of three sequential pilot studies. *Stem Cell Res Ther* 2013;4:48. [PubMed: 23632128]
21. Crain SK, Robinson SR, Thane KE, et al. Extracellular vesicles from Wharton's jelly mesenchymal stem cells suppress CD4 expressing T cells through transforming growth factor beta and adenosine signaling in a canine model. *Stem Cells Dev* 2019;28:212–226. [PubMed: 30412034]
22. IBM SPSS statistics 25 core system user's guide. Armonk, NY: IBM Corp, 2017;1–310.
23. SAS/STAT 9.4 user's guide Cary, NC: SAS Institute Inc, 2013.
24. Levit RD, Landazuri N, Phelps EA, et al. Cellular encapsulation enhances cardiac repair. *J Am Heart Assoc* 2013;2:e000367. [PubMed: 24113327]
25. Menasché P. Stem cells in the management of advanced heart failure. *Curr Opin Cardiol* 2015;30:179–185. [PubMed: 25500893]
26. Qiu G, Zheng G, Ge M, et al. Mesenchymal stem cell-derived extracellular vesicles affect disease outcomes via transfer of microRNAs. *Stem Cell Res Ther* 2018;9:320. [PubMed: 30463593]

Figure 1—.
Photomicrographs of trilineage differentiation of mixed Wharton jelly–derived MSCs. A—Image of MSCs that have differentiated into adipocytes, oil red O stain (left panel; control cells, right panel). B—Image of MSCs that have differentiated into chondrocytes, Alcian blue stain (left panel; control cells, right panel). C—Image of MSCs that have differentiated into osteocytes; Alizarin red stain (left panel; control cells, right panel). Bars = 100 mm.

Figure 2—

Flow cytometric images obtained in the evaluation of mixed Wharton jelly-derived MSCs (mixed WJ). The MSCs are negative for CD34 (panel A) and MHCII (panel B) and positive for CD44 (panel C) and CD90 (panel D). Each MSC sample was incubated with the primary antibody of interest and compared with an unstained sample or a sample incubated with the corresponding isotype (mouse [ms] IgG1-PE for CD34, rat IgG2a-fluorescein isothiocyanate [FITC] for MHCII and CD44, and rat IgG2b-PE for CD90). FL1-A = Fluorescence measured on FL1-A channel. FL2-A = Fluorescence measured on FL2-A channel.

Figure 3—.

Kaplan-Meier curves that compare survival times for dogs in the MSC group ($n = 5$; solid line) with those for dogs in the placebo group (5, dashed line; panel A) and the corresponding hazard curves (panel B). Triangles indicate a censored dog in the MSC group. No significant difference in survival time was found between the 2 groups.

Figure 4—.

Kaplan-Meier curves that compare the time until the first escalation of the diuretic drug dosage for dogs in the MSC group ($n = 5$; solid line) with that for dogs in the placebo group (5, dashed line; panel A) and the corresponding hazard curves (panel B). Triangles indicate censored dogs in the MSC group ($n = 2$), and circles indicate a censored dog in the placebo group (1). No significant difference in time until the first escalation of the diuretic drug dosage was found between the 2 groups.