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Protective effect of fasudil hydrochloride against acute renal injury in septicopyemia rats

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ABSTRACT

Objective: To observe the protective effect of fasudil hydrochloride against acute renal injury in septicopyemia rats.

Methods: A total of 60 Wister rats were included in the study and divided into control group (n = 10), model group (n = 25) and treatment group (n = 25). Model group and treatment group received intraperitoneal injection of endotoxin (ET) to establish acute renal injury models while the control group only received daily intraperitoneal injection of normal saline 1 mL. Five rats were taken out of model group and treatment group respectively at 1 h (T1), 6 h (T2), 12 h (T3), 24 h (T4) and 48 h (T5), for intraperitoneal injection of ET 30 mg/kg. Treatment group received intraperitoneal injection of fasudil hydrochloride 30 mg/kg 1 h before injection of ET. For three groups, 5 mL blood samples were collected from postcava for determination of serum creatinine and urea nitrogen levels at different time points. Concentrations of serum tumor necrosis factor α and ET-1 were determined by using ELISA. The renal pathologic changes were observed under the microscope.

Results: Serum creatinine levels in both model group and treatment group were significantly higher than control group at T2–T5 ($P < 0.05$) while the levels in treatment group were significantly lower than control group at T3–T5 ($P < 0.05$). At T2–T5, blood urea nitrogen levels in model group and treatment group were significantly higher than control group ($P < 0.05$) while the levels in treatment group were significantly lower than model group at T3–T5 ($P < 0.05$). Concentrations of serum tumor necrosis factor α in model group and treatment group were significantly higher than control group at T1–T5 ($P < 0.05$) while the levels in treatment group were significantly lower than model group at T1–T5 ($P < 0.05$). Serum ET-1 concentrations in model group and treatment group were significantly higher than control group at T1–T5 ($P < 0.05$) while the levels in treatment group at T1–T4 were significantly lower than model group ($P < 0.05$). Rats in control group showed no swelling or hyperemia in kidney cells but normal structure and normally arranged renal tubular epithelial cells. Obvious injury was observed in model group at T3 and renal tubular epithelial cells in disorder and at swelling condition, hyperemia and angiectasis in glomerulus, degenerative opacities and vacuolar degeneration, and maximized injury were observed at T4. Injury in renal tissue in treatment group was significantly milder than model group.

Conclusions: Fasudil hydrochloride has the significantly protective effect against acute renal injury in septicopyemia rats.

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1. Introduction

Septicopyemia is the secondary pyogenic infection after severe trauma, with high virulence of pathogenic bacteria, large diversity, fast development and severe impact on patients' life and health [1–3]. The pathogenic bacteria and toxin can spread fast to blood circulation in septicopyemia patients and further induce general infection, among which acute renal function damage is the most severe with the highest mortality rate [4–6]. Septicopyemia is one of the important reasons for causing acute renal injury; clinical researches on septicopyemia therapy are many, but reports on the concomitant acute renal injury are not like that [7]. Fasudil hydrochloride is the Rho kinase selective inhibitor with extensive pharmacological effects and significant functions of vascular dilation and anti-inflammation [8]. Researches show that fasudil hydrochloride can effectively decrease the serum endoxin (ET)-1 concentration in patients with acute renal injury, with significant protective effect on renal tissue during the course of acute renal injury [9–14]. The present study investigates the protective effect of fasudil hydrochloride against acute renal injury in septicopyemia rats. Wister rats were used to establish acute renal injury models through intraperitoneal injection of ET and fasudil hydrochloride was used for intervention treatment 1 h before modeling. The efficacy of fasudil hydrochloride on protection against acute renal injury is observed.

2. Materials and methods

2.1. Experimental animals

A total of 60 male Wister rats aged 8 weeks and weighed (220 ± 10) g were provided by Animal Experimental Center in the hospital. They had a free access to food and water, and raised at (23 ± 3) °C with 50%–55% humidity. All the animal handlings accorded with Laboratory Animal Administration Rules.

2.2. Equipments and reagents

BECKMAN SYNCHRON type fully-automatic blood biochemical analyzer was purchased from USA. Bacterial endotoxin analyzer (BET-24A) was purchased from Tianda Tianfa Technology Co., Ltd (Tianjin, China). BH-2 optical microscope was purchased from OLYMPUS Company, Japan. Fasudil hydrochloride was purchased from Chase Sun Co., Ltd (Tianjin, China) with national medicine permission number of H20040356 (2 mL:30 mg). *Escherichia coli* ET was purchased from Sigma Company, USA. ET-1 ELISA kit was purchased from Blue Gene Company.

2.3. Modeling and animal grouping

Sixty Wister rats were divided into control group (n = 10), model group (n = 25), and treatment group (n = 25). Intraperitoneal injection of ET was given to model group and treatment group to establish the acute renal injury models [15], while intraperitoneal injection of normal saline 1 mL was given to control group every day. Feeding was banned 12 h before modeling in both model group and treatment group. For these two groups, 5 rats were taken out from each group for modeling at 1 h (T1), 6 h (T2), 12 h (T3), 24 h (T4) and 48 h (T5) through intraperitoneal injection of ET 30 mg/kg. For treatment group, intraperitoneal injection of fasudil hydrochloride 30 mg/kg was given 1 h before ET injection.

2.4. Observational items

For all the groups, 5 mL blood samples were collected from postcava at each time point for determination of serum creatinine and blood urea nitrogen levels. Tumor necrosis factor (TNF)-α and ET-1 concentrations were determined by using ELISA. Renal pathologic changes in rats were observed under the microscope.

2.5. Statistical analysis

Data were expressed as mean ± SD and processed by using SPSS13.0 software. ANOVA was used for comparisons among groups while t test was for comparisons between groups. Results with $P < 0.05$ were considered as statistically significant difference.

3. Results

3.1. Comparisons of serum creatinine levels at each time point

At T2–T5, serum creatinine levels in model group and treatment group were significantly higher than control group ($P < 0.05$); while at T3–T5, the levels in treatment group were significantly lower than model group ($P < 0.05$) (Table 1).

3.2. Comparisons of serum blood urea nitrogen levels at each time point

At T2–T5, serum blood urea nitrogen levels in model group and treatment group were significantly higher than control group ($P < 0.05$); while at T3–T5, the levels in treatment group were significantly lower than model group ($P < 0.05$) (Table 2).

3.3. Comparisons of serum TNF-α concentrations at each time point

At T1–T5, serum TNF-α concentrations in model group and treatment group were significantly higher than control group ($P < 0.05$); while at T1–T5, the concentrations in treatment

Table 1
Comparisons of serum creatinine levels at each time point (mmol/L).

Groups	T1	T2	T3	T4	T5
Treatment group	64.69 ± 11.03	78.09 ± 10.54*	84.09 ± 14.17$^{*\#}$	106.06 ± 23.33$^{*\#}$	93.69 ± 22.90$^{*\#}$
Model group	67.09 ± 14.08	81.79 ± 12.49*	143.29 ± 29.59*	163.29 ± 32.50*	115.29 ± 19.56*
Control group	50.69 ± 6.21	50.69 ± 6.21	50.69 ± 6.21	50.69 ± 6.21	50.69 ± 6.21

*: $P < 0.05$ compared with control group; $^{\#}$: $P < 0.05$ compared with model group.

Table 2
Comparisons of serum blood urea nitrogen levels at each time point (mmol/L).

Groups	T1	T2	T3	T4	T5
Treatment group	11.20 ± 3.50	14.07 ± 3.93*	16.65 ± 3.95*#	19.65 ± 4.50*#	12.01 ± 3.65*#
Model group	11.34 ± 4.28	15.07 ± 2.68*	27.81 ± 5.26*	33.59 ± 8.49*	15.67 ± 3.95*
Control group	9.34 ± 4.42	9.34 ± 4.42	9.34 ± 4.42	9.34 ± 4.42	9.34 ± 4.42

*: $P < 0.05$ compared with control group; #: $P < 0.05$ compared with model group.

Table 3
Comparisons of serum TNF-α concentrations at each time point (Pg/mL).

Groups	T1	T2	T3	T4	T5
Treatment group	74.04 ± 3.95*#	93.08 ± 4.12*#	83.08 ± 3.25*#	118.00 ± 6.71*#	76.24 ± 3.75*#
Model group	95.20 ± 3.94*	164.04 ± 6.81*	183.75 ± 5.95*	158.85 ± 7.94*	99.61 ± 3.89*
Control group	51.60 ± 3.36	51.42 ± 2.97	51.26 ± 2.85	51.03 ± 3.43	52.06 ± 3.26

*: $P < 0.05$ compared with control group; #: $P < 0.05$ compared with model group.

Table 4
Comparisons of serum ET-1 concentrations at each time point (ng/mL).

Groups	T1	T2	T3	T4	T5
Treatment group	1.18 ± 0.42*#	1.28 ± 0.40*#	1.77 ± 0.22*#	1.29 ± 0.21*#	0.83 ± 0.25*
Model group	1.74 ± 0.81*	2.01 ± 0.61*	2.46 ± 0.50*	1.63 ± 0.24*	0.94 ± 0.22*
Control group	0.34 ± 0.25	0.34 ± 0.25	0.44 ± 0.15	0.44 ± 0.15	0.44 ± 0.15

*: $P < 0.05$ compared with control group; #: $P < 0.05$ compared with model group.

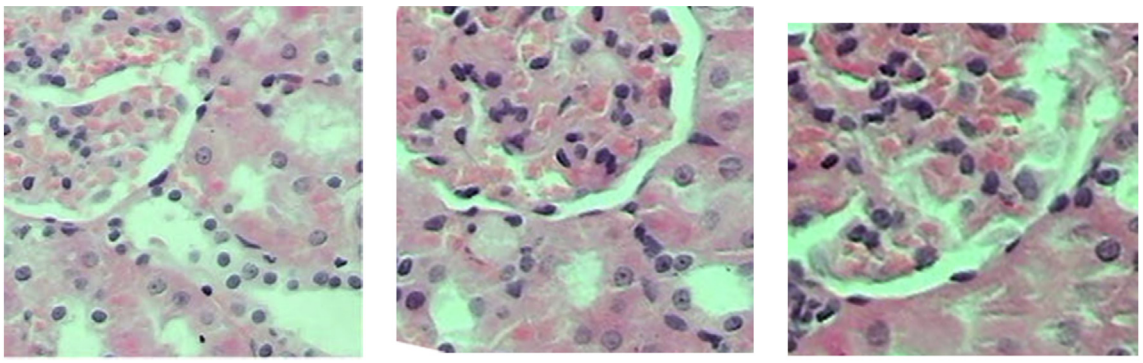

Figure 1. Histological observation of renal tissue in three groups (HE, × 200).

group were significantly lower than model group ($P < 0.05$) (Table 3).

3.4. Comparisons of serum ET-1 concentrations at each time point

At T1–T5, serum ET-1 concentrations in model group and treatment group were significantly higher than control group ($P < 0.05$); while at T1–T4, the concentrations in treatment group were significantly lower than model group ($P < 0.05$) (Table 4).

3.5. Histological observation

In the case of control group, the swelling or hyperemia in kidney cells was not observed except for the normal structure and normally arranged renal tubular epithelial cells. Rats in model group at T3 showed apparent injury and at T4 the renal tubular epithelial cells in disorder and at swelling condition, hyperemia and angiectasis in glomerulus, degenerative opacities and vacuolar degeneration, and maximized injury were revealed. Significantly milder damage in renal tissue than model group at each time point was observed in treatment group (Figure 1).

4. Discussion

Acute renal injury is the clinically common critical disease with urgent onset, fast development and extremely high mortality rate, seriously affecting the prognosis of patients [16]. Septicopyemia caused by infection is one of the important factors leading to acute renal injury [17]. There are researches showing that organism at the condition of septicopyemia can result in involvements of multiple organs, due to that G-bacterium in endotoxemia nidi can release lots of

lipopolysaccharide (LPS) which enters into blood to immediately induce acute renal injury [17–19]. Therefore, the present study establishes acute renal injury models through intraperitoneal injection of ET into Wister rats. The serum blood urea nitrogen and creatinine levels in model group at T2 were significantly higher than control group ($P < 0.05$), suggesting that intraperitoneal injection of ET is fast and efficient in establishment of acute renal injury models.

Fasudil hydrochloride, the new-type medicine with extensive pharmacological effects, is the Rho kinase inhibitor, with functions of dilating blood vessels, improving brain microcirculation, reducing tension of endothelial cells, antagonizing inflammatory factors, protecting nerves and anti-apoptosis, and accelerating nerve regeneration [8]. Researches reveal that fasudil hydrochloride can effectively decrease the serum ET-1 concentration in patients with acute renal injury, with apparent protection effect on kidneys in the course of acute renal injury [8]. In the present study, the serum creatinine and blood urea nitrogen levels in treatment group at T2–T5 were significantly lower than model group, suggesting that fasudil hydrochloride plays a protective effect on kidneys of rats with acute renal injury. TNF is called tumor necrosis factor, and mainly includes TNF-α and TNF-β; TNF-α is mainly produced by macrophages, with functions of immunoregulation, antibacterial, and anti-virus, and abnormal TNF-α concentration may cause a bad effect on organism [20,21]. TNF-α plays a key role in the pathogenic course of infectious diseases; when organisms are stimulated by LPS, *in vivo* TNF-α increase would be induced [22]. In the present study, serum TNF-α concentrations in both model group and treatment group were significantly higher than control group at each time point ($P < 0.05$), suggesting that release of large amount of LPS in endotoxemia nidi into blood makes significant increase in TNF-α concentration *in vivo* and that TNF-α participates in the pathogenic course of acute renal injury. Serum TNF-α concentrations in treatment group at T1–T5 were significantly lower than model group ($P < 0.05$), revealing that fasudil hydrochloride can effectively antagonize inflammatory factors so as to protect renal tissue. ET-1 is the important cytokine in organism, spreading all over the organism and gets involved in regulating a variety of physiological activities and inflammatory reaction though combination with its specific receptors [22,23]. In the physiological status, ET-1 is good for maintaining the stability of organism, however, in the course of endotoxemia, abnormal changes of ET-1 can cause constant destruction in organism and further leads to incidence of acute renal injury [24]. In the present study, serum ET-1 concentrations at T1–T5 in model group and treatment group were significantly higher than control group ($P < 0.05$), suggesting that ET-1 concentrations in rats with acute renal injury are significantly increased, with positive correlation property between concentration change and injury degree. Serum ET-1 concentrations at T1–T4 were significantly lower in treatment group than model group ($P < 0.05$), suggesting that fasudil hydrochloride can effectively regulate the immune response of organism, with significantly protective effect against acute renal injury. In addition, histological observation shows obvious injury in model group at T3, and the renal tubular epithelial cells in disorder and at swelling condition, hyperemia and angiectasis in glomerulus, degenerative opacities and vacuolar degeneration, and maximized injury at T4. Significantly milder injury in kidney tissue in treatment group at each time point than model group was also observed, which can also confirm that fasudil hydrochloride has a significantly protective effect against acute renal injury in septicopyemia rats.

In conclusion, fasudil hydrochloride plays a significantly preventive and therapeutic role in acute renal injury caused by septicopyemia.

Conflict of interest statement

We declare that we have no conflict of interest.

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