Role of Endothelial Progenitor Cell Transplantation in Rats With Sepsis

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ABSTRACT

Objective. This study aimed to investigate the role of endothelial progenitor cells (EPCs) in transplantation in rats with sepsis induced by endotoxins (lipopolysaccharides, LPS).

Methods. EPCs of Sprague-Dawley rats were isolated, cultured, and identified. EPCs were fluorescently labeled with the green fluorescent protein adenoviral transfection method. The rats were divided into normal control group (NC group), endothelial progenitor cell transplantation control group (EPC group), sepsis model group (LPS group), and endothelial progenitor cell transplantation treatment group (LPS+EPC group). The sepsis model was established by intravenous delivery of LPS. Two EPC transplantation groups were injected with a fluorescently labeled EPC suspension via the tail vein. One day and 7 days after transplantation, lung, liver, and kidney tissues were obtained to detect the wet/dry ratio (W/D) and perform hematoxylin and eosin (HE) staining. An ELISA assay was used to detect cytokines interleukin (IL)-6 and IL-10. Real-time polymerase chain reaction was used to detect TLR4 mRNA expression in lung, liver, and kidney tissues.

Results. EPC transplantation can significantly reduce lung, liver, and kidney tissue damage and W/D ratios in septic rats, downregulate TLR4 mRNA expression, and reduce IL-6 and IL-10 levels.

Conclusion. EPC transplantation can significantly alleviate lung, liver, and kidney tissue damage.

SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) is a waterfall-like inflammatory cytokine storm involving a variety of inflammatory mediators and effector cells that cause infection or trauma through the whole body. The SIRS caused by bacteria or highly suspicious foci is called sepsis [1]. Sepsis pathogenesis is unknown, but high morbidity and mortality are associated with sepsis, which is a major cause of death in critically ill patients [2]. In sepsis, bacterial lipopolysaccharide (LPS) and Toll-like receptors (TLRs) combine to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The NF-κB activation generates a large number of inflammatory mediators, such as tumor necrosis factor alpha (TNF-α), cytokines interleukin (IL)-6 and IL-10 through cellular signal transduction, and activated endothelial cells. These mediators cause tissue injury and dysfunction and whole organ dysfunction, as well as activate the inflammatory response, which expands the injury [3,4]. Meanwhile, an imbalance of immune regulation (a suppressed inflammation state) in late sepsis causes increased pathogen infection, leading to multiple organ dysfunction [3]. Thus, repairing endothelial injury and managing the inflammatory response regulating vascular imbalance are the keys to treating sepsis. Endothelial progenitor cells (EPCs) are a class of cells that can differentiate into mature endothelial precursor cells that are involved in blood vessel formation, the process of re-endothelialization, and repair after tissue injury. Recent studies have shown that EPCs play a role in repairing vascular endothelium and immune regulation, as well as in regulation of vascular injury and inflammation [5,6]. In this study, EPC transplantation was used to explore its role in sepsis and its potential therapeutic effects on endothelial repair and immune regulation.

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MATERIALS AND METHODS

Animal Model Preparation and Grouping

Sprague-Dawley (SD) rats with the same genetic background and weighing between 150 and 200 g were provided by the Experiment Animal Center of Inner Mongolia Medical University. Eighty healthy male inbred SD rats were randomly divided into 4 groups: normal group (n = 20), endothelial progenitor cell group (EPC group, n = 15). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Sepsis model group (LPS group, n = 20), and endothelial progenitor cell transplantation treatment group (LPS+EPC group, n = 15).

To prepare sepsis animal models, rats were fixed on an animal laboratory table and intravenously injected with 5 mg/kg LPS (Sigma, St. Louis, MO). The control group was intravenously injected with an equal amount of saline. One day and 7 days after LPS injection, intraperitoneal injections of 10% hydrated pentanal were performed for anesthesia and dissection. Lung, liver, and kidney tissues were collected and fixed with 1% paraformaldehyde.

Isolation, Culture, Identification, and Labeling of Endothelial Progenitor Cells

Mononuclear cells were obtained with a rat lymphocyte separation medium-gradient centrifugation method; 10 μL cell suspension was mixed with 90 μL of 0.4% trypan blue dye solution on a blood count plate for 10 minutes at room temperature. The number of cells stained with trypan blue was counted and expressed as a percentage of all cells in the blood count plate. Expression of surface markers CD133 and CD34 (Beijing Biosynthesis, Beijing, China) were identified by flow cytometery (Becton-Dickenson, Franklin Lakes, NJ).

Progenitor cells were inoculated into two 6-well plates with 1×10^6 cells/mL. Adenovirus with a titer of 10^{10} plaque-forming units/mL was added directly to 1 mL of medium. The medium was changed every 2 hours. Fluorescent-labeled EPCs were injected through the tail vein. Rats were anesthetized with isoflurane. An IVIS Lumina II in vivo imaging instrument (Cold Spring Harbor Biotechnology Company, Chinese Taipei) was used to perform synchronization detection of fluorescent-labeled EPCs in vitro. An inverted fluorescence microscope (Nikon, Tokyo, Japan) was used to perform fluorescent expression of tissue samples (slice thickness of 5 μm).

Pathology and W/D Ratio Identification of Animal Models

Conventional paraffin sectioning and hematoxylin-eosin (HE) staining was performed on the fixed pathological lung, liver, and kidney tissues. Five fields of each organ biopsy were randomly selected for observation. Frozen tissue sections were used to detect fluorescence-labeled EPCs.

The W/D ratio was calculated immediately after removing the left lung, liver, and kidney. After obtaining blood following rat anatomy to perform wet weight, tissues were weighed immediately after dissection to calculate the wet weight. The tissues were then placed in a drying oven at a temperature of 60°C, and dry weight measurements were obtained at 72 and 96 hours. The W/D ratio of various organs was calculated after the weight of the tissues staying still.

IL-6, IL-10, and TLR4 mRNA Detection

An enzyme-linked immunosorbent assay (ELISA) was used to detect inflammatory cytokines IL-6 and IL-10 (Boster, Wuhan, China). After dissection of the rats in each group, whole blood was immediately collected in anticoagulant tubes and centrifuged at 3000 rpm for 10 minutes at 4°C still to stratify. The supernatant was transferred to a clean EP tube and tested via ELISA.

Real-time PCR was used to detect TLR4 mRNA expression. One milliliter of Trizol reagent (TaKaRa, Shiga, Japan) was added to 2 mL of liver tissues to extract total RNA. PrimeScript reagent kit with gDNA Eraser was used to perform reverse transcription to cDNA (TaKaRa). Real-time quantitative PCR amplification was performed with the Roche LightCycler 480 system real-time PCR instrument (Roche, Berlin, Germany). A housekeeping gene of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control. The *TLR4* primer and reference gene, *GAPDH*, primer were synthesized by Beijing Liuhe Genomics Ltd. *TLR4* primer sequences were as follows: upstream 5′-AGGTTGGCACTCTCACTTCC-3′, downstream 5′-TGTAAATGGTGGCAGGGCAG-3′, the amplified product was 149 base pairs (bps). *GAPDH* primer sequence was upstream 5′-ATGAACATGGCTGTGCCTTTG-3′, downstream 5′-AGCACCCCTAACCTCTTGTGC-3′, and the amplified product was also 149 bps. PCR conditions were as follows: 95°C for 30 seconds, 95°C for 5 seconds, 60°C for 20 seconds, 72°C for 30 seconds, for 50 cycles. Four parallel wells were set for each group. Cycle threshold (Ct) values of the *TLR4* gene for each specimen extracted the Ct value of the corresponding reference gene. *GAPDH* was used to normalize expression of *TLR4*.

Statistical Analysis

All measurement data are expressed as mean ± SD. The 2 groups were compared using a Student *t* test. Comparisons of statistical data among multiple groups were performed using 1-way ANOVA analysis. All statistical calculations were performed with SPSS10.0 software.

RESULTS

Isolation, Culture, Identification, and Labeling of Endothelial Progenitor Cells

Freshly isolated mononuclear cells were small and round. Cell viability was confirmed to be 98% by trypan blue staining. Fusiform adherent cells began to appear on day 7. On day 10, fusiform adherent cells were visibly growing from the edge of the cell mass. At this time, the cell morphology became round like small rods and short shuttle-type cells appeared. Ten to 15 days after inoculation, most cells turned into short spindle-type cells, the cell volume increased, adherent cells were close to fuse together, and fusion cells formed cobblestone-like changes (Fig 1).

Identification of EPCs

Flow cytometry analysis of EPC at passage 5 showed that they expressed high levels of surface markers CDl33 and CD34, which had a double marker positive rate of 99.0% (Fig 2).

Twenty-four hours after adenovirus infection, green fluorescent protein (GFP) expression was observed and reached a peak at 36 hours. The fluorescence expression was strong, and it was sustained for 3 weeks without diminishing.