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Effect of urinary trypsin inhibitor on inflammatory cytokines and organ function in patients with cardiopulmonary bypass

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Abstract. – OBJECTIVE: This research is to study the effect of urinary trypsin inhibitor (UTI) on inflammatory cytokines and organ function in patients with cardiopulmonary bypass.

PATIENTS AND METHODS: From February 2015 to February 2016, 40 patients that had undergone cardiopulmonary bypass surgery in our hospital were selected and randomly divided into the observation group and the control group with 20 patients in each group. Patients in the control group were intravenously injected with 5000 U/kg normal saline during the operation and 5000 U/kg·d^{-1} at 1-3 days postoperatively, while the patients in the observation group received intravenous injection of the same amount of UTI at pre-operation (T0), post-anesthesia (T1), after aortic opening (T2), after cardiopulmonary bypass 4h (T3), 8h (T4), 24h (T5), 48h (T6), and 72h (T7). We detected tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) levels in each group, and compared the pre and post-operative alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB) and creatinine level in the two groups of patients.

RESULTS: At the time T3, T4, T5, T6, and T7, TNF-α, IL-1β, IL-6, and IL-8 water in the observation group were significantly lower than those in the control group; the difference was statistically significant ($p < 0.05$). The 24 h postoperative ALT, AST, and TB of two groups were significantly higher than those pre-operatives ($p < 0.05$). The ALT and AST levels in the observation group were significantly lower than those in the control group after 24 h postoperative ($p < 0.05$). The 24 h postoperative TB DB of the two groups had not statistically significant differences ($p > 0.05$). At 24 h postoperative creatinine levels in the two groups were significantly lower than those before operation ($p < 0.05$), and there was no significant difference between the two groups ($p > 0.05$). In the observation group, the duration of ventilation and ICU hospitalization time were significantly lower than that in the control group, and the difference was statistically significant ($p < 0.05$).

CONCLUSIONS: UTI can effectively regulate the inflammatory cytokines and provide protection for organ function during cardiopulmonary bypass surgery, which is conducive to promote the recovery of patients.

Key Words:
Urinary trypsin inhibitor (UTI), Cardiopulmonary bypass, Cardiac surgery, Inflammatory factor.

Introduction

In the case of cardiopulmonary bypass, the heart surgery often leads to an abnormal increase of endotoxin[1]. The increase of endotoxin not only causes damage to the tissue cells of patients, but leads to systemic inflammatory response syndrome (SIRS), further results in failure of the organ function[2]. Therefore, it is of great clinical significance to find an effective method to reduce the damages of patients with cardiopulmonary bypass. Research shows that the main reason for the increase of circulating endotoxin levels *in vitro* is caused by intestinal or organ ischemia, intestinal endotoxin translocation, etc. due to a decrease of the circulation[3]. Urinary trypsin inhibitor (UTI), as a broad spectrum protease inhibitor, was used to provide organ protection for patients with severe acute pancreatitis in the past and excellent therapeutic effect; however, whether it was effective in patients with cardiopulmonary bypass surgery has never been reported[4]. This study was designed to provide an appropriate therapeutic program for clinical treatment by analyzing the inflammatory cytokines and organ

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function in patients with cardiopulmonary bypass surgery with UTI.

Patients and Methods

Patients

From February 2015 to February 2016, 40 cases of patients who had gone the cardiopulmonary bypass surgery in our hospital were selected in this study.

Inclusion criteria[5]: All patients with rheumatic heart disease were treated with artificial heart valve replacement. Patients and their families signed the informed consent.

Exclusion criteria: (1) Patients had valve replacement operation before admission; (2) Patients had autoimmune diseases, the chronic diseases of the digestive system and the systemic infectious diseases.

The patients were randomly divided into the observation group and the control group with 20 cases in each group. There were 12 males and 8 females in the observation group, aged from 46 to 71 years old with an average age of (53.4 ± 5.2) years old with the left ventricular ejection fraction (61.1 ± 6.2)%. In the control group, there were 13 males and 7 females, aged from 45 to 70 years old, mean age (53.2 ± 5.1) years old with the left ventricular ejection fraction (62.3 ± 6.6)% before the operation. The age, gender, preoperative cardiac function and other basic data of the two groups of patients were compared with no difference ($p > 0.05$). The study was approved by the hospital Ethics Committee.

Research Methods

All patients who were given the conventional thoracotomy and the cardiopulmonary bypass surgery were operated with moderate hypothermia and moderate blood dilution condition. All patients were admitted to ICU after the operation. Patients in the control group were intravenously injected with 5000 U/kg of normal saline before cardiopulmonary bypass during the operation, and continued intravenous injection of 5000 U/kg normal saline for 3 days after the operation. In the observation group spontaneously, the intravenous infusion of the same amount of UTI (Guangdong Tianpu Biochem Ltd., Co. Guangdong, Guangzhou, China) was injected. In addition, we extracted 2 ml peripheral blood of each patient at pre-operation (T_0), post-anesthesia (T_1), aortic opening (T_2), 4 h after cardiopulmonary bypass (T_3), 8 h (T_4), 24 h (T_5), 48 h (T_6) and 72 h (T_7) for testing the indicators.

Observation Indicators

The comparison of the levels of tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) in the two groups of patients was made at different time points. The test method was detected by radioimmunoassay (Beijing Kemeidongya Bio-Tech Ltd., Co. Beijing, China); then, we compared the pre- and post-operative liver and renal function index, in which the liver index included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB), renal index including creatinine. Besides, we compared the ventilator-assisted time and ICU hospital stay of the two groups of patients.

Statistical Analysis

All data were analyzed by the SPSS20.0 statistical software (SPSS Inc., Chicago, IL, USA). *Chi-square* test was used to compare the data. The measurement data were expressed as mean plus or minus (s); the data between the two groups were compared by the *t*-test. $p < 0.05$ was considered statistically significant.

Results

Comparison of the Levels of Inflammatory Cytokines in the Two Groups of Patients at Different Time Points

In the observation group at the time T_3, T_4, T_5, T_6, and T_7, the levels of TNF-α, IL-1β, IL-6, and IL-8 water were significantly lower than those of the control group, and the difference was statistically significant ($p < 0.05$), as shown in Table I.

Comparison of Liver Function Indicators of Two Groups at 24h Pre-operation and Post-operation

There was no significant difference between the two groups before the operation ($p > 0.05$); at the 24 h postoperatively, ALT, AST and TB were significantly higher than those pre-operation ($p < 0.05$), and the levels of ALT and AST in the observation group were significantly lower than those in the control group ($p <$

Table I. Comparison of the levels of inflammatory cytokines in two groups of patients at different time points (n=20).

Index	Group	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
TNF-α (ng/ml)	Observation group	1.14 ± 0.20	1.88 ± 0.09	1.92 ± 0.09	1.76 ± 0.11[a]	1.44 ± 0.17[a]	1.37 ± 0.21[a]	1.08 ± 0.11[a]	1.01 ± 0.28
	Control group	1.10 ± 0.17	1.86 ± 0.08	1.78 ± 0.21	1.91 ± 0.12	1.82 ± 0.11	1.65 ± 0.12	1.52 ± 0.32	1.23 ± 0.26
IL-1β (ng/ml)	Observation group	0.19 ± 0.03	0.22 ± 0.11	0.32 ± 0.05	0.28 ± 0.02[a]	0.25 ± 0.02[a]	0.21 ± 0.02[a]	0.19 ±0.02[a]	0.18 ± 0.02[a]
	Control group	0.18 ± 0.03	0.24 ± 0.07	0.33 ± 0.04	0.32 ± 0.04	0.30 ± 0.04	0.27 ± 0.03	0.26 ± 0.03	0.24 ± 0.04
IL-6 (pg/ml)	Observation group	132.12 ± 3.55	134.42 ± 9.33	132.18 ±15.73	159.32 ± 9.11[a]	142.37 ± 8.14[a]	119.44 ± 8.23[a]	107.48 ± 7.83[a]	105.22 ± 4.18[a]
	Control group	133.44 ± 4.11	132.74 ± 11.42	132.38 ± 13.88	167.22 ± 4.72	152.83 ± 8.32	140.38 ± 10.22	132.07 ± 7.44	125.38 ± 7.39
IL-8 (ng/ml)	Observation group	0.16 ± 0.01	0.15 ± 0.02	0.17 ± 0.04	0.22 ± 0.01[a]	0.20 ± 0.01[a]	0.19 ± 0.02[a]	0.16 ± 0.03[a]	0.16 ± 0.03[a]
	Control group	0.16 ± 0.01	0.16 ± 0.03	0.18 ± 0.03	0.25 ± 0.03	0.24 ± 0.02	0.23 ± 0.02	0.21 ± 0.01	0.20 ± 0.01

Note: Comparison with the control group, [a]$p < 0.05$.

0.05). The comparison of DB and TB at 24 h post-operation showed no statistical difference ($p > 0.05$) (Table II).

Comparison of Creatinine Levels between the Two Groups at 24h Pre-operation and Post-operation

The creatinine level at 24 h after operation was significantly lower than that pre-operation ($p < 0.05$), but there was no significant difference between the two groups (Table II).

Comparison of the Ventilator Assisted Time and ICU Hospital Stay Time of Two Groups of Patients

In the observation group, the duration of ventilation and ICU hospitalization time were significantly lower than those in the control group, the difference was statistically significant ($p < 0.05$), as shown in Table III.

Discussion

The patients with cardiopulmonary bypass heart surgery usually suffered by endotoxin translocation and triggering of endotoxin due to low tissue perfusion, organ and intestinal ischemia and hypoxia[6]. Endotoxin can not only cause increased tissue damage, but further trigger cause the release of inflammatory factors, resulting in the systemic inflammatory response syndrome (SIRS), and eventually it cause even the occurrence of multiple organ dysfunction syndrome[7-10]. At present, there has been no effective protective and preventive plan for systemic inflammatory response syndrome. The main treatment is the functional replacement of the organ failure, but the clinical effect is limited[11,12]. If there is an effective treatment of systemic inflammatory response syndrome, it can effectively inhibit the release of inflammatory factors in patients after cardiopulmonary bypass surgery, which is of great significance in the prevention of multiple organ dysfunction syndrome[13]. UTI, as a broad spectrum protease inhibitor, is used to protect the organs of patients with severe acute pancreatitis and has a good therapeutic effect. Some studies suggested that UTI has the function of scavenging oxygen free radicals and stable cell membrane and lysosomal membrane, and it can also inhibit the release of inflammatory mediators[14].

TNF-α is mainly composed of monocytes and macrophages, which can produce a single nuclear

Table II. Comparison of the two groups of preoperative and postoperative 24h liver and kidney function indexes (n = 20).

Group	ALT (U/L)		AST (U/L)		TB (mmol/L)		DB (mmol/L)		Creatinine (mg/dL)	
	Preoperative	Postoperative 24h	Preoperative	Postoperative 24h	Preoperative	Postoperative 24h	Preoperative	Postoperative 24h	Preoperative	Postoperative 24h
Observation group	20.48 ± 7.12	55.43 ± 15.29[a]	21.45 ± 8.15	27.29 ± 6.25[a]	16.38 ± 6.49	34.26 ± 9.51[a]	7.57 ± 4.23	7.45 ± 4.37	107.42 ± 10.38	87.29 ± 14.39[a]
Control grup	20.66 ± 7.49	71.49 ± 16.18[a]	21.38 ± 8.32	38.14 ± 11.24[a]	16.22 ± 6.53	35.11 ± 10.42[a]	7.62 ± 4.19	7.62 ± 4.19	106.83 ± 10.51	96.38 ± 22.42[a]
t-value0.079	3.026	0.027	3.173	0.078	0.269	0.038	0.096	0.179	1.526	
p-value0.938	0.003	0.979	0.001	0.939	0.789	0.970	0.924	0.859	0.135	

Note: Comparison with pre-operation, [a]$p < 0.05$.

factor and the center of the inflammatory reaction and SIRS. Studies showed that TNF-α can further stimulate the release of other cytokines, such as interleukin and adhesion factor, etc.[15], in which IL-1 β, IL-6, and IL-8 were important cytokines in this process. TNF-α can stimulate the white blood cells to release IL-1β, IL-6, and IL-8, and generate prostaglandins, leukotrienes, platelet activating factor, etc., and causing cascade reaction followed in SIRS[16]. In this study, we selected patients with cardiopulmonary bypass surgery for the control study. The results showed that at T_4, T_5, T_3, T_6, and T_7, TNF-α, IL-1β, IL-6, and IL-8 in the observation group were significantly lower than those in the control group, which conformed to the study of Hao et al[16]. It indicated that UTI can effectively regulate inflammatory cytokines and reduce the damage of inflammatory factors in patients with cardiopulmonary bypass surgery[17,18].

The pre and postoperative ALT and AST levels were compared between the two groups of patients. ALT and AST were important indicators of liver function. When the liver cells were impaired and the liver function was reduced, the plasma ALT and AST could be elevated and their levels could reflect the degree of liver function damage[19]. Results of the study showed that the ALT and AST levels in the observation group were significantly lower than those in the control group after 24h post-operation, which was consistent with the research reported by Wang et al[20-22], indicating that UTI had protective effect on liver function postoperatively. To dig up, we believe that the main reasons are as following: (1) reduce the degree of inflammatory reaction, inhibit the secretion and release of inflammatory mediators; (2) inhibit the release of lysosomal enzymes and also stabilize finger films to prevent the fiber adhesion proteins, elastin and collagen and other structures from being destroyed, thus, providing a protective effect for the patients with intercellular matrix, intercellular matrix and endothelial cells; (3) prevent the production of oxygen free radicals so as to alleviate the damage caused by neutrophils to the liver.

In addition, there was no difference of creatinine level between the two groups 24h pre and post-operation. It suggested that the degree of renal damage in patients with cardiopulmonary bypass was not significant, perhaps because of the low sensitivity of serum creatinine. Besides, the results in this study also showed that the duration of ventilation and ICU stay were significantly lower in the observation group than

Table III. Comparison of the two groups of patients with ventilator assisted time and ICU hospital stay.

Group	Number	Ventilator assisted time (h)	ICU stay (d)
Observation group	20	8.2 ± 4.3	2.6 ± 0.5
Control group	20	12.7 ± 5.2	3.4 ± 0.8
t-value	–	2.982	3.292
p-value	–	0.005	0.001

those in the control group. The reason is that UTI can effectively improve the level of inflammatory cytokines in patients after cardiopulmonary bypass surgery and provide protection to organ function, and further promote the early recovery of patients.

Conclusions

UTI, as used in cardiopulmonary bypass surgery, can effectively inhibit the production and release inflammatory cytokines while preventing the organ function of patients from damage at a certain level, which is conducive to promote early postoperative rehabilitation of patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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