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Bone marrow–mesenchymal stromal cell infusion in patients with chronic kidney disease: A safety study with 18 months of follow-up

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Abstract

Background. Chronic kidney disease (CKD) is a progressive loss of kidney function and structure that affects approximately 13% of the population worldwide. A recent meta-analysis revealed that cell-based therapies improve impaired renal function and structure in preclinical models of CKD. We assessed the safety and tolerability of bone marrow–mesenchymal stromal cell (MSC) infusion in patients with CKD. *Methods.* A single-arm study was carried out at one center with 18-month follow-up in seven eligible patients with CKD due to different etiologies such as hypertension, nephrotic syndrome (NS) and unknown etiology. We administered an intravenous infusion ($1–2 \times 10^6$ cells/kg) of autologous cultured MSCs. The primary endpoint was safety, which was measured by number and severity of adverse events. The secondary endpoint was decrease in the rate of decrease in estimated glomerular filtration rate (eGFR). We compared kidney function during the follow-up visits to baseline and 18 months prior to the intervention. *Results.* Follow-up visits of all seven patients were completed; however, we have not observed any cell-related adverse events during the trial. Changes in eGFR ($P = 0.10$) and serum creatinine ($P = 0.24$) from 18 months before cell infusion to baseline in comparison with baseline to 18 months were not statistically significant. *Conclusions.* We showed safety and tolerability of a single-dose infusion of autologous MSCs in patients with CKD.

Key Words: *cell therapy, chronic kidney diseases, clinical trial, mesenchymal stromal*

Introduction

Chronic kidney disease (CKD) is defined as a progressive loss of kidney function and structure over time that affects 11.7–15.1% of the world population [1]. Ageing, hypertension and diabetes are the most common causes of CKD [2,3]. Despite advances in management of CKD by using medications and renal replacement therapies, CKD still remains an important public health issue due to its various complications and huge disease burden.

Patients with final stages of CKD share a common appearance of glomerulosclerosis, vascular sclerosis and tubulointerstitial fibrosis, regardless of underlying disease [4–7]. The appearance that suggests a common final pathway of progressive injury, which is associated with apoptosis, oxidative damage and microvascular rarefaction [8], mesengial and fibroblast activation, renin–angiotensin–aldosterone system (RAAS) activation, various cytokines and growth factors production, epithelial-mesenchymal transition (EMT), and monocytes, macrophages and T-cell infiltration [4,5,9,10]. These pathways can be attenuated.

Actually, the kidney has regenerative capacity, which leads to organ recovery [11]. Unfortunately, this ability is limited and usually inefficient to prevent fibrosis [12].

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Importantly, CKD may finally progress toward end-stage renal disease (ESRD). Therefore, novel therapies to stop or retard the kidney damage process are required.

Following promising results of stem cell transplantation in CKD models in recent decades, this method is taken into consideration in pre-clinical and clinical setting. Mesenchymal stromal cells (MSCs) are undifferentiated adult stem cells of mesodermal origin that were originally identified in the bone marrow (BM) stroma by Friedenstein *et al.* [13]. They are plastic-adherent cells that express CD105, CD73 and CD90, and lack CD45, CD34, CD14 or CD11b, CD79 alpha or CD19 and HLA-DR surface molecules. Also, these cells are able to differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* [14]. They are renoprotective cells that act mainly in a paracrine manner by releasing some proteins and hormones, transferring extracellular vesicles and mitochondria through tunneling nanotubes or microvesicles [15], which eventually impact on apoptosis, fibrosis, inflammation and microvascular rarefaction that make them a proper option for treating CKD [16–19].

A systematic review and meta-analysis revealed that cell-based therapies, mostly MSCs, improved impaired renal function and structure in preclinical models of CKD [20]. Previously we reported that gentamicin nephrotoxicity could be ameliorated by human MSC–conditioned medium (MSC-CM) [21]. Furthermore, we stated that intrarenal arterial infusion of BM-MSCs improved renal function and structure in an acute kidney injury (AKI) model [22] and a CKD model of rhesus *Macaca mulatta* monkey [23].

Moreover, an Egyptian group showed that infusion of MSCs in patients with CKD was promising; however, they did not report and discuss any safety issues [24,25]. Both their trials had short-term follow-up periods (3 and 6 months) and they recruited a limited number of CKD patients with heterogeneous etiologies. Packham *et al.* reported that allogeneic BM-derived mesenchymal precursor cells (MPCs) were safe in patients with diabetic nephropathy (DN) [26], but the safety of an autologous source of MSCs in other types of CKD has been still an issue that is addressed by this article. Recently, we showed the safety of MSCs in autosomal dominant polycystic kidney disease patients [27]. Here, we tried to evaluate safety and tolerability of autologous MSCs in nondiabetic CKD patients with long-term follow-up evaluation.

Materials and methods

Study design and enrollment criteria

The study was an open-label, single-arm trial in a single center that was designed to evaluate safety and tolerability of an autologous MSC infusion in CKD patients.

Inclusion criteria were as follows: male or female patients; presence of CKD confirmed with serum and urine analysis; a glomerular filtration rate (GFR) of 25–60 mL/min/1.73 m^2; age between 25 and 60 years old and ability to understand and willingness to sign consent form. Exclusion criteria were as follows: being pregnant or lactating; underlying diseases such as diabetes and malignancy; having hematologic or liver diseases; having a past history of chronic transplant rejection; being unable to follow postoperative exercise regimen or return for evaluations. All subjects had a medical file at the clinic for at least 18 months prior to enrollment. We recommended the participants continue medication and follow a low-salt and low-protein diet during the study. All patients gave their written informed consent prior to enrollment.

We conducted the study in accordance with current International Conference on Harmonisation—Good clinical practice (ICH-GCP) guidelines and the Declaration of Helsinki. The Ethics Committee of Royan Institute and the Institutional Review Board (IRB) approved this study. A trial monitor and Data Safety Monitoring Board (DSMB) observed the whole trial to ensure the safety of participants. The trial schedule is shown in Figure 1. The trial was registered on www.ClinicalTrials.gov (NCT02195323).

Primary endpoint: safety and tolerability

Primary endpoint was the safety issue so the number, type and grade of adverse events (AE) and serious adverse events (SAE) related to cell infusion were assessed throughout the study according to common terminology criteria for adverse events (CTCAE) version 4.0. We also evaluated clinical parameters (physical examination and blood pressure changes) and paraclinical changes (complete blood count [CBC], fasting blood sugar [FBS], hemoglobin A1c [HbA1c], serum electrolytes, serum albumin, blood lipid profile, uric acid, liver function tests, erythrocyte sedimentation rate [ESR], parathyroid hormone [PTH], thyroid-stimulating hormone [TSH], dipstick proteinuria and urine culture).

Secondary endpoint

The secondary endpoint was decrease in the rate of decrease in estimated glomerular filtration rate (eGFR), which was evaluated by comparing eGFR decrease between two 18-month periods before and after cell infusion (baseline to 18 months after cell infusion versus 18 months before the infusion to baseline). The eGFR was calculated usingy the Modification of Diet in Renal Disease (MDRD) formula and diethylenetriamine pentaacetate (DTPA) scan. We also

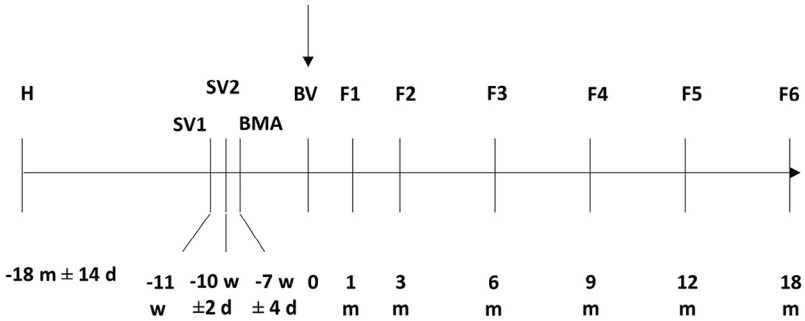

Figure 1. Assessment schedule timeline. SV1, Screen visit 1; SV2, Screen visit 2; BMA, bone marrow aspiration; BV, baseline visit; F, follow-up; H, 18 months prior to cell infusion; M, month; W, week; D, day. SV1: informed consent, physical examination, vital signs and medical history. Laboratory assessment including serum creatinine, eGFR, fasting blood sugar and hemoglobin A1c. Viral tests (human immunodeficiency virus, hepatitis C virus, hepatitis B virus, human T-lymphotropic virus) and kidney diethylenetriamine pentaacetate (DTPA) scan scheduled for next day for primary eligible patients. SV2: final eligibility criteria assessment was performed 7 ± 2 days after SV1. BMA was performed for eligible patients around 3 weeks following SV. BV: cell infusion. Baseline parameters were assessed exactly before cell infusion. Patients were followed up 1, 3, 6, 9, 12 and 18 months after cell infusion. BV, F1, F2, F3, F4, F5 and F6: physical examination, adverse events, laboratory parameters assessment including the following: complete blood count, fasting blood sugar, sodium, potassium, calcium, phosphorus, magnesium, albumin, triglycerides, total cholesterol, low-density lipoprotein, uric acid, alanine aminotransferase, aspartate aminotransferase; alkaline phosphatase, erythrocyte sedimentation rate and dipstick proteinuria. H, BV, F1, F2, F3, F4, F5 and F6: Systolic and diastolic blood pressure, blood urea nitrogen, serum creatinine and eGFR. SV, F3 and F6: Thyroid-stimulating hormone, parathyroid hormone, hemoglobin A1c and urine culture. SV2, F3 and F6: DTPA renal scan.

assessed blood pressure, blood urea nitrogen (BUN) and serum creatinine (SCr) at follow-up visits and compared them with those of baseline and 18 months before infusion.

MSC isolation and characterization

An oncologist performed bone marrow aspiration (BMA) from the iliac crest of informed CKD patients under local anesthesia (2% lidocaine) and sedation, which was induced by intravenous infusion of midazolam (0.1 mg/kg) and fentanyl (25–50 mg/100 mm). The sample was transferred to a clean room and mononuclear BM cells were isolated under sterile conditions according to the density gradient strategy by Ficoll-Paque open system (Lymphodex, Inno-TRAIN, REF: 002041600). Isolated mononuclear BM cells were rinsed in phosphate-buffered saline (PBS; Miltenyi Biotech GmbH, REF: 700-25, 1:1) and antibiotics including penicillin and streptomycin were added to culture medium. Cell viability was determined using trypan blue staining and confirmed using the NucleoCounter system (ChemoMetec A/S). Mononuclear cells (MNCs) were cultured under standard culture conditions consisting of MEM Alpha Medium 1X (Gibco, catalog number 22571) supplemented with 10% fetal bovine serum, Pharma Grade (PAA, catalog number A15-512), and were then seeded at 1×10^6 MNCs/cm^2 in Millicell HY Flasks (Millicell HY Flask T-600, catalog number PFHYS0616) for primary culture. Flasks were incubated with 5% CO_2 at 37 C.

The medium was transferred to new flasks after the initial 3–4 days to give enough time for attachment of floating cells. After 3–4 days, culture medium was changed for elimination of nonadherent cells. This process was repeated every 3 days. Following one or two passages, when well-developed colonies of fibroblast-like cells appeared, the cells were trypsinized and passaged into new flasks for the next expansion. Cell viability was determined using trypan blue staining as well as by the NucleoCounter system before infusion. To determine the expression of cell surface markers, flow cytometry analysis was performed. The cells were characterized through expression of surface markers using monoclonal antibodies, including CD105-phycoerythrin (PE) Endoglin (BD PharmingenTM, catalog number 560839), CD73-PE (BD PharmingenTM, catalog number 550257), CD90-fluorescein isothiocyanate (FITC) (EXBIO, catalog number 1F-652-T100),CD44-FITC (BD PharmingenTM, catalog number 555478) and CD45FITC-CD34PE (BD PharmingenTM, catalog number 341071), and isotype controls, including MultiMixTM FITC Mouse immunoglobulin (Ig)G1, PE-Mouse IgG1 (X0932, Dako), FITC-Mouse IgG2b (Millipore, catalog number MABC006F) and PE-conjugated Mouse IgG1k (BD PharmingenTM, catalog number 551436). Then, cells were fixed using 4% paraformaldehyde and immunophenotyping analysis was performed using a BD FACS Calibur flow cytometry system (BD Biosciences). Finally, cells were resuspended in 7 mL of normal saline supplemented with 2% human serum albumin (Octalbin, Octapharma, AG).

MSCs were washed with PBS and trypsinized with trypsin/ethylenediaminetetraacetic acid (EDTA; 0.05%,

Gibco, Germany, catalog number 25300-062). Next, cells were suspended in normal saline at a density of 100/mL medium and loaded into 10 mL sterile syringes. For each patient, about $1–2 \times 10^6$ cells/kg were prepared and kept in the operating room in a cold box at 4 C.

MSC administration

We administered $1–2 \times 10^6$/kg autologous MSCs to the patients through intravenous infusion according to our infusion protocol.

Follow-up of patients

Follow-up visits were done 1, 3, 6, 9, 12 and 18 months after MSC infusion. Evaluations during each visit included a physical examination and blood pressure (BP) assessment, as well as laboratory tests such as renal function tests (BUN, SCr, eGFR), urine analysis (U/A), FBS, HbA1C, liver function tests, serum electrolytes and lipid profile tests. DTPA renal scans were conducted at baseline and 6 and 12 months after MSC therapy.

Using their medical files available at the clinic, we collected the data of BP, BUN and SCr of the patients from 18 months before baseline.

DTPA kidney scan

This information is previously described [27].

Statistical analysis

Data are expressed as mean ± standard deviation (SD). According to the small sample size and in order to find differences in mean of laboratory parameters during all follow-up visits (baseline, 1, 3, 6, 9, 12 and 18 months), a longitudinal Bayesian generalized linear mixed effects model was used. By using informative prior distributions as well as proper link functions such as logit for binary, probit for ordinal and identity for continuous outcomes, Bayesian approaches are assumed to result in more valid estimations when the sample size is relatively small [28]. The mixed effects nature of the model guarantees the difference among patients in the study. The model estimates the slope of the variables over the time points with a 95% highest posterior density confidence interval (HPD CI). If the interval includes zero then it shows no statistical difference longitudinally. The comparison between these two differences (baseline to 18 months) versus (18 months to baseline) was performed using paired-sample t test. Two-sided $P < 0.05$ is considered statistically significant. We used statistical programming software R version 3.3.1 to analyze the data.

Results

Patient characteristics

Initially, we evaluated 55 patients with CKD for enrollment in the trial. Finally, seven eligible patients, two females and five males, enrolled in the trial between June 2014 and January 2015. These seven patients suffered from CKD with different etiologies including hypertension, nephrotic syndrome, focal segmental glomerosclrosis (FSGS) and unknown etiology. Demographic data and patient characteristic at the time of enrollment are shown in Table I. Cell characteristics and parameters are shown in Supplemental Table S1 and Supplemental Figure S1.

Primary endpoint: safety and tolerability

We did not record any AE related to BMA and intravenous catheterizing for cell infusion. These sites were healed without any complications. All patients received MSC infusion and completed follow-up visits. In total, 37 AEs and 5 SAEs were recorded and sent to the DSMB (Supplemental Table S2). They reported that AEs were probably due to the usual progression of kidney disease or the underlying disease. All patients experinced nausea, headache and dizziness right after firsrenal DTPA scan procedure . So that, eventually, the DSMB suggested cancelation of the 12-month DTPA scan. There was no AE and SAE related to cell infusion. We observed no significant differences in mean estimated difference of safety laboratory parameters in any follow-up visits compared with baseline (Table II).

Patient 1 received living kidney transplantation 4 months after study completion without any previous hemodialysis. Patient 3 underwent hemodialysis twice a week, 14 months after enrollment in the trial. She had a history of coronary heart disease (CHD) and we referred her to a cardiologist due to cardiac symptoms exacerbation that resulted in coronary artery bypass grafting (CABG) in month 15. She received a kidney transplant in month 27. Patient 5 used a single-dose Ibuprofen tablet 3 months after the intervention due to headache and creatinine increase (SCr: 4.77 mg/dL) (Supplemental Table S3), which reached its previous level (3.7 mg/dL) in 2 weeks. He has been under hemodialysis twice a week in month 15. Patient 6 underwent peritoneal dialysis quater in die for 3 months from month 13 to month 15 and his SCr levels were stabilized (SCr: 3.3 mg/dL).

Secondary endpoint

eGFR, SCr levels and bp.

The mean eGFR value of 48.1 ± 7.3 mL/min/1.73 m^2 18 months before cell infusion decreased to

Table I. Patient characteristics and demographic data at the time of enrollment.

Patient number	1	2	3	4	5	6	7
Sex (m/f)	M	F	F	M	M	M	M
Age (years)	29	42	60	34	29	29	51
Race	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Educational level	Graduate studies	Completed high school	Completed primary school	Completed high school	Bachelor student	Completed high school	Doctorate
Marital status	Married	Married	Married	Married	Married	Married	Married
Number of children	2	2	6	2	0	0	3
Employment	Full-time employment	Homemaker	Homemaker	Full-time employment	University student	Retired due to CKD	Full-time employment
BMI (kg/m^2)	25.86	21.30	29.90	28.07	30.10	18.99	28.34
Blood group	A+	A+	AB+	A-	A+	AB+	0-
BP sitting position (mm Hg)	110/78	135/85	145/91	130/80	127/75	110/80	130/80
eGFR (ml/min/1.73m^2)	25	25	25	44	30	28	33
GFR DTPA SCAN	25	25	30	37	25	29	42
Serum creatinine (mg/dl)	3.1	2.1	2.02	2.17	2.88	2.89	2.3
Dipstick proteinuria[a]	+	Trace	+	++	++	Trace	Neg
Past medical history (yes/no)							
DM	N	N	N	N	N	N	N
HTN	Y	Y	Y	Y	Y	Y	Y
Other (name)	Appendectomy, Thyroid nodule	Behcet's syndrome, Hypothyroidism, Appendectomy, Colon diverticulum	Kidney stone, Coronary heart disease	-	Pyelonephritis	-	Kidney stone
Diagnosis of HTN, age	21	37	40	33	27	26	49
Duration of CKD at enrollment (estimation by month)	168	49	39	13	128	36	22
Etiology of CKD (name)	Unknown	Probably CIN and HTN	Unknown, probably HTN	CIN-FSGS	Nephrotic syndrome	FSGS	Unknown , probably HTN
Smoking history (yes/no)	N	N	N	N	N	N	N
Alcohol consumption history (yes/no)	N	N	N	N	N	N	N
Family history of CKD (yes/no)	N	Y	Y	N	Y	Y	Y
Family history of disease (name/relation)	-	HTN in mother, DM in father	HTN and CVA in mother	-	DM in mother and father	Kidney TX in sister and nephew due to HTN	Dialysis in mother following hypertensive CKD
Under controlled diet[b] **(yes/no)**	Y	Y	Y	Y	Y	Y	Y
Drug list at enrollment (name/dose)	Losartan 12.5mg/bid, Allopurinol 100mg/daily, Calcitrol I in 5 days of week, Folic acid I daily, Nephro-Vite I daily, Calcium carbonate I daily	Allopurinol 100mg/daily , Calcitrol I daily Nephro-Vite I daily, Calcium carbonate I daily, Omega-3 I daily, Ferrosulfat I daily	Atorvastatin 20mg/daily, Amilodipin 5mg/bid, valsartan 80mg/bid, spironolactone 50 mg/daily, Lasix 80 mg/daily, Aspirin 80mg/daily	Amilodipin 5mg/bid, Losartan 25 mg/daily, Allopurinol 100 mg/daily, Nephro-Vite I daily, calcium-D I daily	Amilodipin 5mg/bid, Diltiazem 60mg/daily Allopurinol 100mg/daily Nephro-Vite I daily, Calcium carbonate I daily	Losartan 25mg ½/ daily, Amilodipin 5mg½/bid, Allopurinol 100 mg ½/daily, Calcitrol I daily, Nephro-Vite I daily, Calcium I daily, Folic acid I daily	Atorvastatin 10 mg/daily, Diltiazem 60 mg/daily, Allopurinol 100mg/daily Enalapril 5mg/daily, Nephro-Vite I daily

M, male; F, female; BMI, body mass index; DM, diabetes mellitus; Neg, negative; HTN, hypertension; CHD, coronary heart disease; CIN, chronic interstitial nephritis; FSGS, focal segmental glomerulosclerosis; TX, transplantation; N, no; Y, yes.

[a]Dipstick proteinuria range is defined as follows: Neg, 0 mg/dL; trace, 15–30 mg/dL; +, 30–100 mg/dL; ++, 100–300 mg/d; +++, 300–1000 mg/dL; ++++, >1000 mg/dL.

[b]Under controlled diet is defined as follow: no, on regular diet; yes, patients have been under the recommendation of nutritionist by taking low protein, low salt diet, with low caffeine.

Table II. Laboratory parameters differences among baseline, 1, 3, 6, 9, 12 and 18-month follow-up.

Patient parameters	Normal range	Mean estimate difference baseline,1,3,6,9,12,18	Coefficient (HPD CI 95%)
Leukocytes (*10^3/uL)	4–10	6.4	0.04 (–0.022, 0.07)
Hemoglobin (g/dL)	12–16	12.7	0.05 (–0.05, 0.04)
HCT (%)	38–47	36	–0.00 (–0.09, 0.16)
MCV (fl)	80–96	83.2	–0.01 (–0.09, 0.08)
Platelets (*10^3/uL)	150–450	213.5	0.43 (–1.20, 1.39)
FBS (mg/dL)	60–105	91.5	0.06 (–0.17, 0.40)
HbA1c (%)[a]	4–6	5.3	–0.01 (–0.02, 0.01)
Sodium (mEq/L)	134–148	138.7	0.07 (–0.08, 0.18)
Potassium (mEq/L)	3.5–5.5	4.4	0.01 (–0.01, 0.03)
Calcium (mg/dL)	8.6–10	9.6	–0.01 (–0.03, 0.02)
Phosphorus (mg/dL)	2.6–4.5	4.3	–0.01 (–0.02, 0.02)
Magnesium (mg/dL)	1.6–2.4	2.1	–0.01 (–0.02, 0.01)
Albumin (g/dL)	3.5–5.2	4.4	0.00 (–0.01, 0.02)
Triglycerides (mg/dL)	<200 desirable	124.8	2.11 (–0.23, 4.04)
Total cholesterol (mg/dL)	<200 desirable	162.7	–0.76 (–1.79, 0.05)
LDL cholesterol (mg/dL)	<100 low risk	88.5	–0.23 (–1.35, 0.32)
Uric acid (mg/L)	2.6–6	8.9	0.37 (–0.77, 1.14)
AST (U/L)	0–31	18.8	–0.12 (–0.29, 0.05)
ALT (U/L)	0–40	23.1	–0.05 (–0.230, 0.182)
Alkaline phosphatase (U/L)	0–240	157.1	–1.13 (–3.239, 0.428)
ESR 1h (mm/h)	3–20	22.7	–0.47 (–0.862, 0.043)
PTH (pg/mL)[a]	15–65	111.8	–0.92 (–3.43, 2.34)
TSH (MIU/mL)[a]	0.2–5.0	2.8	–0.02 (–0.04, 0.01)
Dipstick proteinuria[b]	Negative	2.5	–2.83 (–0.62, 0.73)
Urine culture[a]	Negative	Negative	NA

HPD CI, highest posterior density confidence interval; HCT, hematocrit; MCV, mean corpuscular volume; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AlK-p, alkaline phosphatase; NA, not assessed.

[a]Assessed at baseline, 6 and 18 month visits.

[b]1, 0 mg/dL; 2, 15–30 mg/dL; 3, 30–100 mg/dL; 4, 100–300 mg/d; 5, 300–1000 mg/dL; 6, >1000 mg/dL.

30 ± 6.9 mL/min/1.73 m^2 at baseline (Figure 2) and this trend continued till 18 months of follow-up to 20.1 ± 10.7 mL/min/1.73 m^2. The mean SCr level of 1.6 ± 0.1 mg/dL 18 months before cell infusion increased to 2.5 ± 0.4 mg/dL at baseline and reached to 4.2 ± 1.9 mg/dL in 18 months of follow-up. There were no significant differences between the two periods (–18 month to baseline versus 18 month to

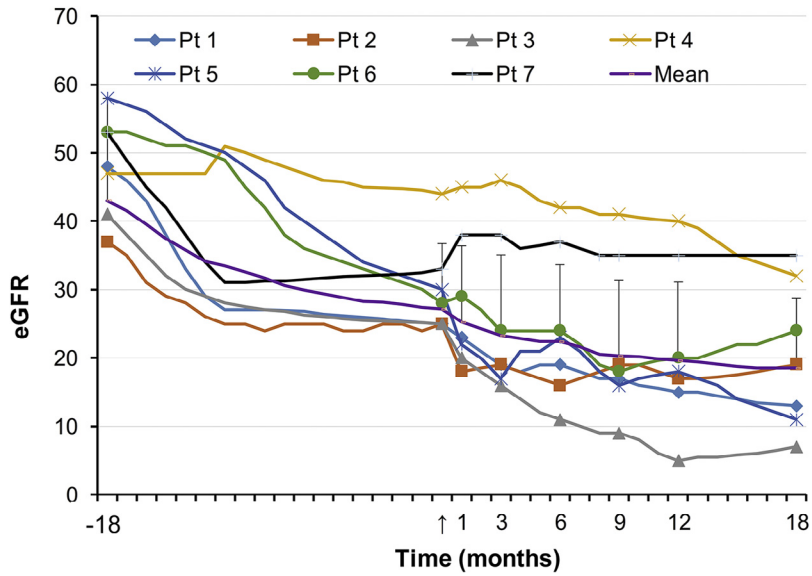

Figure 2. eGFR changes in CKD patients 18 months before MSC infusion up to 18 months after the infusion for each patient. Arrow shows cell transplantation time. 0: baseline, Pt: patient.

Table III. Changes in renal parameters in CKD patients following MSC infusion at 18 months before, baseline, and 12-month and 18-month follow-up.

					Mean difference		
Patient parameters	Normal range	–18 mo	BV	18 mo	18, BV	BV,–18	*P*
SBP (mm Hg)	<120	128.3 ± 8	126.7 ± 12.8	124.6 ± 9.4	–2.1 ± 5.7	–1.5 ± 7.9	0.90
DBP (mm Hg)	60–80	83.1 ± 6.1	81.3 ± 5.2	82.8 ± 8.1	1.6 ± 7.5	–1.8 ± 3.9	0.43
BUN (mg/dL)	7–20.6	45.7 ± 20.8	49.8 ± 22.1	53.5 ± 22.2	3.7 ± 23.5	–4.1 ± .7.8	0.96
SCr (mg/dL)	0.4–1.4	1.6 ± 0.1	2.5 ± 0.4	4.2 ± 1.9	1.7 ± 1.6	0.8 ± 0.4	0.24
eGFR[a]	90–120	48.1 ± 7.3	30 ± 6.9	20.1 ± 10.7	–9.8 ± 7.2	–18.1 ± 8.6	0.10

BV, baseline visit; HP DCI, highest posterior density confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a]MDRD study formula (mL/min/1.73 m^2).

baseline) in eGFR ($P = 0.10$), SCr, Bun and BP (Table III).

Discussion

To the best of our knowledge this is the longest follow-up period trial that showed the safety and tolerability of a single intravenous infusion of autologous MSCs in patients with CKD. Comparing eGFR decrease between two 18-month periods, before and after cell infusion, we observed a nonsignificant reduction in the rate of eGFR decrease. However, we could not assess the efficacy due to our study design. The limitations of our trial were limited sample size and lack of a control group.

There are a limited number of trials assessing MSC impact on kidney diseases [26,29,30]. However, safety assessment of autologous MSC in nondiabetic CKD patients has not been reported yet. Also, a systematic review and meta-analysis report has supported the safety of systematic MSC infusion in different conditions. This report indicated that there was no acute infusional toxicity, organ system complication, infection, death or malignancy following intravenous infusion of MSCs [31]. These findings are consistent with the safety results of the current trial using comprehensive assessment of AE, and clinical and para-clinical parameters.

Stem cell–based therapy, mainly MSCs, has been the focus of limited clinical studies for treatment of CKD (Table IV). A recent dose-escalating randomized clinical trial (RCT) showed allogeneic MSC infusion in DN patients was safe and tolerable in a 15-month follow-up period [26], which is in line with our result, noting that the cell source of our trial was not from healthy donors and were obtained from each patient (autologous transplantation). They also reported that GFR was stabilized in the MSC group compared with the control. However, the difference was not statistically significant. Similarly, we recorded less decrease in eGFR, which was not significant. Packham *et al.* have shown that patients with baseline eGFR >30 responded to the intervention significantly; likewise, our study showed a better trend of eGFR in three patients with baseline eGFR >30 (Supplemental Figure S2), although it is not of great importance because we had a limited number of patients.

Similarly, Saad *et al.* showed that autologous adipose-derived MSCs (ADMSCs) are safe and well tolerated in patients with renovascular disease. They reported that eGFR stabilized in the MSC group compared with the control after 3 months [32]. On the other hand, a RCT revealed that 2 SAEs occurred following two doses of allogeneic umbilical cord mesenchymal stromal cells in systemic lupus erythematous (SLE) patients. Meanwhile, this cell infusion was not effective [33]. However, four open-label single-arm trials that were conducted by Sun *et al.* in SLE patients indicated that allogeneic umbilical cord mesenchymal stromal cells and MSCs are safe and tolerable as well as induce significant reduction in 24 h proteinuria, BUN and SCr [35–38].

Administration of umbilical cord MSC-derived extracellular vesicles (MSC-EVs) in 20 CKD patients improved kidney function compared with the control [34]. This supported the anti-inflammatory effect of MSC-EVs in CKD. They used a cell-free product, so their report of safety is considerable but is not applicable for supporting our result.

Moreover, El-Ansary *et al.* reported that MSC infusion in 20 CKD patients (10 with glomerulonephritis due to SLE and 10 kidney transplantation group) improved both SCr and creatinine clearance compared with 10 patients in the control group [24]. Furthermore, they illustrated that MSC infusion improved creatinine and creatinine clearance in 10 CKD patients of stage II and III. In addition, the levels of both vascular endothelial growth factor (VEGF) and insulin growth factor-1 (IGF-1) showed an overall increase during the first week after transfusion [25]. However, the design of both studies limits us to come to a conclusion on efficacy. Meanwhile, they had not reported

Table IV. Clinical trials using MSC-based products in CKD patients.

Reference (y)	Study design	Underlying disease	Number of patients /control group	eGFR at enrollment	Intervention	Dose	Number of infusions/ route	Follow-up	Result
Our trial	Open-label, single-arm trial	HTN, NS, CIN, UN	7/-	25–60	Autologous BMMSC	$1–2 \times 10^6$ /kg BW	Singe infusion/ IV	18 mo compared with baseline and 18 mo before the cell infusion	Safe and tolerable Nonsignificant reduction in the rate of eGFR decrease
Saad *et al.* (2017) [32]	Dose-escalating open-label trial	RVD	14/14	30–75	Autologous ADMSC	7 patients received 1×10^5 /kg BW 7 patients received 2.5×10^5 /kg BW	Singe infusion/ IRA	3 mo compared with baseline and control	Safe and tolerable Cortical perfusion and RBF increased after 3 mo GFR stabilized
Makhlough *et al.* (2017) [27]	Open-label, single-arm trial	ADPKD	6/-	25–60	Autologous BMMSC	$1–2 \times 10^6$ /kg BW	Singe infusion/ IV	12 mo compared with baseline and 12 mo before the cell infusion	Safe and tolerable A significant reduction in the rate of SCr decrease
Deng *et al.* (2017) [33]	Randomized controlled trial	SLE (LN)	12/6	70–140	Allogeneic UCMSC	2×10^8	Two doses 1 week apart/IV	12 mo compared with baseline and control	2 SAEs in UCMSC group and 2 in control group 75% remission in UCMSC group versus 83% remission in control Similar improvement in SLEDAI, BILAG, Serum albumin and renal function of both groups
Packham *et al.* (2016) [26]	Dose-escalating randomized controlled trial	DM	20/10	20–50	Allogeneic BMMSC	10 patients received 150×10^6 10 patients received 300×10^6	Singe infusion/ IV	15 mo follow-up compared with baseline and control	Safe and tolerable GFR was stabilized in MSC group compared with control (not statistically significant) eGFR >30 responded to the intervention significantly
Nassar and El-Ansary *et al.* (2016) [34]	Randomized controlled trial	DM, HTN, SLE, CIN	20/20	15–60	Allogeneic umbilical cord MSC-EV	100 ug/kg/dose	Two doses 1 week apart/IV and IRA	12 mo compared with control	Safe and tolerable Improvement in GFR, SCr and BUN TGF b and IL-10 increased, TNF-α decreased
Saadi and El-Ansary *et al.* (2016) [25]	Randomized controlled trial	Not mentioned	10/12	Not mentioned (CKD stage II, III)	Allogeneic BMMSC	15×10^6	Single infusion/ IV	3 mo compared with baseline	Safety issues were not reported 14% decrease in SCr, 23% increase in creatinine clearance VEGF and IGF-1 increased during the first week
Saadi and El-Ansary *et al.* (2012) [24]	Open-label controlled trial	Group I: 10 nonactive SLE Group II: 10 RT with BPCAN Control: 10 other types	20/10	Not mentioned	BMMSC (group I: autologous, group II: allogeneic)	$0.7–1 \times 10^6$/kg BW	Two does a week apart/IV	6 mo compared with control	Safety issues were not reported Decrease in SCr and increase in creatinine clearance levels compared with control ($P < 0.05$)
Wang and Sun *et al.* (2014) [35]	Open-label, single-arm trial	40 active refractory SLE (39 LN)	40/-	Not mentioned	Allogeneic UCMSC	1×10^6/kg BW	Double infusion a week apart/IV	12 mo compared with baseline	Safe and tolerable Improvement in SLEDAI, BILAG, anti-ds DNA ab, serum albumin Significant reduction in 24-h proteinuria at 9 and 12 mo BUN and SCr decreased significantly in 6 mo
Wang and Sun *et al.* (2013) [36]	Open-label, single-arm trial	87 active refractory SLE (73 LN)	87/-	<30– > 100	Allogeneic BMMSC or allogeneic UCMSC	1×10^6/kg BW	69 patients received single infusion and 18 received multiple infusions/IV	Mean follow-up time: 27 mo, compared with baseline	Safe and tolerable Improvement in SLEDAI, serum autoantibodies, serum complement and serum albumin Significant reduction in 24-h proteinuria, BUN and SCr
Sun and Liang *et al.* (2010) [37]	Open-label, single-arm trial	16 active refractory SLE (15 LN)	16/-	Not mentioned	Allogeneic UCMSC	1×10^6/kg BW	Single infusion/ IV	Mean follow-up time: 8.25 mo, compared with baseline	Safe and tolerable Improvement in SLEDAI score, Serum ANA, anti-dsDNA ab, serum albumin Significant reduction in 24-h proteinuria at 3 and 6 mo BUN and SCr decreased in 6 patients
Liang and Sun *et al.* (2010) [38]	Open-label, single-arm trial	15 active refractory SLE	15/-	10–70	Allogeneic BMMSC	1×10^6/kg BW	Single infusion/ IV	Mean follow-up time: 17.2 ± 9.5, compared with baseline	Safe and tolerable Reduced 24-h proteinuria, SLEDA and anti-dsDNA ab GFR improved in 2 patients SCr improved in 4 patients

DM, diabetes mellitus; HTN, hypertension; RVD, renovascular disease CIN, chronic interstitial nephritis; NS, nephrotic syndrome; UN, unknown; ADPKD, autosomal polycystic kidney disease; SLE, systemic lupus erythematous; LN, lupus nephritis; BPCAN, biopsy-proven chronic allograft nephropathy; RT, renal transplantation; BMMSCs, bone marrow mesenchymal stromal cells; ADMSCs, adipose-derived mesenchymal stromal cells; MSC-EVs, mesenchymal stromal derived extracellular vesicles; UCMSC, umbilical cord mesenchymal stromal cells; BW, body weight; **µg**, micrograms; IV, intravenous; IRA, intra renal artery; RBF, renal blood flow; SAE, serious adverse events; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; SCr, serum creatinine; SLEDAI, systemic lupus erythematosus disease activity index; BILAG, British Isles lupus assessment group; ANA, antinuclear antibodies;TGF b, transforming growth factor b; IL, interleukin; TNF-α: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor, IGF-1: insulin growth factor-1.

safety issues. In brief, our comprehensive safety result is in line with most of the trials using MSCs in CKD.

While limited clinical trials have assessed the effect of MSCs on CKD, several pre-clinical studies have reported the safety and efficacy of MSCs in different models of CKD [20,35,39]. A systematic review and meta-analysis reported that MSCs improve renal structure and function in CKD models. MSCs and intravenous (IV) were optimal source and route for infusion [20], therefore we subsequently used this source and route as well.

CKD progression is associated with a common appearance of glomerulosclerosis, vascular sclerosis and tubulointerstitial fibrosis regardless of underlying cause of the disease [4–7]. It has been indicated that MSCs have the potential to attenuate renal function and structure through decreasing apoptosis, oxidative stress, microvascular rarefaction, fibrosis and inflammation as well as stimulating endogenous regeneration [16–19,39]. Villanueva *et al*. showed MSCs and ADMSCs resulted in kidney regeneration by reducing tissue damage factors (Alpha-smooth muscle actin [α-SMA], ED-1), stimulating angiogenesis (via increase in VEGF) and an increase in renal developmental markers (bone morphogenetic protein 7 [BMP7], Pax2) in a rat CKD model [40,41]. Furthermore, MSCs improved glomerular sclerosis and reduced transforming growth factor beta (TGF-β) and proteinuria [42,43], as well as reduced glomerular damage through an increase in nephrin, CD2-associated protein (CD2AP) and VEGF, and reduction in macrophage infiltration in a CKD model [44]. Likewise, MSCs decreased pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 and increased IL-4 and IL-10, which led to renal function restoration [42,45] and improved renal structure via modification in EMT, TGF-β, tissue inhibitor of metalloproteinases (TIMP) and matrix metallopeptidase 2 (MMP2) in rat CKD models [46].

Conclusion

In conclusion, this phase 1 trial showed the safety and tolerability of an IV infusion of autologous MSCs in CKD patients for at least 18 months. This approach is well tolerated, which provides an important foundation for future clinical trials to assess the efficacy of autologous MSCs in CKD.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jcyt.2018.02.368.