



HUMAN CLINICAL ARTICLE

Acute kidney injury successfully treated with autologous granulocyte colony-stimulating factor-mobilized peripheral blood CD34-positive cell transplantation: A first-in-human report

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Abstract

A 36-year-old man with severe acute kidney injury (AKI) was admitted to Shonan Kamakura General Hospital in Japan. He was diagnosed with refractory hypertension based on a severely elevated blood pressure of 224/116 mmHg and retinal, cardiac, and brain damage revealed by electrocardiogram, fundoscopy, and magnetic resonance imaging, respectively. Although hemodialysis was withdrawn following strict blood pressure control by an angiotensin receptor blocker, severe kidney insufficiency persisted. Therefore, we performed an autologous granulocyte colony-stimulating factor-mobilized peripheral blood CD34-positive cell transplantation. Collected CD34-positive cells were directly infused to both renal arteries. The patient's general condition was unremarkable after intervention, and the serum creatinine level gradually improved to 2.96 mg/dL 23 weeks after cell therapy. Although transient fever and thrombocytosis were observed after intervention, no major adverse events were observed. This patient is the first case in a phase I/II clinical trial of autologous granulocyte colony-stimulating factor-mobilized peripheral blood CD34-positive cell transplantation for severe AKI with a CD34-positive cell dose-escalating protocol (trial number jRCTb030190231).

KEYWORDS

CD34+, clinical trials, granulocyte-colony stimulating factor (G-CSF), hematopoietic stem cells (HSCs), kidney, stem cell transplantation

Lessons learned

- This clinical trial made use of autologous granulocyte colony-stimulating factor-mobilized peripheral blood CD34-positive cells for the treatment of severe acute kidney injury.
- Transplantation of autologous CD34-positive cells into renal arteries was safely performed.
- This cell therapy might have a therapeutic potential as a new strategy for severe acute kidney injury.

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Significance statement

This article reports a first-in-human case in a phase I/II clinical trial of autologous granulocyte colony-stimulating factor-mobilized peripheral blood CD34-positive cell transplantation for severe acute kidney injury. Cell therapy was safely performed, and the patient showed improvement of kidney function without major adverse events during the observation period.

1 | INTRODUCTION

The high prevalence of acute kidney injury (AKI) and its impact on the prognosis of critically ill patients is one of the major clinical problems in the field of critical care nephrology. Although the mortality rate for severe AKI is high (50% or more) in many countries all over the world,¹ there is currently no established treatment to promote kidney repair in these patients. An effective therapy for AKI is thus urgently needed.

We recently reported the efficacy of CD34-positive cell-enriched human peripheral blood mononuclear cell transplantation in an animal model of ischemia/reperfusion AKI.² Cell therapy dramatically improved kidney function and restored peritubular capillary loss due to ischemia. Other researchers have used other cells as the source of regenerative therapy, including mesenchymal stem cells derived from bone marrow,³ umbilical cord,⁴ and adipose tissue⁵ for AKI treatment. Although the

findings of these basic studies suggested the potential usefulness of regenerative therapy for AKI, clinical trials have not yet been performed.

Here, we present the first case of granulocyte colony-stimulating factor (G-CSF)-mobilized autologous CD34-positive cell transplantation for AKI in which the severe AKI needed to be transiently treated by hemodialysis.

2 | CASE REPORT

A 36-year-old man was admitted to Shonan Kamakura General Hospital because of heart failure, lung edema, and a severely elevated blood pressure (BP) of 224/116 mmHg. Although he had a multiyear history of hypertension, sufficient BP control was not achieved until admission. His serum creatinine level was 1.5 mg/dL 10 months before admission.

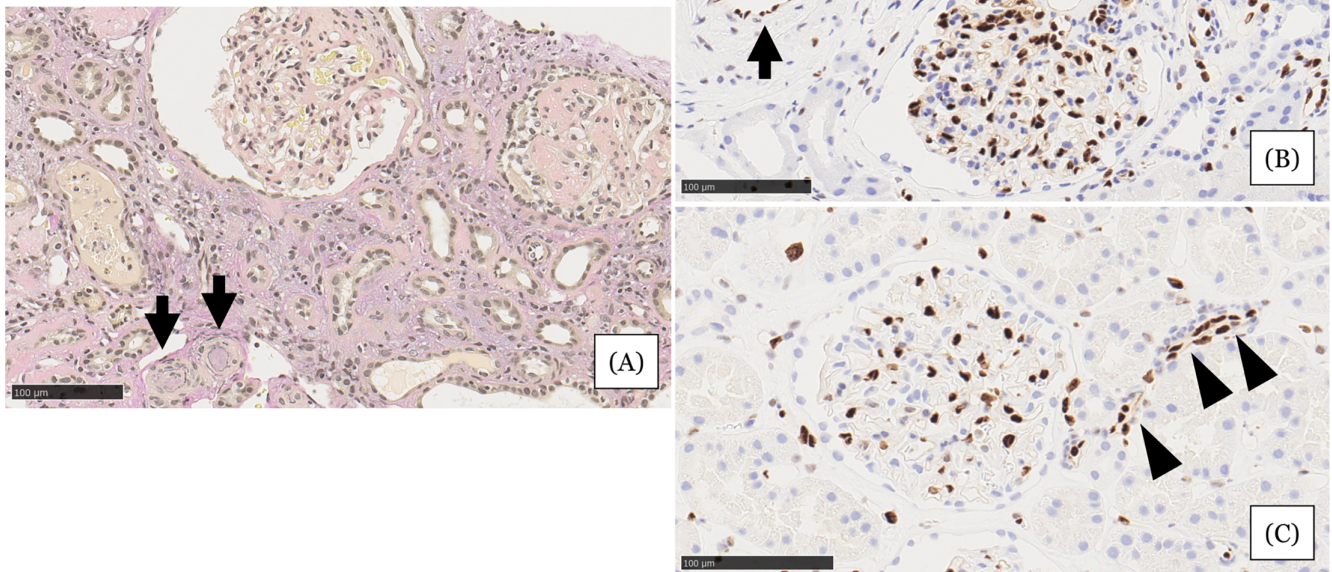


FIGURE 1 Kidney biopsy findings taken on the 31st hospital day. A, Small arteries clogged with mucinous intimal thickening (arrows), shrunken glomerulus with wrinkling of the capillary walls, and severe interstitial mononuclear cell infiltration with tubular damage are shown (Elastica van Gieson stain). B, Nuclei of vascular endothelial cells (ECs) were stained using anti-Ets-related gene (ERG) antibody (clone EP111; Nichirei, Biosciences Inc., Tokyo, Japan). The paucity of EC nuclei is prominent in an arteriole (arrowhead). Irregular nuclei of varying size are noted in an adjoining arteriole (arrow). C, Isometric nuclei of ECs form an orderly line in an arteriole in a kidney from a normotensive kidney donor. Scale bar = 100 μ m

Laboratory tests showed serum creatinine of 7.56 mg/dL, lactate dehydrogenase of 1366 U/L, and hemoglobin of 8.2 g/dL. Peripheral blood smear showed schistocytes. An abnormal signal in the cerebrum in T2-weighted magnetic resonance imaging and Keith-Wagener stage IV retinopathy were also noted. Kidney biopsy revealed severe narrowing or occlusion of small arteries with severe endothelial damage as well as shrunken glomeruli and interstitial cell infiltration (Figure 1A). Compared with normotensive normal kidney tissue that showed isometric nuclei of vascular endothelial cells (ECs) forming an orderly line (Figure 1C), varying sized EC nuclei were prominent, and there were fewer nuclei within the arterioles in the patient (Figure 1B), indicating severe vascular EC damage due to severely elevated blood pressure. Based on the above findings, we diagnosed this patient's AKI as acute with chronic ischemic renal damage due to refractory hypertension with microangiopathic hemolysis. He was intensively treated by intermittent hemodialysis, and his heart failure and accompanying pulmonary congestion gradually improved.

Although BP control by nicaldipine and olmesartan allowed hemodialysis withdrawal after six treatment sessions, the serum creatinine level remained high. After written informed patient consent, we performed autologous CD34-positive cell transplantation for AKI. This was the first case in a phase I/II clinical trial of autologous G-CSF-mobilized peripheral blood CD34-positive cell transplantation for severe AKI with a CD34-positive cell dose-escalating protocol (trial number jRCTb030190231) to evaluate the safety and efficacy as primary and secondary endpoints, respectively. In this trial, three doses (into one renal artery) were set: low dose (5×10^5 /kg body weight), middle dose (1×10^6 /kg body weight), and high dose (2×10^6 /kg body weight) for three patients in each group. After safety was confirmed, the dose of CD34-positive cells was increased to the next higher dose. The low starting dose (5×10^5 /kg body weight, ie, 1×10^6 /kg body weight, bilateral renal arteries) was based on a consideration of safety in previous reports that used CD34-positive cells for other diseases, including critical limb ischemia⁶ and liver cirrhosis.⁷ This dose-escalating protocol was approved by the special committee for class 2 regenerative medicine certified by the Ministry of Health, Labor, and Welfare in Japan (SKRM-2-003). After 800 µg/d (400 µg/m² body surface area) of G-CSF treatment for 5 consecutive days, leukapheresis (COMTEC, Fresenius Kabi Japan Co., Tokyo, Japan) was performed to harvest peripheral blood mononuclear cells on day 5. Magnetic separation of CD34-positive cells using a CliniMACS instrument was performed on day 6 using anti-CD34 antibody-labeled magnetic nanobeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Immediately after CD34-positive cells were separated and the viability (98.1%) and purity (95.6%) of collected cells were confirmed, 90 million CD34-positive cells were dissolved in 100 mL saline. A guiding catheter was placed in either renal artery through right femoral artery by an interventional radiologist. Cells were directly administered into each renal artery (45 million CD34-positive cells in 50 mL saline) at a rate of 150 mL/h (90 million CD34-positive cells in total) using a syringe driver.

The patient's general condition was unremarkable except for a transient elevation in body temperature to 38.1°C 5 days after transplantation. Platelet count and serum levels of interleukin (IL)-6 and IL-8

TABLE 1 Laboratory findings before and after CD34-positive cell transplantation

	1	4	10	15	30	40	44	45	50	59	73	129	180	208
Days from admission														
Days of G-CSF														
Days from cell transplantation						1	5	1	5	14	28	82	133	161
sCr (mg/dL)	7.56	9.22	7.51	6.94	5.87	4.70	5.17	5.15	4.79	3.87	3.32	3.31	3.03	2.96
eGFR (mL/min/1.73 m ²)	7.6	6.1	7.6	8.3	10	12.8	11.5	11.5	12.5	15.8	18.7	18.7	20.6	21.2
WBC (/µL)	14,300	15,300	8,900	7,000	6,700	5,600	49,300	57,800	9,000	8,200	6,600	9,000	7,700	8,100
PLT ($\times 10^4$ /mL)	11.5	26.3	42.9	44.9	36.2	31.1	29.9	25	19.9	60.1	34.9	32.3	31.8	30.8
CRP (mg/dL)	1.039	0.881	0.703	0.202	0.039	0.610	0.410	0.441	7.545	1.704	0.036	0.041	0.043	0.040
CD34 (%)						0.2	1.8	2.0	0.6		0.1	0.6		
Thrombopoietin (fmol/mL)					0.41		1.05		0.97	0.51				
HGF (ng/mL)					0.39		1.82		0.49	0.40		0.31		
IL-6 (pg/mL)					5.7		11.8		56.3	9.1		1.6		
IL-8 (pg/mL)					<2.0		<2.0		2.5	<2.0		<2.0		

Abbreviations: CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; IL, interleukin; PLT, platelet; sCr, serum creatinine; WBC, white blood cell.

transiently increased during the early post-treatment course (Table 1). The patient was discharged to home 10 days after cell transplantation.

Twenty-three weeks after cell therapy, his serum creatinine level was 2.96 mg/dL. Blood pressure was well controlled with 60 mg of nifedipine and 20 mg of olmesartan.

3 | DISCUSSION

Here, we present a case of AKI due to refractory hypertension in which G-CSF-mobilized autologous CD34-positive cell transplantation was safely and successfully performed. No major adverse events (AEs) were observed during the entire treatment course. To the best of our knowledge, this is the first case of autologous CD34-positive cell transplantation for human AKI.

CD34-positive cells have angiogenic potential as they have lineage to vascular endothelial progenitor cells,⁸ but they do not play a role in tubulogenesis in kidney injury.⁹ In a parabiosis experiment, blood-derived hematopoietic cells did not contribute to tubulogenesis but were instead engrafted in renal interstitial spaces as monocyte/macrophage lineage.¹⁰ CD34-positive cells also have anti-inflammatory potential.¹¹ Therefore, in consideration of the ischemic and inflammatory nature of AKI, CD34-positive cell transplantation might be considered as a new therapeutic tool for AKI. Indeed, inflammatory processes other than ischemia have been proposed as mechanisms of AKI in refractory hypertension.¹² Thus, CD34-positive cells might exert renoprotection through angiogenic and anti-inflammatory potential but not tubulogenesis. In addition to AKI, several types of ischemic organ damage, including critical limb ischemia,¹³ myocardial ischemia,¹⁴ and stroke,¹⁵ have been successfully treated by CD34-positive cell transplantation. Not all patients would benefit from cell therapy, however.¹⁶ Identifying individuals with a higher response to cell therapy would be of great interest in future trials.

A mild elevation in the patient's body temperature and transient thrombocytopenia were noted 5 and 14 days after cell therapy, respectively. We speculated that elevated cytokine levels in the sera (as shown in Table 1) were suggestive of an association with these symptoms. The secretion of IL-6, IL-8, tumor necrosis factor- α , hepatocyte growth factor, and many other factors also act as stimulatory regulators of angiogenesis,¹⁷ which appears to be beneficial for the treatment of ischemia. In fact, CD34-positive cell transplantation-related fever or thrombocytopenia occurs in 45.5% and 36.4% of patients after transplantation, respectively.¹⁸ The elevated cytokine level in relation to symptoms needs to be clarified further.

Yang et al have recently provided important clinical trial evidence of intrarenal arterial CD34-positive cell transfusion for patients with chronic kidney disease (CKD) in a randomized controlled study.¹⁹ Although kidney function had not improved after CD34-positive cell therapy in patients with CKD by 12 months, unfavorable clinical outcomes (dialysis and death) at 1 year were significantly lower in the cell therapy group compared with the control group.¹⁴ In contrast, our patient showed improvement of kidney

function during the observation period. As for the differences between the pathophysiology of AKI and CKD, inflammatory processes other than ischemia from vascular lesion were proposed as mechanisms of AKI from refractory hypertension,¹² whereas multiple factors such as immunological, metabolic, hemodynamic, and systemic interplay other than BP are also involved in CKD progression. Furthermore, intervention might be initiated at an earlier time of kidney injury in AKI than in CKD.

We found that the estimated glomerular filtration rate after hemodialysis withdrawal was 0.11 mL/min/1.73 m²/d on average from day 15 to day 44, and it improved to 0.25 mL/min/1.73 m²/d after the procedure from day 45 to day 73, suggesting that cell transplantation might have affected the recovery of kidney injury. Because the precise mechanisms against AKI remain unknown, useful clinical parameters to evaluate the efficacy of AKI treatment by cell therapy need to be further clarified.

4 | CONCLUSION

We present the first case of severe AKI treated with G-CSF-mobilized autologous peripheral blood CD34-positive cell transplantation. No severe AEs were observed during the first 23 weeks after cell therapy, and the patient's serum creatinine level continues to improve gradually. These first-in-human data are of great importance in the field of cell transplantation for AKI treatment. Because it is a single case report, this study's major limitations include a lack of generalizability to all patients with AKI from various etiologies and the impossibility of establishing a cause-and-effect relationship. Nevertheless, our data shed new light on a care regimen for AKI when no active therapy to restore injured kidneys previously existed. The accumulation of clinical and biological data is necessary to establish this cell therapy as a new therapeutic strategy for the treatment of AKI.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

H.S.: manuscript writing, provision of study material or patient, collection and assembly of data, reviewed the manuscript; T.O.: conception and design, patient recruiting and observation, CD34+ cells quality control, manuscript writing, reviewed the manuscript; T.T.: administration of CD34+ cells; M.M., K.I., H.M., Y.M.: provision of study material or patient, collection and assembly of data; S.H.: provision of study material or patient, collection and assembly of data, CD34+ cells characterization; T.S.: provision of CD34+ cells for the transplantation;

T.A.: conception and design, reviewed the manuscripts; S.K.: principal investigator, conception and design, data interpretation, final approval of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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