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LABORATORY STUDY

Effect of Stem Cell Factor and Granulocyte-Macrophage Colony-Stimulating Factor-Induced Bone Marrow Stem Cell Mobilization on Recovery from Acute Tubular Necrosis in Rats

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Abstract

Background: Acute tubular necrosis (ATN) is the most common reason for acute kidney injury (AKI), and there is still an absence of effective therapies. *Objective*: To assess the value of bone marrow cell mobilization by stem cell factor (SCF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy in rats with gentamicin-induced ATN. *Methods*: ATN was induced in male Sprague–Dawley (SD) rats with five daily high-dose intraperitoneal injections of gentamicin. Subcutaneous injections of SCF and GM-CSF were administered simultaneously and these cytokines were observed on days 2, 5, 10, 17, 24, and 31. Peripheral blood and renal tissue CD34+ cell count, mortality rate, blood urea nitrogen (BUN), serum creatinine (SCr), creatinine clearance rate (CCr), and histopathologic lesion scores were determined. Twelve hours after bone marrow ablation (BMA) by lethal X-ray radiation, specific pathogen-free (SPF) ATN rats were given five daily injections of SCF and GM-CSF. BUN, SCr, and histopathologic lesion scores were evaluated on days 2, 5, and 10. *Results*: Peripheral blood CD34+ cell count increased significantly in ATN rats between 2 and 10 days after SCF and GM-CSF injection. Mortality was reduced from 34.7% in the ATN group to 18.6% in the ATN+CSF. In addition, cytokines administration significantly decreased SCr and BUN. Moreover, cytokines rapidly ameliorated tubular injury. There was no significant effect on ATN rats after BMA. *Conclusions*: This study demonstrated that SCF and GM-CSF effectively mobilized bone marrow cells in ATN rats, and cytokines administration partially prevented gentamicin-induced ATN. These results suggest that bone marrow stem cell (BMSC) mobilization may be an effective therapy for ATN.

Keywords: acute tubular necrosis, bone marrow stem cells, stem cell factor, granulocyte-macrophage colony-stimulating factor, irradiation

INTRODUCTION

Acute tubular necrosis (ATN) is the most common reason for acute kidney injury (AKI), accounting for 75–80% of all AKI.[1] The mortality of ATN patients has increased to nearly 60% in hospital settings. While epidemiological investigation has shown that ATN morbidity is increasing, there is still an absence of effective therapies. Since the 1990s, stem cell therapy has become a central area of clinical research.[2]

At present, there are no reliable methods available to obtain sufficient numbers of well-characterized renal stem cells that could be applicable in such a stem cell therapy approach.[3–8] However, bone marrow stem cells (BMSCs) were suggested to have high plasticity and can differentiate into various tissue cells by crossing lineage boundaries for the regeneration of damaged tissues. In damaged non-hematopoietic organs, such as vascular tissue,[9,10] myocardium,[11] brain,[12,13] liver,[14,15] lung,[16,17] and skin,[18] homing and engraftment of BMCs have been observed and were suggested to contribute to the wound-healing process and improve organ function. Recent studies have shown that transplanted BMSCs can differentiate into renal tubular epithelial cells that will promote recovery from ATN.[19–22] However, all therapies focusing on renal damage repair by BMSCs are based on in vitro-cultured

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cell transplantation, which has a longer preparatory period, involves many stages, and is not feasible in clinical treatment of acute renal failure. A clinically attractive approach is to use a mobilizer to mobilize bone marrow cells to the circulation. It has been reported that autologous BMSC mobilization can also heal damaged tissues.[23–26] Above studies provides a proof that autologous BMSC mobilization can also restore renal tubular structure and improve renal function.

Gentamicin is a very effective aminoglycoside antibiotic in treating gram-negative infections. Aminoglycoside drug could induce renal tubular cell injury such as derangement of lysosomal, mitochondrial, and plasma membrane structure. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. In this study, we constructed a rat model of gentamicin-induced ATN[27,28] and mobilized BMSCs with stem cell factor (SCF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Our aim was to explore the capacity of mobilized BMSCs to promote ATN recovery.

MATERIALS AND METHODS

Materials

SCF was purchased from Chengdu DI'AO Pharmaceutical Group (Chengdu, PR China), GM-CSF was purchased from Harbin Pharmaceutical Group (Harbin, PR China), and fluorescein isothiocyanate (FITC)-CD34+ monoclonal antibodies were obtained from Jingmei Biotech (Shenzhen, PR China). Six- to eight-week adult male Sprague–Dawley (SD) rats were purchased from the Experimental Animal Center at Nanhua University (Hunan, PR China). All studies were performed with the approval of the experimental animal committee at our university. The animals were housed under standard conditions with free access to standard diet pellets and tap water.

Mobilization of BMSCs

The rats were randomly divided into four groups: a control group ($n = 48$) that received five daily intraperitoneal injections with normal saline (NS) (6 mL/kg), an ATN group ($n = 73$) that received five daily intraperitoneal injections with gentamicin (250 mg/kg), a ATN+SCF group ($n = 59$) that received five daily intraperitoneal injections with gentamicin (250 mg/kg) in addition to daily subcutaneous administration of SCF (200 μg/kg) and GM-CSF (200 μg/kg),[24,29] and a CSF group ($n = 48$) that only received daily subcutaneous administration of SCF (200 μg/kg) and GM-CSF (200 μg/kg). Eight rats in each group were killed on days 2, 5, 10, 17, 24, and 31. Blood, urine, and renal tissue were harvested.

Bone Marrow Ablation

Twenty-six rats were given a sublethal radiation dose of 11 Gy, using an X-ray irradiator (1 Gy/min) to induce bone marrow ablation (BMA)[19]; 12 h after irradiation, the rats were randomly divided into three groups: a BMA group ($n = 6$) that received five daily intraperitoneal injections with NS (6 mL/kg), a BMA+ATN group ($n = 10$) that received five daily intraperitoneal injections with gentamicin (250 mg/kg), and a BMA+ATN+CSF group ($n = 10$) that received five daily intraperitoneal injections with gentamicin (250 mg/kg), in addition to five daily subcutaneous injections of SCF (200 μg/kg) and GM-CSF (200 μg/kg). The irradiated rats were housed under specific pathogen-free (SPF) conditions and two rats in each group were killed on days 2, 5, and 10. Blood and renal tissues were harvested.

Cytologic and Biochemical Examination

Harvested peripheral blood was diluted and leukocytes were counted using a full automatic blood cell counter. Wright's stained peripheral blood smears were observed under an optical microscope and the percentage of mononuclear cells was quantitated. All rats were individually housed in metabolic cages to collect 24 h urine samples; 24 h urine protein was measured using the Coomassie Brilliant Blue method. Serum creatinine (SCr), blood urea nitrogen (BUN), and urine creatinine were measured at various time points using a Full Automatic Chemical Analyzer (Bechman, NJ, USA). Endogenous creatinine clearance rate (CCr) was calculated by the standard formula. Urine *N*-acetyl-*β*-D-glucosidase (NAG) was determined using the colorimetric method and results were corrected by Cr(Ucr) to account for changes in urine volume.[30]

Histology, Renal Function, and Immunohistochemistry

Kidney tissues were fixed in 10% formalin, embedded in paraffin, and cut into 3μm samples for staining with hematoxylin and eosin stain (H&E) and periodic acid Schiff stain (PAS). The slides were observed using an optical microscope and pathologic renal classification was performed as described by Miller et al.[31] Twelve microscopic fields of slides of different points in all groups were chosen randomly to be scored. The pathologic scoring grades are as follows: (i) *epithelial calcification*: 0, no epithelial calcifications; 1, one to four fields with epithelial calcifications; 2, five to eight fields with epithelial calcifications; 3, nine to twelve fields with epithelial calcifications; (ii) *tubular dilatation*: 0, normal by H&E and PAS; 1, suggestive tubular dilatation by H&E with partial loss of PAS staining along the brush border; 2, unequivocal tubular dilatation by H&E with widespread loss of PAS staining along the brush border; 3, unequivocal tubular dilatation by H&E with loss of tubular specificity and total absence of PAS staining along the brush border; (iii) *proximal tubular papillary proliferation*: 0, no proliferation; 1, one to four fields with proliferative changes; 2, five to eight fields with proliferative changes; 3, nine to twelve fields with proliferative changes; (iv) *interstitial infiltration*: 0, no infiltrate; 1, one

to four fields with an infiltrate; 2, five to eight fields with an infiltrate; 3, nine to twelve fields with an infiltrate. The total score for each kidney examined was divided by 8 to give a final score of 0–3.

Immunohistochemistry

Renal tissue CD34+ immunohistochemical staining kit was performed according to the supplied protocols. Stained sections were randomly taken from each 10,400-fold magnification field, counting the number of CD34+ cells per field, count the average (excluding the positive staining of vascular endothelial cells).

Proliferating Cell Nuclear Antigen

Renal tubular epithelial expression of proliferating cell nuclear antigen (PCNA) was performed according to the supplied protocols. Double-blind method was used to calculate the renal tubular epithelial cell proliferation index (PI). Randomly taken 5 high-power microscope (10×40) field, count 100 renal tubular epithelial cells each, was used to calculate the percentage of expression of PCNA-positive cells.

Flow Cytometry

Mononuclear cells were washed with phosphate-buffered saline (PBS), resuspended to a concentration of 1×10^6 cells/mL, and incubated with FITC-CD34+ monoclonal antibodies in the dark for 25 min at room temperature. The cells were washed twice with PBS and resuspended in 1% paraformaldehyde. Flow cytometry was performed using a fluorescence-activated cell sorter (FACS) Vantage flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). A minimum of 10,000 cells were acquired from each sample. Data were analyzed using CellQuest software (Becton Dickinson), and the percentage of CD34+ cells was determined.

Statistical Analysis

All data were expressed as ($\bar{x} \pm$ SD) and analyzed using the statistical package SPSS 10.0 (Department of Statistics, Public Health School, Centre South University, Changsha, Hunan, PR China) for Windows. One-way analysis of variance (ANOVA) was used to determine statistically significant differences between the groups, and the mean of every two different groups was detected using the Student's least significant difference (LSD) t-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Cytokines Administration Reduced Mortality

In the control and CSF groups, the rats had lustrous hair, agile responses, and normal appetites. In the ATN and ATN+CSF groups, the rat had lusterless vertical hair, dull responses, reduced motion, and anorexia. The mortality of the rats was 0% in the control group, 34.7% in the ATN group, 18.6% in the ATN+CSF group, and 4% in the CSF group.

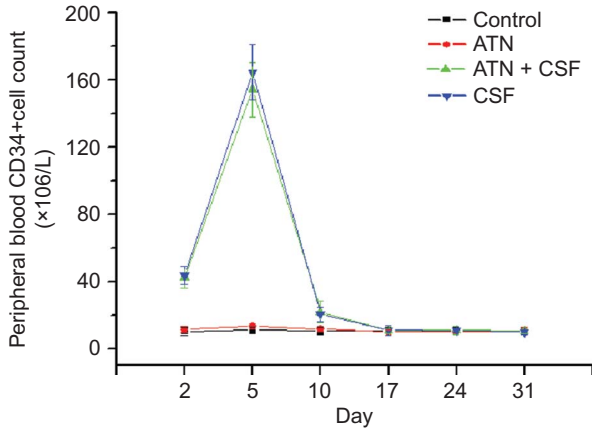

Figure 1. Peripheral blood CD34+ cell count in different time points.

BMSC Mobilization by Cytokines

It had been reported that GM-CSF and SCF can mobilize BMSCs into the peripheral blood and CD34 usually was regarded as a marker of stem cells and precursor cells. Therefore, we examined the number of CD34+ cells as a result of the administration of GM-CSF and SCF. As showed in Figure 1, peripheral blood CD34+ cell count increased on day 2, peaked on day 5, and remained high on day 10 in both the ATN+CSF and CSF groups, with significant differences between the control and ATN groups ($p < 0.05$). CD34+ cells decreased to a normal level in all treatment groups by day 17. These data showed cytokines administration efficiently mobilized BMSCs into the peripheral blood in our study.

Cytokines administration partially inhibited deterioration of renal function and accelerated renal function recovery.

We determined rat weight, 24 h urine volume, urine protein content, Scr, BUN, CCr, and urine NAG content at different time points to quantify the contribution of cytokines administration to ameliorated renal function (Table 1).

Rat weight and urine volume did not change significantly in either the control or CSF groups. In the ATN and ATN+CSF groups, it increased on day 2, peaked on day 10, decreased on day 17, and returned to normal levels by day 24. There were significant differences between these groups and the control ($p < 0.05$).

Rat urine protein content increased on day 2 in the ATN and ATN+CSF groups, differing significantly from the control and CSF groups ($p < 0.05$). Urine protein peaked on day 10, decreasing to normal by day 31 in the ATN group, and peaked on day 5, decreasing to normal by day 24 in the ATN+CSF group. Rat urine protein content in the ATN and ATN+CSF groups was significantly different on days 10, 17, and 24 ($p < 0.05$). Urine protein was slightly higher in

Table 1. Renal injury index in each stem cell mobilizer group ($\bar{x} \pm SD$).

Group	Time	Weight (g)	24 h UV (mL/day)	Upro. (mg/day)	Cr (μmol/L)	CCr (mL/min)	NAG (U/μmolCr)
Control	2d	219.02 ± 33.64	5.41 ± 0.89	10.53 ± 2.45	54.30 ± 8.50	0.201 ± 0.033	13.90 ± 3.62
	5d	239.31 ± 36.43	6.2 ± 0.99	10.50 ± 2.20	49.91 ± 9.32	0.225 ± 0.032	14.71 ± 2.94
	10d	263.45 ± 37.39	7.05 ± 1.06	10.63 ± 1.31	51.53 ± 3.90	0.223 ± 0.019	13.27 ± 2.67
	17d	288.62 ± 45.75	7.3 ± 1.18	9.63 ± 1.69	53.01 ± 10.59	0.224 ± 0.024	13.62 ± 1.84
	24d	300.43 ± 54.88	7.78 ± 1.37	9.89 ± 1.73	52.22 ± 9.66	0.223 ± 0.029	13.44 ± 2.64
	31d	311.80 ± 62.87	8.39 ± 1.64	9.75 ± 1.49	52.30 ± 15.54	0.229 ± 0.034	14.03 ± 3.05
ATN	2d	198.02 ± 24.84	12.41 ± 2.29*	22.38 ± 5.15*	64.47 ± 3.57	0.199 ± 0.022	33.82 ± 6.32*
	5d	201.31 ± 26.43*	14.21 ± 2.93*	27.25 ± 7.23*	225.55 ± 29.13*	0.169 ± 0.024*	227.66 ± 43.41*
	10d	190.45 ± 24.39*	18.05 ± 3.76*	37.50 ± 8.89*	365.45 ± 33.51*	0.084 ± 0.020*	297.59 ± 57.87*
	17d	218.62 ± 35.75*	16.3 ± 3.13*	29.88 ± 8.22*	170.77 ± 8.41*	0.060 ± 0.019*	198.96 ± 21.51*
	24d	230.43 ± 44.88*	12.78 ± 2.73*	18.47 ± 3.65*	75.55 ± 6.91*	0.184 ± 0.017*	35.96 ± 11.94*
	31d	264.80 ± 62.87*	11.39 ± 1.84*	14.13 ± 5.08*	59.23 ± 6.31	0.224 ± 0.037	13.16 ± 3.36
ATN + CSF	2d	201.72 ± 31.14	11.41 ± 2.89*	23.37 ± 4.66*	59.27 ± 14.67	0.200 ± 0.032	35.41 ± 7.74*
	5d	197.37 ± 32.49*	13.32 ± 2.99*	27.07 ± 6.76*	154.90 ± 29.37*,**	0.171 ± 0.025*	242.25 ± 52.11*
	10d	213.23 ± 36.51*,**	15.05 ± 1.06*,**	30.57 ± 6.82*,**	93.013 ± 13.74*,**	0.093 ± 0.035*	202.32 ± 67.06*,**
	17d	258.54 ± 43.14*,**	10.3 ± 1.18*,**	18.25 ± 3.69*,**	57.48 ± 9.32**	0.196 ± 0.047**	71.97 ± 28.99*,**
	24d	279.29 ± 54.36**	8.58 ± 1.37**	11.50 ± 1.41	61.69 ± 12.20**	0.208 ± 0.057	20.84 ± 9.01**
	31d	298.84 ± 59.59**	8.99 ± 1.84**	10.87 ± 2.53	50.20 ± 9.51	0.220 ± 0.025	12.40 ± 2.44
CSF	2d	212.32 ± 31.46**	5.13 ± 0.69**	11.04 ± 2.62**,***	53.01 ± 10.89	0.213 ± 0.035	15.00 ± 3.27**,***
	5d	238.31 ± 34.53**	6.12 ± 0.94**	10.75 ± 2.71**,***	54.72 ± 3.47**,***	0.218 ± 0.032**,***	16.64 ± 2.32**,***
	10d	262.49 ± 35.69**	7.35 ± 1.12**	11.63 ± 3.42**,***	49.81 ± 7.41**,***	0.223 ± 0.035**,***	15.41 ± 3.63**,***
	17d	282.64 ± 44.63**	7.86 ± 1.23**	10.50 ± 3.93**,***	53.27 ± 9.71**	0.214 ± 0.027*,**	13.48 ± 4.17**,***
	24d	302.43 ± 54.88**	7.25 ± 1.45**	9.88 ± 1.96**	55.31 ± 4.77**	0.221 ± 0.031	13.08 ± 3.24**
	31d	310.93 ± 52.58**	7.97 ± 1.58**	10.00 ± 2.51	53.77 ± 5.36	0.219 ± 0.031	16.64 ± 2.32

Notes: NAG, *N*-acetyl-*β*-D-glucosidase; ATN, acute tubular necrosis; CSF, colony-stimulating factor.
*$p < 0.05$ versus control group; **$p < 0.05$ versus ATN group; ***$p < 0.05$ versus ATN+CSF group.

the CSF than the control group, although this was not statistically significant.

Rat SCr increased on day 5 in the ATN and ATN+CSF groups, differing significantly from the control and CSF groups ($p < 0.05$). SCr peaked on day 10, decreasing to normal by day 31 in the ATN group, and peaked on day 5 (though at a significantly lower level; $p < 0.05$), decreasing to normal by day 24 in the ATN+CSF group. SCr levels were significantly different between the ATN and ATN+CSF groups on days 5, 10, and 17 ($p < 0.05$). SCr did not undergo much change in the CSF group and control group.

Rat endogenous CCr decreased on day 5 in the ATN and ATN+CSF groups and differed significantly from the control and CSF groups ($p < 0.05$). Clearance was at a minimum level on day 17, returning to normal by day 31 in the ATN group, and was at a minimum level on day 10, returning to normal by day 24 in the ATN+CSF group. On day 17, there were significant differences between the ATN and ATN+CSF groups ($p < 0.05$). Endogenous CCr did not change significantly in the CSF group and control group.

Gentamicin-induced nephrotoxicity is mostly characterized by direct tubular necrosis. Urine enzyme activities are used as sensitive and early indicator of tubular injury in different experimental AKI models. As a marker of tubular function, NAG is a hydrolytic lysosomal enzyme, which is released after renal tubular damages. As shown in Table 1, NAG content increased on day 2 in the ATN and ATN+CSF groups, differing significantly from the control and CSF groups ($p < 0.05$). NAG peaked on day 10, decreasing to a normal level by day 31 in the ATN group, and peaked on day 10, decreasing to a normal level by day 24 in the ATN+CSF group. There were significant differences on days 10, 17, and 24 between the ATN and ATN+CSF groups ($p < 0.05$). NAG content was slightly higher in the CSF than the control group although they were not statistically significant. This data showed cytokines administration accelerated renal tubular function recovery.

Cytokines administration induced morphological changes and pathological scores of rat renal tissue.

Gentamicin-induced nephrotoxicity is mostly characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. Histopathological examination of kidney showed severe and extensive damage after gentamicin injection. The characteristic morphology is extensive necrosis of epithelial cells in the proximal convoluted tubules of the cortex, decreased karyocyte numbers, extensive drop-off of the brush borders, absence of cytoplasm, and separation of the basilar membranes, but the tubule walls did not crack or subside, and the basilar membrane remained intact. However, there were nidus inflammatory cell infiltration at the renal cortex and epiopticon area (Figure 2C). With the passage of course, tubule cells began regeneration and necrosis disappeared (Figure 2D). Our study showed the regeneration of proximal convoluted tubules in ATN+CSF group was ahead of that in ATN (Figure 2A–D). The morphology of the renal tissue did not change significantly in either the control or CSF groups.

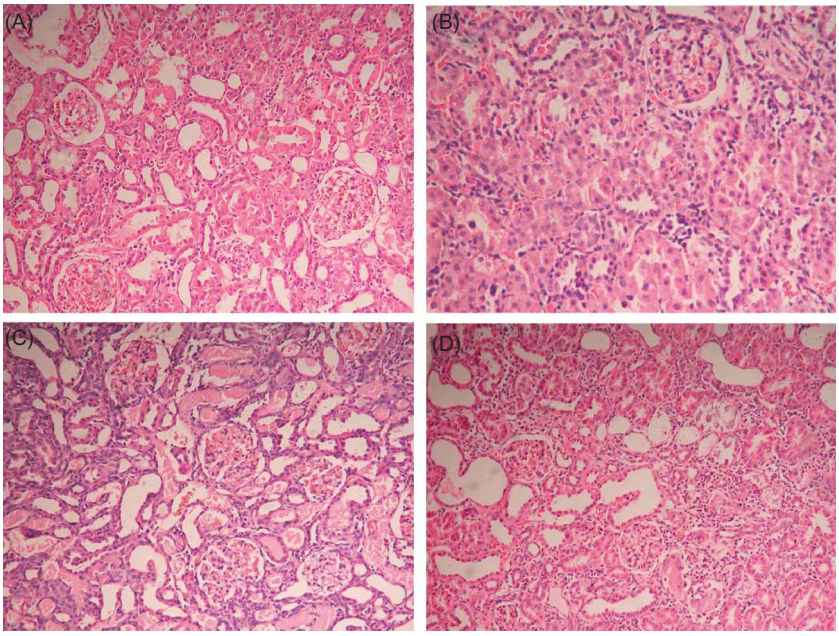

Figure 2. The morphology of the renal tissue in the ATN+CSF group and in the ATN group. (A) Kidney pathological changes on day 10 in ATN+CSF group. The necrotic substance in tubular cavity decreased gradually and proximal convoluted tubule regenerated evidently. Some epithelial cell hyperplasia of proximal convoluted tubules protruded into tubular cavity in a papillary way and inflammatory cell infiltration was seen. (B) Kidney pathological changes on day 24 in ATN+CSF group. The morphological structure basically restored to normal. (C) Kidney pathological changes on day 10 in ATN group. There were flat epithelial cells which began to cover the basal membrane of some proximal convoluted tubules, the renal tubular cavity showed cystic dilation, and there was evident inflammatory cell infiltration. (D) Kidney pathological changes on day 24 in ATN group. The necrotic substance in tubular cavity disappeared, some epithelial cells were still flat, some renal tubular cavities showed cystic dilation, and there was still inflammatory cell infiltration.
Note: ATN, acute tubular necrosis; CSF, colony-stimulating factor.

CD34+ staining cells were seen in endothelial cells. There was no expression of CD34+ cells in other parts in the control group and the CSF group at all time points. CD34+ cells could also be found in the damaged tubular site of ATN+CSF group on days 2, 5, and 10, but not seen in the non-damaged tissue of the distal convoluted tubule and collecting duct area nor other time points (Figure 3).

PCNA test showed that the control group and CSF group had no significant positive staining cells whereas ATN and ATN+CSF groups had PCNA-positive cells after gentamicin-induced damage. The PI reached the peak on day 17, continuing to day 31 in ATN group. And in ATN+CSF group the PI peaked on day 5 and was close to normal on day 31 (Figure 4).

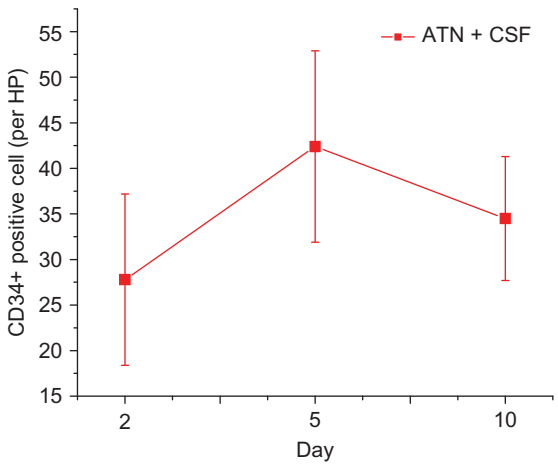

Figure 3. Counts of CD34+ cells in renal tissue in ATN+CSF group at different time points.
Note: ATN, acute tubular necrosis; CSF, colony-stimulating factor.

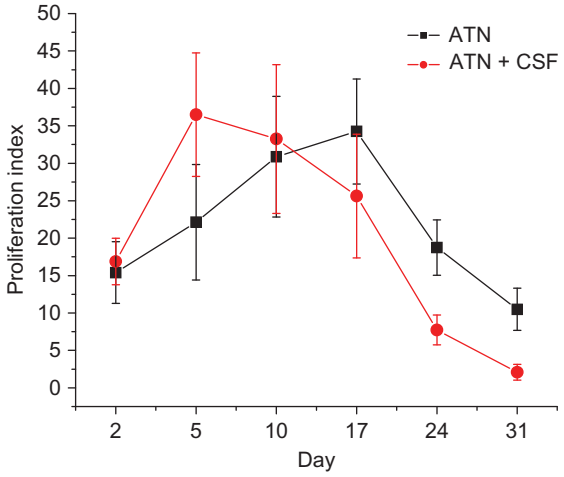

Figure 4. The changes of PI in ATN and ATN+CSF group at different time points.
Note: PI, proliferation index; ATN, acute tubular necrosis; CSF, colony-stimulating factor.

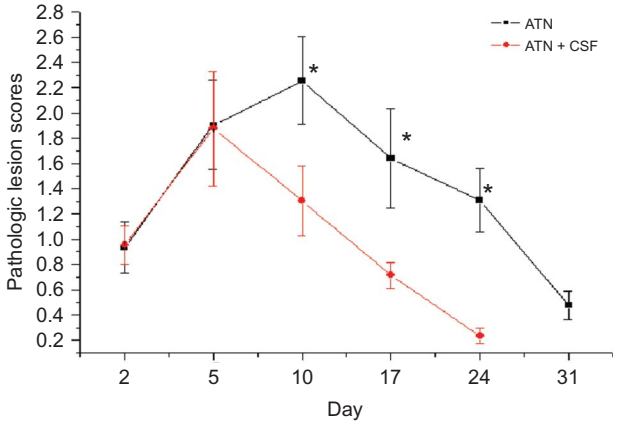

Figure 5. The pathologic lesion scores of ATN and ATN+CSF group.
Notes: ATN, acute tubular necrosis; CSF, colony-stimulating factor.
*$p < 0.05$.

As shown using the Miller pathological grading system, the pathological score peaked on day 10 in the ATN group and on day 5 in the ATN+CSF group. It was restored to a normal level earlier in the ATN+CSF group than in the ATN group (Figure 5).

These data showed cytokines administration can reduce renal damage and promote the regeneration of tubules.

Cytokines Administration Had No Effect on Renal Functions After BMA

It has been reported that BMSCs help repair injured renal tubules and our data showed cytokines have the ability to mobilize BMSCs into the peripheral blood in gentamicin-induced AKI and partially protect renal tissues. Next, we examined whether protection effect are due to BMSC mobilization through BMA. SCr and BUN levels and pathological scores were determined. Data were showed in Table 2. The BUN and SCr content did not differ significantly between the BMA and control groups on days 2, 5, 10. Means were higher in the BMA+ATN and BMA+ATN+CSF groups than in the ATN and ATN+CSF groups at the same time point. Pathology was not evident in the BMA group, and the mean pathological score was higher in the BMA+ATN and BMA+ATN+CSF groups than in the ATN and ATN+CSF groups at the same time point. These data show that after the ablation of bone marrow, SCF and GM-CSF can no longer promote the regeneration and repair of ATN; BMCs mobilized by cytokines can be attributed to the acceleration of renal damage recovery induced by gentamicin.

Table 2. Renal injury index in BMA groups.

Group	Time after GM injection (d)	BUN (mmol/L)	Cr (μmol/L)	Lesion scores
BMA (mean)	2 ($n = 2$)	7.8	56.7	–
	5 ($n = 2$)	8.2	55.6	–
	10 ($n = 0$)	–	–	–
BMA+ATN (mean)	2 ($n = 2$)	14.70	68.34	0.925
	5 ($n = 2$)	43.00	252.60	2.175
	10 ($n = 2$)	64.30	397.57	2.410
BMA + ATN + CSF (mean)	2 ($n = 2$)	13.90	63.07	0.941
	5 ($n = 2$)	47.50	241.24	1.993
	10 ($n = 1$)	62.50	411.00	2.320

Note: GM, granulocyte-macrophage; BMA, bone marrow ablation; BUN, blood urea nitrogen; ATN, acute tubular necrosis; CSF, colony-stimulating factor.

DISCUSSION

The vast majority of metabolic products are excreted through the kidney, so renal tubule epithelial cells are particularly susceptible to damage caused by nephrotoxicity factors that induce ATN. ATN is treated mainly by targeting ATN-inducing factors, hemodialysis, and other supporting therapies that will promote the self-regeneration of renal tubule epithelial cells and the restoration of kidney function. Dialysis can eliminate toxins, correct water and electrolyte disorders, and replace the filtration function of the renal glomerulus, but cannot improve the reabsorption, endocrine, concentrating, and diluting functions of the renal tubules. Cellular therapies are a central area of current research. Embryonic stem (ES) cells have the ability to generate all the cell types in the adult body but face ethical and political hurdles for human use.[31,32] As distinct from other tissues, such as bone marrow, it has been difficult to isolate or confirm the existence of stem cells in the kidney, although several studies have suggested the existence of stem cells in the adult renal tissues.[3–8,33,34] Evidence for bone marrow-derived stem cell contributions to renal repair represents a fascinating new approach for the management of renal diseases. Some researchers believe that bone marrow provides the common stem cell bank for tissue and organ regeneration and that tissue-specific adult stem cells (ASCs) originate from a continuous supplementation of germinal BMSCs.[35–40] BMSCs differentiate into the cells required for metabolism and tissue regeneration in mammals.

Our study showed cytokines administration partially prevented gentamicin-induced renal structural and functional damage and decreased ATN-induced mortality. But the effect disappeared after BMA. It has been reported that the transplantation of BMSCs can restore renal function and structure.[19–22] Sufficient stem cell numbers and a tissue damage microenvironment are both required for the BMSCs to promote tissue repair. Our study showed leukocyte numbers and CD34+ cell count in the peripheral blood were slightly higher in the ATN group than in the NS control group at the acute stage (days 2, 5, and 10), with the peripheral blood CD34+ cell peaking on day 5, but the mobilization was weakened and there were no statistically significant differences ($p > 0.05$). Thus, although BMSCs

help to replace old tissue cells and repair mild damage, they fail to meet the requirements for tissue regeneration and repair in cases of serious renal damage. BMSC mobilizers can significantly increase the number of BMSCs in the peripheral blood and renal tissue, which may be equivalent to or even superior to the bone marrow itself. As shown by Bodine et al.,[40] a splenomectomy together with a subcutaneous injection of recombinant human G-CSF and recombinant mouse SCF resulted in a dramatic increase in the number of hematopoietic stem cells (HSCs) in the peripheral blood of an C57BW/6J mouse. In our study, peripheral blood leukocytes and CD34+ cells were significantly higher in cytokines administration groups than the saline control group, indicating that BMSC mobilizers can effectively mobilize BMSCs. The expression of CD34+ cells and PCNA-positive cells in damaged tubular site in ATN+CSF group on days 2, 5, and 10 showed that the mobilized BMSCs can reach the damaged tissue and promote the regeneration of renal tubular epithelial cells that will promote recovery from ATN.

Data of BMA showed that after the ablation of bone marrow, SCF and GM-CSF can no longer promote the regeneration and repair of ATN, which indicates that BMSCs are required to repair renal damage and it is unlikely that mobilizer directly stimulates renal regeneration to improve the morphologic and functional recovery. The primary role of these cytokines is to mobilize bone marrow-derived cells.

More and more studies have confirmed that bone marrow-derived cells contribute to renal repair[41–43]; however, the mechanism underlining this beneficial effect is still a matter of debate. Several studies show that multiple differentiation of bone marrow-derived cells plays a predominant role in the tubule-regenerative process.[19–22] Lin first reported that HSCs can differentiate into renal tubular cells after ischemia-reperfusion (I/R) injury through detecting the characteristic indicator. We used autologous BMC mobilization, so we could not directly detect specific marker of BSC differentiation. But other studies have demonstrated that stem cells contribute to the regenerative process by producing protective and regenerative factors rather than directly using their differentiated progeny to replace damaged cells.[44–47]

Our data indicate that cytokine-induced mobilization is effective in gentamicin-induced ATN, but our current study could not tell that the partial morphologic and functional improvements were due to multiple differentiation of bone marrow-derived cells or secretion of stimulatory or regenerative factors. Meanwhile, bone marrow contains various types of stem cells. All of them can be mobilized by mobilizer. At present, no studies confirm which types of stem cells contribute to renal repair. The molecular mechanism behind the observed protection and duration needs to be further explored.

In summary, BMSC mobilizers, like GM-CSF, G-CSF, M-CSF, and SCF, have excellent safety and validity records that have been verified by multi-year clinical applications. This study indicates a clinically applicable protocol for the use of BMC mobilization to ameliorate ATN. Stem cell mobilization avoids the processes of surgical operation and prior collection and doping of stem cells. Moreover, treatment with BMSC mobilizers is convenient, safe, effective, noninvasive, and free of immunological rejection reactions caused by cell transplantation. Thus, BMSC mobilizers have a greater potential for clinical application than BMSC transplantation.

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