Original Article

Bone marrow-derived mesenchymal stem cells alleviates renal injury in severe acute pancreatitis via RhoA/Rho kinase

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Abstract: Acute pancreatitis (AP) is a common acute abdominal disease, 10%-20% of which can evolve into severe acute pancreatitis (SAP). SAP causes significant morbidity and mortality. RhoA/Rho kinase is activated in SAP. Bone marrow-derived mesenchymal stem cells (BMSCs) have been demonstrated to be a therapeutic role in SAP, but the underlying molecular mechanism is still unclear. This study was designed to determine whether RhoA/Rho kinase involved in APS, and the specific mechanism of BMSCs in APS. We validated that BMSCs could promote renal repair, reduce the ratio of wet to dry kidney weight, renal EB concentration, pancreatic edema and serum amylase, Cr, BUN and systemic TNF-α, IL-6 levels. BMSCs also reduce ROCK I and increase ZO-1 protein levels in APS, but the effects are inhibited by RhoA/Rh0 promoter CNF1. These results indicated that BMSCs can alleviate SAP rat kidney injury by inhibiting the RhoA/Rho kinase signaling pathways, increase the ZO-1 expression, reduce capillary permeability, blood capillary leakage and improve renal function.

Keywords: BMSCs, SAP, RhoA/Rho kinase, renal injury

Introduction

Severe acute pancreatitis (SAP) is characterized by acute disease, rapid progress, difficulty in treatment and the risk of multiple organ failure [1]. The incidence of renal impairment is high in SAP merged important organs damage. The mortality of SAP patients can be up to 80% when renal damage evolve into acute renal failure [2, 3]. It is demonstrated that the over-release of inflammatory factors and the destruction of endothelial barrier of capillaries play important roles in SAP [4]. Remolding of F-actin, increasing of the interval of ECs and the endothelial permeability in APS resulted in tissue and organ edema and dysfunction.

RhoA/Rho kinase (ROCK) signaling pathway is widespread expressed in human, which regulates the reconstruction of endothelial cell actin cytoskeleton. ZO-1 is an important protein closely connecting between endothelial cells and cooperated with F-actin regulating endothelial cell reconstruction. We hypothesized that capillary leakage and renal damage in SAP were regulated by RhoA/ROCK signaling.

Bone marrow-derived mesenchymal stem cells (BMSCs) inhibit the excessive inflammatory responses and repair the damaged pancreas [5]. It is reported that BMSCs can improve capillary leaks, reduce blood creatinine, urea and amylase concentration, reduce the renal tissue damage through migrating to kidney in SAP. However, the potential mechanism is remain unknown.

We hypothesized that RhoA/ROCK signaling participated in the therapeutic effects of BMSCs in SAP. This study was designed to explore the underlying molecular mechanism of BMSCs in the treatment of SAP.

Results

CNF-1 inhibited the improvement of renal injury treated with BMSCs in SAP rats

The levels of serum amylase, Cr, BUN and the ratio of wet to dry kidney weight, renal EB concentration were increased in SAP rats, BMSCs reduced them. Cytotoxic necrotizing factor 1 (CNF1), a 114 kDa protein toxin produced by
Escherichia coli, permanently activates RhoA, Rac1 and Cdc42 in intact cells [6]. Single administration of CNF1 inhibited the decrease of serum amylase, Cr, BUN and the ratio of wet to dry kidney weight and renal EB concentration (Figure 1A-E). Edema increased in SAP rats, BMSCs attenuated edema significantly, the effects were interrupted by CNF1 (Figure 1F).

*CNF-1 inhibited the decrease of inflammatory factors treated with BMSCs in SAP rats*

The levels of inflammatory factors TNF-α and IL-6 were increased in SAP rats, BMSCs reduced their levels. CNF1 interrupts the ameliorative effects and increase TNF-α and IL-6 levels (Figure 2).

*CNF-1 inhibited the decrease of ROCK I treated with BMSCs in SAP rats*

Immunohistochemistry showed that ROCK I expressed widely in kidney malpighian tube skin cells and renal tubular capillary endothelial cells. ROCK I protein level was increased in SAP rats, which was prevented by interfering with BMSCs for 24 h. CNF1 inhibited the effects of BMSCs as well. Western blot showed that BMSCs attenuated the increased ROCK I induced by SAP, but the effect was prevented by CNF1 (Figure 3).

*CNF-1 inhibited the increase of ZO-1 treated with BMSCs in SAP rats*

Immunohistochemistry showed that ZO-1 widely expressed in kidney malpighian tube skin cells and renal tubular capillary endothelial
cells. ZO-1 protein level was reduced in SAP rats, which was prevented by interfering with BMSCs for 24 h. CNF1 inhibited the decrease as well. Western blotting tests showed that BMSCs attenuated the reduced ZO-1 induced by SAP, but the effect was prevented by CNF1 (Figure 4).

Material and methods

All experiments adhered to the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication, 8th edition, 2011) and approved by the Experimental Animal Care and Use Committee of Fujian Medical University.

Rat model of SAP

Acute pancreatitis was induced by retrograde injection of 4% sodium taurocholate transduodenally into the biliopancreatic duct (1 mL/kg bodyweight) at a constant infusion rate [1]. During the injection, a clamp was used across the proximal hepatic duct.

ELISA

Commercial ELISA kits (Uscn Life Science (Houston, TX, USA) were used for the measurement of IL-6 and TNF-α in according to the manufacturer’s instructions. Briefly, the samples were collected and added into the pre-coated ELISA plate (100 μl per well). Plates were incubated at 37°C for 2 h, washed and then incubated with conjugated solution for 1 h at 37°C. Finally, the reactions were stopped with stop solution, and optical density was determined by use of a microplate reader (ELX800, BioTek, Vermont, USA) at 450 nm.

Western blot analysis

ROCK I and ZO-1 were determined with Western blot analysis. Briefly, kidney or plasma were sonicated in RIPA lysis buffer and homogenized. The debris was removed, and the supernatant was obtained by centrifugation at 4°C. Total or nuclear proteins were extracted using commercially available kits (Beyotime Biotechnology, Shanghai, China) according to the manufacturer’s protocol. Equal amounts of protein were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membrane. Blocking was made at room temperature with 5% nonfat milk powder prepared in Tris-buffered saline containing 0.1% Tween 20. Then, membranes were incubated overnight at 4°C with the primary antibodies followed by incubation with appropriate HRP-linked secondary antibody. The protein expressions were visualized by enhanced chemiluminescence (Millipore, Billerica, MA, USA). Primary antibodies of ROCK I, ZO-1 and GAPDH and secondary antibodies were purchased from Abcam (Cambridge, MA, USA).
Immunohistochemistry

The kidney was fixed in 4% paraformaldehyde for immunohistochemistry and then embedded in paraffin. Kidney was cut into 25 μm-thick sections and then were permeabilized using Triton X-100 for 10 min after deparaffinization and rehydration. The sections were washed with PBS and blocked in 10% goat serum for 1 h before incubated with anti-ZO-1 or anti-ROCK antibody overnight at 4°C. After washing for three times, sections were incubated with the secondary antibody for 1 h at room temperature. The slide was reviewed under light microscope at 40× magnification.

Statistical analysis

Differences among groups were made by One-way analysis of variance (ANOVA) or Student’s t test by using Graph-Pad Prism analysis software. All data were expressed as mean ± SME. A value of P<0.05 was considered statistically significant.

Discussion

A large number of inflammatory mediators are released on the early state of acute pancreatitis, which cause the whole blood capillary endothelial barrier damage, serious capillary leak syndrome (CLS) [4, 7], plasma protein and water leakage from blood vessels, insufficient circulating blood volume, damaged systemic viscera function. The incidence of renal damage is increased in SAP merged pancreatic function [8]. The study determined that serum amylase, Cr, BUN, IL-6, TNF-α, EB concentration in kidney were elevated in SAP. Kidney edema and increased ratio of dry to wet indicates kidney blood capillary leakage and renal function damage. The release of inflammatory mediators lead to remolded F-actin, destroyed connection between endothelial cells, expanded intercellular space, which are the important pathologic basis of blood capillary leakage [9]. But the molecular mechanisms are still unclear.

Rho is one of the Ras family, RhoA is the main isomer with GTP enzyme. Rho kinase (ROCK) is the most important downstream effector of Rho [10], it includes ROCK I and ROCK II. Rock I mainly expresses in skeletal muscle, cardiac muscle, pancreas and kidney, ROCK II mainly expresses in the nervous system. Inflammatory cytokines and angiotensin take participate in pathophysiological processes of numerous diseases by activating the RhoA/ROCK signaling pathway [11, 12]. Recently, it has reported that RhoA/ROCK was a key factor in regulating vascular endothelial permeability. Carles found it could cause higher pulmonary capillary endothelial permeability and pulmonary edema after becativated [13]. The endothelial leakage of pulmonary vascular endothelium in the SAP rats is also associated with the RhoA/ROCK signaling pathway [14]. One of the prime causes for endothelial permeability of capillaries is the
reconstruction of endothelial cell skeleton F-actin, in which intercellular connections were damaged and the intercellular space was widened. Closely link protein ZO-1 is needed to maintain the integrity of the connection between the endothelial cells, it keeps the close connection between the vascular endothelial cells by interacting with F-actin [15, 16]. These study indicated that the activated RhoA/ROCK signaling influence F-actin reconstruction via regulating the expression of ZO-1. In this study, we found ROCK I in SAP rats is higher but ZO-1 is lower than that in control rats. The level of inflammatory factors increased in SAP rats. We speculated that the decreased ZO-1 caused by activated RhoA/ROCK signaling that lead to damaged connections between cells, the intercellular space was widened and capillary permeability was increased, which resulted in blood capillary leakage and damaging the vis cerea function.

BMSCs, a class of pluripotent stem cells located in mesodermal layer, can induce differentiation into multiple tissue cells. Increasing animal and clinical experiments have determined that injected BMSCs can migrate to damaged tissues [17, 18], repair the damaged tissue and restrain inflammation [19, 20]. It reported that BMSCs could improve SAP by inhibiting inflammation and repairing damaged pancreas tissue [5, 21-23]. BMSCs can also reduce the concentration of blood creatinine, urea nitrogen and amylase and attenuate renal damage caused by SAP [24]. We hypothesize that BMSCs alleviates renal injury in SAP via RhoA/Rho kinase. In this study, we validated that BMSCs could promote renal repair, reduce the ratio of wet to dry kidney weight, renal EB concentration, pancreatic edema and serum amylase, Cr, BUN and systemic TNF-α, IL-6 level. BMSCs also reduce rock and increase zo-1 protein levels in APS, but the effects are inhibited by RhoA/Rho promoter CNF1.

In conclusion, BMSCs can alleviate SAP rat kidney injury by inhibiting the RhoA/Rho kinase signaling pathways, increase the ZO-1 expression, reduce capillary permeability, reduce blood capillary leakage and improve renal function.

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Disclosure of conflict of interest

None.

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