MicroRNA-143 inhibitor protective sepsis induced renal injury

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Abstract: The aim of this study is to evaluate the effects of miRNA-143 inhibitor in rats sepsis induced renal injury. In the experiment, the 27 male rats were divided into 3 groups: Sham operation (sham) group (n=9), cecal ligation and puncture group (CLP) group (n=9) and miRNA (CLP+miRNA inhibitor) group (n=9). After rat model establishment 48 h, measuring the creatine clearance (CrCl), renal blood perfusion unit, renal pathological chances and renal tubular injury score; renal tubular cell apoptosis was determined by terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) method and integrated optical density (IOD) was calculated. The protein expressions of Akt, Bcl-2 and Bax were detected by Western-blotting. The CrCl and renal blood perfusion unit had significant differences in these 3 groups (P <0.05). The CrCl and renal blood perfusion unit in the miRNA group were significantly increased compared with CLP group (P <0.05, respectively). The renal tubular damage scores of miRNA group was significantly down-regulation compared with CLP group (P <0.05). The IOD of miRNA group was significantly lower than that of CLP group (P <0.05). Compared with CLP group, The Bcl-2 and Akt protein expression were significantly stimulated, The Bax protein expression was suppressed in the miRNA group (P <0.05, respectively). In conclusion, miRNA-143 inhibitor might improve renal dysfunction and the pathological changes, reduce the tubular cell apoptosis, regulate Akt, Bcl-2 and Bax and relieve renal impairment in rat model of sepsis-induced acute kidney injury.

Key words: Sepsis; Apoptosis; miRNA-143; acute kidney injury
Introduction

Severe sepsis and septic shock are still the leading cause of death in patients with ICU, despite the rapid development of critical care medicine. The mortality rate is 20.9% - 56.8% (1). Because of the cascade effect of a large number of inflammatory cytokines in the cell pathway, conventional therapies often have little effect. Recently, the abnormal regulation of apoptosis cells and has been considered as the major causative of sepsis and the cause of death, which is often caused by severe sepsis and multiple organ dysfunction, activation of the apoptotic pathway is considered to be an important cause of early acute septic renal injury (2). Small RNA (miRNA, microRNA) is a class of about 22 (17 ~ 25) nucleotide non encoding single stranded RNA, widely exist in eukaryotes, are highly conserved, temporal and tissue-specific evolution in species. Uncontrolled expression of miRNA can lead to the destruction of the environment, and may cause disease. In the function, miRNA may induce the degradation of target mRNA and/or translation inhibition mediated post transcriptional gene silencing, regulate the expression of proteins, the regulation for the specific research on the organization of some pathological diseases and disease mechanism plays an important role. Studies have shown that miRNA plays an important role in the regulation of embryonic development, cell differentiation, tissue development, angiogenesis, and tumor (3-7). There is no report on the study of miRNA in renal disease, especially the renal injury caused by sepsis. In our study, we discussed the miRNA-143 over-expression had effects to improve sepsis induced renal injury.

Materials and methods

27 clean male Sprague Dawley (SD) rats, the body weight (200±20) g, provided by the laboratory animal center of Nanjing University School of medicine and feeding. Adaptive feeding for one week before experiment. The sepsis model was made by cecal ligation puncture (CLP) (8). The rats were randomly divided into 3 groups: Sham operation group (Sham group), cecal ligation puncture (CLP group) and cecal
ligation puncture and transfection miRNA-143 inhibitor (miRNA group). After modeling, 0.5 h rats were used to determine the success of the animal model, such as weakness of the spirit, reduction of activity, the disorder of hair color and luster, the slight stimulation of respiration, the preference of drinking water and the elimination of watery stools.

The rats of Sham group were anesthetized and then turned to open the intestine, then closed the abdomen. The CLP group and miRNA group were injected with normal saline (4 ml / kg) or miRNA-143 injection (ml / kg) after 0.5 h of CLP. All the rats in the postoperative routine injection of saline (30 ml /kg) for fluid resuscitation, after every 12 hours of CLP group and miRNA group were given saline or miRNA-143 injection 4 ml / kg intravenous drip treatment.

**Target detection and specimen retention**

**Detecting biochemical indexes**

The urine volume of 48 h rats in each group was collected after operation, Observation with pentobarbital (50 mg / kg) of the rats were anesthetized with intraperitoneal injection of brokenabdominal, exposure to the kidney, by laser Doppler flowmetry (ML191, Australia ADI) determination of renal blood flow, record blood perfusion, and the blood was collected from the abdominal aorta and renal tissue samples were collected. Measuring the creatinine clearance (CrCl) by CrCl=UV/P.

**Histopathological observation**

Using conventional 10% formalin fixed, dehydrated, paraffin embedding, sectioning and hematoxylin - eosin on renal tissue (HE) staining. The pathological results were observed under the light microscope, and the renal tubular injury score was observed. Renal tubular injury is defined as tubular degeneration, vacuolar degeneration, tubular formation, tubular necrosis and inflammatory infiltration. Standard for evaluation: 0: normal tissue; 1 points: single cell, focal necrosis; 2: renal tubular damage area is less than or equal to 25%; 3: 25 %< renal tubular damage area is less than or equal to 50%; 4: 50 %< renal tubular damage area is less than or equal
to 75%; 5 points: renal tubule damaged area > 75% (9).

**Apoptotic cell detection**

In situ end labeling (Terminal - deoxynucleoitidyl transferase mediated nick end labeling, TUNEL) method for detection of apoptosis, TUNEL Kit (Roche, Germany Mannhein company) and operate according to the kit instructions. The paraffin sections were purified by dewaxing, incubated with protease K, labeling, signal conversion and analysis.

**Relative protein expression by WB assay**

Using Western blotting method, from fresh rat kidney medulla 40 mg precipitation in the lysate of radioimmunoassay of 400 l (Radioimmunoprecipitation assay RIPA) in the homogenate, then 12000 RPM centrifugation for 15 min two times under 4 degrees, the centrifugal radius is 6 cm, removal of unruptured cells, nuclei and mitochondria. The cell supernatant was transferred to a new tube. All cell supernatants were adjusted to the same protein concentration, and twelve sodium (dodecyl sulfonate, SDS) buffer was heated at a temperature of 65 DEG C for 15 min, and kept at a temperature of. Finally SDS- polyacrylamide gel electrophoresis (polyacrylamide gel-electrophoresis, PAGE), transfer film with an anti-Akt, anti-Bax (1: 1000, the United States Santa Cruz), anti-Bcl- 2 (1: 1000, SantaCruz) and reference beta actin (beta -actin) (1: 2000, Santa Cruz) were incubated overnight finally, with two against 2 h incubation by enhanced chemiluminescence (enhanced, chemiluminescence, ECL) reagent, JY-Clear ECL chemiluminescence gel imaging analysis system for imaging.

**Statistical analysis and methods**

Statistical analysis was performed using SPSS 18 software, the quantitative data of normal distribution with mean standard deviation of that of each index were analyzed using two factor analysis of variance, a further 22 compared with SNK-q test, P < 0.05, the difference was statistically significant.

**Results**
Changes of CrCl and renal hemodynamics in rats

Compared with CLP group, the CrCl concentration of miRNA group was significantly down-regulation ($P < 0.05$); However, the blood perfusion unit (BPU) of miRNA group was significantly increased ($P < 0.05$). Meanwhile, there were no significantly differences between miRNA and Sham groups in CrCl concentration and BPU. The relative data was shown in Figure 1.

![Figure 1. CrCl and renal hemodynamics concentration of difference groups](image)

1A. The CrCl concentration in difference groups
1B. The Blood perfusion unit in difference groups

***: $P < 0.001$, compared with CLP group

Pathological changes of kidney and renal tubular injury in rats

48 h after the operation, the pathological tissue of group Sham was normal, CLP group can see obvious renal tubule degeneration, vacuolar degeneration and necrosis in renal medulla, and tube type or transparent tube with a large amount of cells infiltrating mononuclear cells, miRNA group of renal tubular vacuolization decreased, mononuclear cell infiltration was not obvious the increase, as shown in Figure 2. Compared with CLP group, the renal injury score of miRNA group was significantly decreased ($P < 0.05$), however, there were no significantly differences between miRNA and Sham groups. The data was shown in Figure 3.
Figure 2. The Pathological of difference groups

2A. Pathological of Sham group

2B. Pathological of CLP group

2C. Pathological of miRNA group

Figure 3. The renal injury scores of difference groups

***: P<0.001, compared with CLP group
Detection of apoptosis cells in renal tubules of rats

The IOD value of miRNA group was significantly difference compared with CLP group \( (P < 0.05) \), however, there were no significantly differences between Sham and miRNA groups. The results were shown that apoptosis rate of miRNA group was significantly suppressed. The data was shown in Figure 4.

![Figure 4](image)

**Figure 4.** The positive cell apoptosis of difference groups

4A. The TUNEL of Sham group  
4B. The TUNEL of CLP group  
4C. The TUNEL of miRNA group  
4D. The analysis the TUNEL of difference groups

\***: P<0.001, compared with CLP group

The relative protein expression in difference groups

The Bax protein expressions of miRNA group was significantly inhibited compared with CLP group \( (P < 0.05) \). However, Akt and Bcl-2 protein expression of
miRNA group were significantly up-regulation compared with CLP group ($P < 0.05$). There were no significantly differences between Sham and miRNA groups in Akt, Bcl-2 and Bax protein expressions. The relative data was shown in Figure 5.

Figure 5. The relative protein expression of difference groups

***: $P < 0.001$, compared with CLP group

**Discussion**

Sepsis is a systemic inflammatory response syndrome after infection, and the resulting organ dysfunction; severe sepsis leads to multiple organ dysfunction, including septic patients with acute kidney injury mortality rate increased even as high as 75% (10, 11). Although antibiotics upgrading and improvement of therapeutic strategies, including renal replacement therapy such as technology development, not only increases the cost of medical care, but did not significantly reduce the mortality of sepsis, which activate apoptosis pathway is considered to be an important cause of early septic acute kidney injury (12, 13). A large body of evidence has shown that miRNA is involved in the physiological and pathological processes of various diseases, such as ischemia and tumor. The studies also suggested that miRNAs may be involved in the regulation of angiogenesis by regulating the expression of target genes involved in angiogenesis.

In the early stage of sepsis, the hemodynamics is often high and low, with the further development of the disease (14). In this study, CLP group of early renal blood flow increased, CrCl had increased; with the passage of time, sepsis, blood flow
velocity, blood stasis, CrCl decreased rapidly, which is consistent with the actual clinical observation. However, The BPU of miRNA group was up-regulation. At present there are reported in the literature in early stage of septic acute kidney injury is not only reducing blood flow, at the same time because of waterfall effect produces a large number of cellular pathways of inflammatory cytokines and cell abnormal regulation and apoptosis, so the traditional treatment methods often have little effect (15, 16). In this study, we further study the mechanism of apoptosis of renal tubular cells by miRNA-143 inhibitor. Apoptosis is programmed cell death, which is a self-protective mechanism for the removal of necrotic and abnormal cells. TUNEL method is a commonly used method for detection of apoptosis cell, the positive cells in the CLP group increased along with the development of sepsis, and positive cells in the miRNA group after 48 h compared with the CLP group decreased significantly, and the difference was statistically significant, suggesting that apoptosis of miRNA-143 inhibitor can inhibit renal tubular cells. In order to further explore the mechanism of miRNA-143, the present study was conducted to study the early apoptosis of a series of proteins (17-19).

Akt/Bcl-2/Bax was an important signaling pathway in the process of apoptosis (20-22). The Bcl-2 family as apoptosis regulating protein, can regulate the permeability of the cell membrane, the Bcl - 2 is an anti-apoptotic protein, Bcl-2 X protein, Bax is a pro apoptotic protein. The balance of Bcl-2 and Bax in the cell determines the cell apoptosis and survival, and plays an important role in the process of cell apoptosis (23). In our present study, we found that miRNA-143 inhibitor had anti-apoptosis by regulation Akt/Bcl-2/Bax.

References


