ORIGINAL ARTICLES

Influence of thymopentin-5 on renal pathology and relevant indexes of serum in rats with acute kidney injury caused by sepsis

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Abstract

Objective: To discuss thymopentin-5 on renal pathology and relevant indexes of serum with sepsis-caused acute kidney injury (AKI) caused by cecal ligation and puncture (CLP).
Methods: 90 cases of healthy and male SD rats were randomly divided into 3 groups: control group (C group), sepsis group (S group), thymopentin group (T group). These groups were divided into five time point including 1 h, 6 h, 12 h, 24 h and 48 h with 6 rats in each time point. The sepsis model was made by CLP. The blood and kidney tissue were collected in each time point. Changes of renal pathology were observed under light microscope and relevant indexes like serum creatinin (Cr), blood urea nitrogen (Bun), tumor necrosis factor α (TNF-α), interleukin-10 (IL-10), $CD4^+/CD8^+$ were tested and analyzed.
Results: In T group, concentrations of Cr, Bun in 6 h after CLP started to rise, reached peak in 24 h and decreased in 48 h, which were all lower than S group ($p < .05$) and higher than C group ($p < .05$). Compared with C group, concentrations of TNF-α in 1 h significantly improved, reached peak in 12 h and decreased in 24 h, which were all lower than S group ($p < .05$); and concentrations of IL-10 in 1 h significantly decreased, reached peak in 12 h and rised in 24 h, which were all more than S group ($p < .05$). In T group, $CD4^+/CD8^+$ ratio in 6 h after CLP started to decrease, reached the lowest in 24 h and rised in 48 h, which were all lower than S group ($p < .05$) and significantly lower than C group ($p < .05$).
Conclusions: Thymopentin-5 plays the role of renal protection in AKI caused by sepsis.

Key Words: Thymopentin-5, Sepsis, Acute kidney injury

Sepsis is a life-threatening condition that arises when the body's response to infection causes injury to its own tissues and organs. The fatality rate is significantly increased when the kidney is injured (acute kidney injury, AKI).[1] Early prevention and treatment of AKI caused by sepsis is of great significance. Thymopentin-5 (TP-5) has been widely used in severe burns, chronic hepatitis and other immune regulation in recent years.[2] This study was designed to establish an AKI model in rats with sepsis caused by cecal ligation and puncture (CLP), and to investigate the effect of TP-5 on inflammatory response and immune function in sepsis rats, so as to provide the basis for rational use of sepsis.[3]

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1 Material and method

1.1 Animal group

90 healthy adult male SD rats, weighing 220-250 g, were provided by the Experimental Animal Center of Inner Mongolia University. After 1 week of adaptive feeding, the rats were divided into 3 groups (random number table method). Control group: After incision of the abdominal cavity of rats, a thread was placed under the cecum, and the abdominal cavity was closed layer by layer without ligation of the cecum. After regaining consciousness, the rats were kept in cage. Sepsis group: Intraperitoneal injection of saline (1 ml) was performed after the model was completed. The next step was the same as control group. Thymopentin group: After completion of the model, intraperitoneal injection of TP-5 (batch number: 20130602, Hainan pharmaceutical company) 0.35 mg/kg,[4] diluted with physiological saline to 1 ml was performed. The next step was the same as control group.

1.2 Establishment of rat sepsis models

The operation process of the experiment strictly follows regulations for the protection of experimental animals. Rats were fasted overnight before operation but allowed water. After anesthetization by intraperitoneal injection of 10% pentobarbital sodium (3.5 ml/kg), rats were fixed on the operating table with prone position. After iodine and alcohol sterilization, a 2-cm midline incision was made in abdominal wall. The cecum was ligated below the ileocecal valve with silk suture and punctured twice with an 12-gauge needle, indwelling 1 piece with 1 cm in length and 2 mm in width through the cecum. Then the cecum was put back to the abdomen and the incision was closed layer by layer. The rats were moved to the cage after waking up and numbering.

1.3 Sample collection

Time was initiated after suturing of abdominal wall. At 1 h, 6 h, 12 h, 24 h and 48 h, 6 rats were taken respectively. Under the condition of deep anesthesia, the abdominal cavity was cut and the left kidney tissue was fixed in 10% paraformaldehyde. The intestinal canal was poked in order to expose the inferior. The blood of the inferior vena cava was collected by a single syringe at 5 ml, and the rats were killed immediately.

1.4 Index measurement

The abdominal cavity of anesthetized rats was cut open with the left kidney tissue in 10% formalin fixed for 24 h, followed by conventional gradient ethanol dehydration, transparent and embedded in paraffin sections for histological examination (HE staining and immunohistochemical staining). Blood samples were collected from the inferior vena cava at 5 ml and centrifuged at 3,000 r/min for 15 min, and the content of urea nitrogen and creatinine was measured by 722 spectrophotometer. Tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) were also determined by ELISA. After heparin anticoagulation, 1.5 ml inferior vena blood was diluted to 3 ml with saline, and then added to the lymphocyte separation by pipette. After centrifugation, the lymphocyte liquids were sucked out by a pipette and transferred to a new centrifuge tube, then diluted with physiological saline to 10 ml, centrifuged 2 times. After discarding the supernatant, 0.5 ml liquids were dropped to the test tube respectively, and $CD4^+$-PE and $CD8^+$-Percp fluorescent labeled antibodies (purchased from American Becton company, Dickinson) were added as required for quantitative detection by flow cytometry (US Becton Dickinson).

1.5 Statistical analysis

SPSS 16 statistical software was used for analysis. The measurement data were expressed by mean $\pm$ standard deviation ($\bar{x} \pm$ s), and single factor analysis of variance was used in the comparison between groups. The LSD method was adopted for comparison between two groups, with $p < .05$ was considered to be statistically significant.

2 Results

(1) Pathological changes of kidney in rats of three groups (see Figure 1): Under light microscope, the renal tissue cells of the control group showed complete structure, the tubular epithelial cells arranged in neat with no swelling and congestion. In sepsis group, damage began to occur at 12 h and peaked at 24 h. The blood vessels were dilated and congestive, the renal tubular epithelial cells were dilatated and arranged irregularly, accompanied with turbid degeneration and vacuolar degeneration. Vasodilatation and congestion was also observed at the glomerulus in thymopentin group. The renal tubular epithelial cells were swollen and arranged irregularly, but they were significantly less than those in sepsis group.

(2) Comparison of blood urea nitrogen (Bun) in three groups (see Table 1): The concentration of Bun began to increase at 6 h, reached peak at 24 h, and decreased at 48 h in sepsis group and thymopentin group, and was significantly higher than the control group ($p < .05$). Compared with the sepsis group, the concentration of Bun was significantly lower at 12 h, 24 h, 48 h ($p < .05$) in thymopentin group.

(3) The changes of serum creatinin (Cr) in three groups of rats (see Table 2): The concentration of Cr began to rise at 6 h, reached peak at 24 h, and decreased at 48 h in sepsis

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group and thymopentin group, and was significantly higher than the control group ($p < .05$). Compared with the sepsis group, the concentration of Cr was significantly lower at 12 h, 24 h, 48 h ($p < .05$) in thymopentin group.

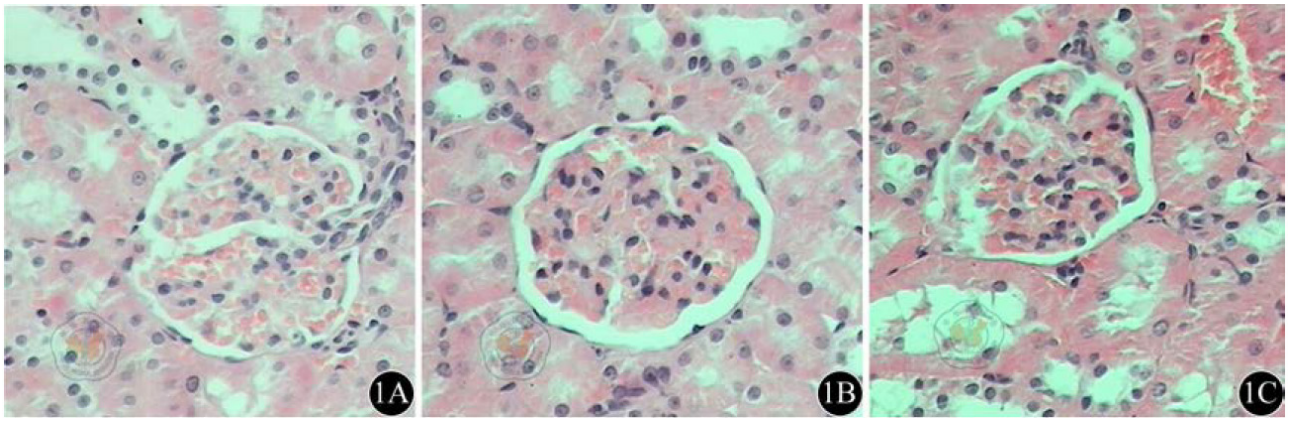

Figure 1: Pathological changes of kidney in rats of three groups (HE staining, × 200)
(1A) Control group, (1B) Sepsis group, (1C) Thymopentin group

Table 1: Changes of Bun after modeling in three groups (mmol/L, $\bar{x} \pm$ s, n = 6)

Groups	Bun				
	1 h	6 h	12 h	24 h	48 h
Control group	8.14 ±3.15	8.61 ±3.32	8.97 ±3.28	8.16 ±3.14	8.87 ±3.44
Sepsis group	11.28 ±3.21	15.65 ±3.63*	27.22 ±5.86*	32.21 ±5.24*	20.34 ±4.31*
Thymopentin group	9.32 ±3.23	12.83 ±3.56$^{*\#}$	16.24 ±4.21$^{*\#}$	19.18 ±4.24$^{*\#}$	13.17 ±3.73$^{*\#}$

Note. Compared with the control group, $^{*}p < .05$; Compared with the sepsis group, $^{\#}p < .05$

Table 2: Changes of Cr after modeling in three groups (mmol/L, $\bar{x} \pm$ s, n = 6)

Groups	Cr				
	1 h	6 h	12 h	24 h	48 h
Control group	43.24 ±6.13	44.61 ±7.22	42.97 ±6.98	45.16 ±7.14	43.87 ±7.84
Sepsis group	65.68 ±11.21	82.65 ±12.23*	141.27 ±29.86*	156.21 ±31.22*	106.34 ±21.37*
Thymopentin group	51.82 ±8.23	68.83 ±8.56$^{*\#}$	85.24 ±17.22$^{*\#}$	102.11 ±15.28$^{*\#}$	89.17 ±11.13$^{*\#}$

Note. Compared with the control group, $^{*}p < .05$; Compared with the sepsis group, $^{\#}p < .05$

Table 3: Changes of TNF-α after modeling in three groups (pg/ml, $\bar{x} \pm$ s, n = 6)

Groups	TNF-α				
	1 h	6 h	12 h	24 h	48 h
Control group	51.71 ±3.25	51.53 ±2.86	51.37 ±2.74	51.04 ±3.32	52.07 ±3.15
Sepsis group	95.31 ±3.83*	164.15 ±6.70*	183.86 ±5.84*	158.96 ±7.83*	99.72 ±3.78*
Thymopentin group	74.15 ±3.92$^{*\#}$	93.09 ±4.01$^{*\#}$	83.09 ±3.14$^{*\#}$	118.11 ±6.60$^{*\#}$	76.35 ±3.64$^{*\#}$

Note. Compared with the control group, $^{*}p < .05$; Compared with the sepsis group, $^{\#}p < .05$

(4) The changes of TNF-α in three groups of rats (see Table 3): compared with the control group, the concentration of TNF-α began to increase from 1 h in sepsis group and thymopentin group. And, concentration at each time point was significantly higher than the control group ($p < .05$). Compared with the sepsis group, the concentration of TNF-α in the thymopentin group was significantly lower at each time point ($p < .05$).

(5) The changes of IL-10 in three groups of rats (see Table 4): Compared with the control group, the concentration of IL-10 began to increase from 1 h in sepsis group and thymopentin group, significantly higher than the control group ($p < .05$). Compared with the sepsis group, the

concentration of IL-10 in the thymopentin group was significantly lower at each time point ($p < .05$).

(6) The changes of $CD4^+/CD8^+$ in three groups of rats (see Table 5): In sepsis group and thymopentin group, $CD4^+/CD8^+$ ratio began to decline, reached the lowest at 24 h, but increased at 48 h, and significantly lower than the control group ($p < .05$). Compared with sepsis group, $CD4^+/CD8^+$ ratio was significantly higher ($p < .05$) in thymopentin group at 6 h, 12 h, 24 h, 48 h.

Table 4: Changes of IL-10 after modeling in three groups (pg/ml, $\bar{x} \pm$ s, n = 6)

Groups	IL-10				
	1 h	6 h	12 h	24 h	48 h
Control group	21.23 ±4.25	21.21 ±3.63	19.24 ±3.14	20.18 ±4.13	20.35 ±4.27
Sepsis group	40.01 ±3.43*	63.96 ±5.44*	135.43 ±8.62*	99.77 ±6.31*	70.23 ±5.41*
Thymopentin group	31.68 ±3.61$^{*\#}$	45.52 ±7.20$^{*\#}$	73.37 ±9.96$^{*\#}$	64.19 ±8.43$^{*\#}$	43.22 ±4.13$^{*\#}$

Note. Compared with the control group, $^{*}p < .05$; Compared with the sepsis group, $^{\#}p < .05$

Table 5: Changes of $CD4^+/CD8^+$ after modeling in three groups ($\bar{x} \pm$ s, n = 6)

Groups	$CD4^+/CD8^+$				
	1 h	6 h	12 h	24 h	48 h
Control group	1.74 ±0.35	1.61 ±0.32	1.87 ±0.28	1.76 ±0.31	1.67 ±0.34
Sepsis group	1.62 ±0.31	1.05 ±0.33*	0.75 ±0.16*	0.61 ±0.14*	1.04 ±0.31*
Thymopentin group	1.82 ±0.23	1.43 ±0.26$^{*\#}$	1.34 ±0.21$^{*\#}$	1.21 ±0.28$^{*\#}$	1.37 ±0.23$^{*\#}$

Note. Compared with the control group, $^{*}p < .05$; Compared with the sepsis group, $^{\#}p < .05$

3 Discussion

Sepsis is a systemic inflammatory response caused by infection or suspected infection syndrome (SIRS) with a high mortality rate. In sepsis, uncontrolled inflammation, immune disorders, high metabolism and multiple organ damage occurs due to endotoxin during pathological processes of multiple organ dysfunction syndrome (MODS).[5] Sepsis complicated with AKI not only imposes greater challenge to clinical treatment but also carries with poor prognosis. In this study, the classical CLP model of AKI was applied. Meanwhile, pathological changes and the indexes of renal function were used to reveal the extent of renal injury. The results showed that kidney damage appeared at 12 h in the sepsis group, and was significant at 24 h. After CLP, the concentration of Bun and Cr began to rise at 6 h, and reached the peak at 24 h and decreased at 48 h. Our study also revealed renal pathological changes under light microscope in TP-5 group. Under light microscope, there were mononuclear macrophage infiltration, edema of interstitial tissue, vacuolar degeneration, swelling and abscission of renal tubular epithelial cells, and partial renal tubule occlusion, which were significantly less than those of the control group at the same time point. After modeling, the Bun and Cr of blood were significantly lower than those of the control group at 12 h, 24 h and 48 h.

TP-5, a short-chain polypeptide derived from the luteinizing hormone II, is also the active center of luteinizing hormone II. It has a good regulatory effect on the decreased immunological activity.[6] The imbalance between inflammatory and anti-inflammatory factors is another important factor contributes to sepsis injury. As a signal of cellular immunity and inflammation mediator, cytokines are endogenous peptides produced by many cells, and play an important role in the pathogenesis of multiple organ failure caused by shock and sepsis.[7] The effect of TNF-α ranks the first. After being affected by pathogenic factors, it is produced then at once, reaches the peak, and can stimulate other kinds of proinflammatory factors such as IL-1, IL-6, IL-8, PAF and so on. It is an important factor to cause the formation of SIRS, imposing extensive biological effect of induction of endothelial cell activation and leukocyte migration, neutrophil degranulation and fibrinolysis reaction inhibition, increasing vascular permeability and promoting thrombosis. TNF-α is also a cytokine with multiple biological activities, mainly derived from monocytes and macrophages. It is an important inflammatory promoting factor and participates in the reperfusion injury of many tissues, and plays an important role in the early stage of injury.[8] During sepsis, the release of proinflammatory factors also leads to the release of endogenous anti-inflammatory mediators, with IL-10 being one of the most typical factors. IL-10, also refers to cytokine synthesis inhibitory factor (CSIF), is a molecular weight equivalent to 18-21 kD cell factor, and is a kind of T helper cell factor 2 (Th2), produced by a variety of cells. It inhibits the inflammatory response directly or indirectly by inhibiting the production of TNF-α, IL-1, IL-12, granulocyte macrophage colony-stimulating factor (GM-CSF), chemokines and prostaglandin E2 in monocytes and macrophages. It is also able to downregulate the expression of molecules that are very important for the antigen presentation, such as class II major

histocompatibility antigen molecule (MHC-n) and adhesion molecule B7, thereby inhibiting antigen presenting functions of macrophages. IL-10 also inhibits the activation of interferon gamma on macrophages by production of nitric oxide.[9] When a large number of inflammatory mediators caused by severe tissue damage slip into the blood (waterfall release), and endogenous anti-inflammatory mediators are not enough to offset the effects of cytokines, the function of cytokines changes from protection to their own destruction, which not only does it damage tissue cells, but also attacks remote organs and eventually leads to the development of MODS.[10] In our study, the concentrations of TNF-α and IL-10 were significantly lower than those in the control group at the same time point. The pathological changes and renal function changes of TP-5 group were obviously alleviated, which played a very good role in renal protection, and the mechanism may be related to inhibiting the release of inflammatory factors and mediators.

In recent years, the $CD4^+/CD8^+$ ratio measured by flow cytometry proves to be a more effective way to measuring immunity than conventional laboratory tests. Under normal conditions, the number of T lymphocytes and their subsets in the surrounding tissues is in the status of dynamic equilibrium.[11] $CD4^+/CD8^+$ ratio is one of the most commonly used indexes to reflect the immune function in clinic. The ration is lower than normal generally attributes to the increasing levels of $CD8^+$. The results of this study suggested that sepsis could lead to marked cellular immune dysfunction. The number of $CD4^+$ T and $CD8^+$ T lymphocytes both raised in the course of the recovery of cellular immune function, while the absolute number of $CD4^+$ T lymphocytes increased more obviously, causing the ratio to rise. In this study, the recovery rate of $CD4^+/CD8^+$ in TP-5 group was significantly faster than that in sepsis group, indicating that TP-5 could promote the recovery of cellular immune dysfunction caused by sepsis.

In summary, the study of sepsis AKI model established by CLP, using the TP-5 intervention showed a good protective effect on the kidney. It may help restore normal immune function, maintain the stability of the body environment, and block the waterfall-like chain reaction. TP-5 is supposed to play a role in the mechanism of immune regulation and inhibiting the release of inflammatory factors, but the specific mechanism still needs further exploration.

Conflicts of Interest Disclosure

The authors have no conflict of interest related to this article.

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