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Combined Treatment of Ulinastatin and Tranexamic Acid Provides Beneficial Effects by Inhibiting Inflammatory and Fibrinolytic Response in Patients Undergoing Heart Valve Replacement Surgery

Ting-ting Chen, MD,[1] Jiandong-Liu, MM,[2] Gang Wang, MD,[1] Sheng-li Jiang, MD,[1] Li-bing Li, MD,[1] Chang-qing Gao, MD[1]

[1]Department of Cardiovascular Surgery, Chinese PLA General Hospital, Beijing, China; [2]The 175th Hospital of PLA (Affiliated Dongnan Hospital of Xiamen University), Department of Anesthesiology, Zhangzhou, China

ABSTRACT

Objective: To investigate the effect of ulinastatin and tranexamic acid administered alone or in combination on inflammatory cytokines and fibrinolytic system in patients undergoing heart valve replacement surgery during cardiopulmonary bypass (CPB).

Background: CPB-induced fibrinolytic hyperfunction and systemic inflammatory response syndrome (SIRS) are the leading causes responsible for the occurrence of postsurgical complications such as postsurgical cardiac insufficiency and lung injury, which may lead to an increase in postsurgical bleeding, prolongation of hospital stay, and increased costs.

Methods: One hundred twenty patients undergoing heart valve replacement surgery during CPB were randomly assigned into 4 groups of 30 patients each: blank control group (Group C), tranexamic acid group (Group T), ulinastatin group (Group U), and tranexamic acid–ulinastatin combination group (Group D). Physiological saline, tranexamic acid, ulinastatin, and a combination of tranexamic acid and ulinastatin were given to each group, respectively. Arterial blood was collected from the radial artery at 4 time points: after induction of anesthesia (T1), unclamping the ascending aorta (T2), and at 1 hour (T3) and 24 hours (T4) after CPB. The levels of plasma tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), neutrophil elastase (NE), and the concentrations of tissue plasminogen activator (t-PA) and α2-antiplasmin (α2-AP) were detected. The changes in the volume of pericardial mediastinal drainage after surgery were observed and recorded.

Results: The plasma TNF-α, IL-6, and NE levels significantly increased in patients from all 4 groups at time points of T2, T3, and T4 in comparison to those before CPB ($P < .05$), and the plasma TNF-α and IL-6 levels in groups U and D were significantly lower than those in the other 2 groups ($P < .05$). The plasma t-PA, α2-AP, and D-dimer concentrations significantly increased in patients from all 4 groups at T2 and T3 compared with those before CPB ($P < .05$), and the plasma t-PA and D-dimer concentrations were significantly lower in groups T and D than those in groups U and C ($P < .05$) at T2 and T3. The plasma α2-AP concentrations in groups T and D were significantly higher than those in Group C at T3 ($P < .05$). The volumes of pericardial mediastinal drainage per body surface area were significantly lower in groups T and D than those in Group C 6 hours after the surgery ($P < .05$).

Conclusions: Ulinastatin inhibits the release of inflammatory medium and reduces the inflammatory response during CPB. Tranexamic acid can effectively inhibit the fibrinolytic hyperfunction caused by CPB and thus decreases postsurgical bleeding. In addition, it exhibits a minor anti-inflammatory response. As a consequence, a combined treatment of ulinastatin and tranexamic acid reduces postsurgical bleeding and shortens postoperative hospital stay in patients undergoing heart valve replacement surgery.

INTRODUCTION

Cardiopulmonary bypass (CPB) is a prerequisite to performing open heart surgery. Multiple physical, chemical, and biological stimuli in CPB may cause activation of the complement system and the fibrinolytic system and aggregation of neutrophils and thrombocytes, along with release of oxygen free radicals, various proteases, and inflammatory media inducing the production of a large number of inflammatory cytokines that cause systemic inflammatory response syndrome (SIRS) [Wan 1997; Massoudy 2001]. CPB-induced fibrinolytic hyperfunction and SIRS are the leading causes responsible for the occurrence of postsurgical complications such as postsurgical cardiac insufficiency and lung injury, which may lead to an increase in postsurgical bleeding, prolongation of hospital stay, and inceased costs [Endo 1993; Hirose 1998; Aosasa 2001; Nakatani 2001]. Therefore, development of a strategy to effectively inhibit the activation of fibrinolysis, alleviate inflammation, and reduce postsurgical bleeding is a hot topic of research in heart surgery during the peri-CPB period.

For a long time, aprotinin was widely used to inhibit fibrinolysis in clinical practice, which not only decreased bleeding, but also reduced the need for blood transfusion during

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Correspondence: Gang Wang, Department of Cardiovascular Surgery, Chinese PLA General Hospital, Beijing Fuxing Road 28, China, 100853; 010-66938136 (email: beijing-baoding@sohu.com).

CPB in addition to alleviating the inflammation caused by CPB [Nakahama 1996; Aihara 1998; Cao 2000]. However, the high cost and potential adverse effects of the drug, such as allergic reaction, have caused the use of aprotinin to be banned in some European countries; use of the drug has also been terminated by the State Food and Drug Administration of China since 17 December 2007 [Hirose 1998]. Therefore, a search for a safe and effective alternative to aprotinin in the post-Aprotinin era has been given high priority in clinical studies. Since then, protease inhibitors and antifibrinolytic drugs are now widely used.

Ulinastatin is a broad spectrum protease inhibitor that not only exhibits inhibitory effects against multiple proteases and hydrolytic enzyme, but also inhibits the release of the inflammatory medium [Sablotzki 2002; Santos 2007; Karkouti 2010]. Ulinastatin has been widely used for treatment of pancreatitis and circulatory failure in clinical practice and has recently been applied in cardiac surgery with CPB [Sato 2000; Giomarelli 2003]. Studies have demonstrated that the drug could effectively inhibit the release of a series of enzymes and inflammatory media induced by CPB and protect important organs such as the liver, kidney, lung, and myocardium [Ren 2006; Bingyang 2007].

Tranexamic acid, an antifibrinolytic drug that reduces bleeding, is an amino acid compound that shows competitive inhibition on plasmin by blocking the binding of fibrinogen and fibrin to the lysine on thrombocyte receptors and protects thrombocytes through reducing the degradation of membrane glycoprotein receptors on thrombocytes by plasmin [Sugita 2002; Nakanishi 2006]. This results in a decrease in postsurgical bleeding and the need for blood transfusion, reduces cost, and increases safety and effectiveness, thereby making the drug widely recognized by clinicians for application in CPB surgery.

It is hypothesized that combined treatment of tranexamic acid and ulinastatin may effectively inhibit fibrinolytic hyperfunction and the inflammatory response. Therefore, the present study was undertaken to investigate the effect of ulinastatin and tranexamic acid administered alone or as a combined treatment on inflammatory cytokines and fibrinolytic indexes in valve replacement surgery during the peri-CPB period by comparing the effects on postsurgical bleeding volumes.

MATERIALS AND METHODS

Selection of Subjects

A total of 120 cases of patients with rheumatic heart disease and New York Heart Association (NYHA) cardiac function of Grade II and III undergoing open heart valve replacement surgery were involved in the present study (54 men and 66 women, ages 33 to 72 years). Single valve replacement was performed in 88 cases, and double valve replacement was performed in 32 cases. The exclusion criteria were 1) Age greater than 80 years; 2) re-operation; 3) use of hormone and antibiotics 1 week prior to the surgery; 4) preoperative examinations that revealed severe coagulation abnormalities such as significant prolongation of prothrombin time and significant reduction in thrombocytes; 5) severe liver and renal failure; 6) detection of pericardial adhesions during surgery; 7) receipt of treatment with recombinant human coagulation factor VII during and after surgery.

Groups

The subjects were randomly assigned into 4 groups using the random number method (computer randomization): the blank control (Group C), tranexamic acid group (Group T), ulinastatin group (Group U), and tranexamic acid–ulinastatin combination group (Group D), with 30 patients in each group. The set of surgeons in each group were the same to rule out operator bias. The patients in Group T were intravenously injected with tranexamic acid (tranexamic acid sodium chloride injection, Beijing Join Chain Medicine Co., Ltd., 1 g/100 mL) at a dose of 15 mg/kg body weight prior to the surgery and then maintained by intravenous infusion of tranexamic acid at a dose of 15 mg/kg body weight per hour until the termination of the surgery. After induction of anesthesia, the patients in Group U were intravenously injected with half of ulinastatin (ulinastatin for injection, Guangdong Techpool Bio-Pharma Co., Ltd., 100,000 U) at a dose of 1.2×10^4 U/kg body weight via the internal jugular vein prior to the surgery; another half was added into the CPB priming solution. The cases in Group D were intravenously administered with tranexamic acid and ulinastatin at the aforementioned doses, and the same volume of physiological saline was given to patients in the control group.

This study was approved by the Ethics Review Committee of the General Hospital of the Chinese People's Liberation Army. Informed consent was obtained from all participants following a detailed description of the purpose and potential benefits of the study.

Anesthesia and CPB

All patients underwent routine fasting for 8 hours before surgery and were intramuscularly injected with 10 mg morphine and 0.3 mg scopolamine 30 minutes before surgery. Electrocardiography (ECG), arterial blood oxygen saturation (SpO_2), and non-invasive blood pressure (NBP) were monitored once the patients were admitted to the operating room. The venous pathway was opened, and continuous monitoring of arterial pressure was done following radial artery puncture and tube implantation under local anesthesia. The patients were intravenously anesthetized with midazolam at a dose of 0.03 to 0.05 mg/kg body weight, etomidate at a dose of 0.3 mg/kg body weight, sufentanil at a dose of 0.6 to 1 μg/kg body weight, and pipecuronium bromide at a dose of 0.15 mg/kg body weight. Following tracheal intubation, mechanical ventilation with pure oxygen was performed; the respiratory rate was 10 to 12 times/minute, the tidal volume was 8 to 10 mL/kg, and the end-tidal carbon dioxide (PETCO2) was sustained at 35 to 45 mmHg. The double-lumen central venous catheters and 6-lumen Swan-Ganz catheters were implanted into the right internal jugular vein in parallel, which were connected with the Edwards Vigilance continuous cardiac output (CCO)/mixed venous oxygen saturation (SvO2) monitoring system (Edwards Lifesciences Corporation, Irvine, CA, USA) to continuously measure the hemodynamics and

Table 1. Comparison of General Clinical Characteristics of Patients in the 4 Groups

Index	Group C	Group T	Group U	Group D
Age, y	50.4 ± 10.0	50.4 ± 9.7	49.8 ± 10.8	49.5 ± 11.5
Gender (male/female)	12/18	12/18	14/16	16/14
Body weight, kg	58.3 ± 10.0	58.2 ± 9.5	57.1 ± 8.3	57.8 ± 8.9
Height, cm	161.3 ± 6.9	161.1 ± 7.1	161.8 ± 8.0	162.9 ± 7.2
Body surface area, m^2	1.61 ± 0.2	1.6 ± 0.2	1.6 ±0.2	1.6 ± 0.1
Type of valve replacement (single/double valve)	22/8	20/10	24/6	20/10
Duration of operation, min	207.0 ± 24.3	216.0 ± 28.0	214.0 ± 28.5	212.0 ± 24.6
Duration during bypass, min	99.5 ± 22.6	103.5 ± 22.5	102.5 ± 20.7	105.9 ± 20.2
Duration in blockage, min	69.0 ± 20.4	72.4 ± 21.0	74.7 ± 20.5	80.1 ± 19.6
Duration of removal of tracheal catheter, h	16.1 ± 3.5	16.5 ± 3.1	14.4 ± 4.1	13.8 ± 4.2
Length of stay in intensive care unit, d	5(2)	5(2)	5(2)	4.3 ± 1.2*

*P < .05 vs. Group C.

changes in oxygen metabolism. Total intravenous anesthesia was performed. Sufentanil was intravenously injected on an intermittent basis, maintained by pump infusion of propofol at a dose of 5 mg/kg body weight per hour, followed by pipecuronium bromide for continued muscle relaxation. Vasoactive drugs such as atropine, dopamine, and phenylephrine were used during surgery to regulate the changes in heart rate and blood pressure.

Extracorporeal circulation was instituted with the membrane oxygenator, primed mainly with Lactated Ringer's solution, Voluven injection (Fresenius Kabi AG, Richmond Hill, ON, CA), human serum albumin, 5% $NaHCO_3$, and 20% mannitol injection. Heparin at a dose of 3 mg/kg body weight was intravenously injected before bypass to make activated clotting time (ACT) longer than 480 seconds. The high-potassium oxygenated cold blood cardioplegia (4:1) was intermittently perfused through the aortic root for myocardial protection. The blood was moderately diluted, after which the pH value of arterial blood was sustained between 7.35 and 7.45, the perfusion flow was maintained at a range of 60 to 70 mL/kg body weight per minute, the arterial pressure was maintained between 60 and 90 mmHg, and the nasopharyngeal temperature was sustained between 28°C and 32°C. After cardiac resuscitation and vena cava opening, dopamine at a dose of 3 to 10 μL/kg per minute was used for the assistance of circulation stability. Protamine was used after the termination of CPB.

Sample Collection and Determination

Collection of 5.4 mL of arterial blood via the radial artery occurred after the induction of anesthesia (T1), at the time of unclamping the ascending aorta (T2), 1 hour after termination of CPB (T3), and 24 hours after termination of CPB. After the hematocrit (HCT) was measured, the blood was transferred into 2 anticoagulant tubes containing 0.3 mL of sodium citrate. The D-dimer concentration in 1 tube of blood was determined using the immune turbidimetric test (determined by the clinical laboratory of the General Hospital of Chinese People's Liberation Army). The blood in the other tube was centrifuged at 3000 r/min for 10 minutes. The upper plasma was collected and stored at –20°C. The plasma tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and neutrophil elastase (NE), as well as the concentrations of fibrinolytic indexes including tissue plasminogen activator (t-PA) and α2-antiplasmin (α2-AP), were detected with the enzyme-linked immunosorbent assay (ELISA) (R&D Systems; detection by the Sun Biomedical Technology (Beijing) Co., Ltd.). The changes in postsurgical pericardial mediastinal drainage were observed and recorded. Considering that the blood dilution continuously changed during CPB, all data were corrected to eliminate the effect of blood dilution on the determination value using the following formula: corrected value = real measured value (presurgical HCT/the HCT when sampling).

Statistical Analysis

All statistical analyses were performed using the statistical software SPSS version 17.0 (IBM Corp, Armonk, NY, USA). All measurement data were tested for normality; the normally distributed data were expressed as mean ± standard deviation (SD), and the non-normally distributed data were described as median (quartile interval). If the statistical variables exhibited normal distribution and homogeneity of variance, comparisons within groups were done using the analysis of variance (ANOVA) for randomized block design and completely random design; the differences between groups were tested for statistical significance with the ANOVA for completely random design. When the data were non-normally distributed or present with heterogeneity of variance, a nonparametric test (rank sum test) was used to compare the differences. A P value < .05 was considered statistically significant.

RESULTS

Clinical Data of Patients

There were no significant differences in age, sex, height, body weight, body surface area, type of surgery (single/double valve, position of valve replacement), duration of operation, duration

Table 2. Plasma Tumor Necrosis Factor (TNF)-α Levels in Patients from the 4 Groups at Different Time Points (pg/mL)

Group	T1 (before induction of anesthesia)	T2 (unclamping the ascending aorta)	T3 (1 hour after cardiopulmonary bypass)	T4 (24 hours after cardiopulmonary bypass)
C	2.40 ± 0.71	4.96 ± 0.81*	19.95 ± 4.79*	6.66 ± 1.71*
T	2.46 ± 0.71	4.74 ± 0.85*	14.98 ± 3.86*†	6.43 ± 1.67*
U	2.49 ± 0.68	3.92 ± 0.85*†	7.98 ± 1.94*†	4.22 ± 1.23*
D	2.42 ± 0.77	3.82 ± 0.78*†	7.56 ± 1.91*†	4.05 ± 1.14*

*P < .05 vs. T1; †P < .05 vs. Group C.

of bypass, duration of blockage, and duration of removal of tracheal catheter in patients among the 4 groups (P > .05). The length of stay in the intensive care unit (ICU) in Group D was shorter than that in Group C (P < .05), but there was no significant difference among groups T, U, and C (P > .05) (Table 1).

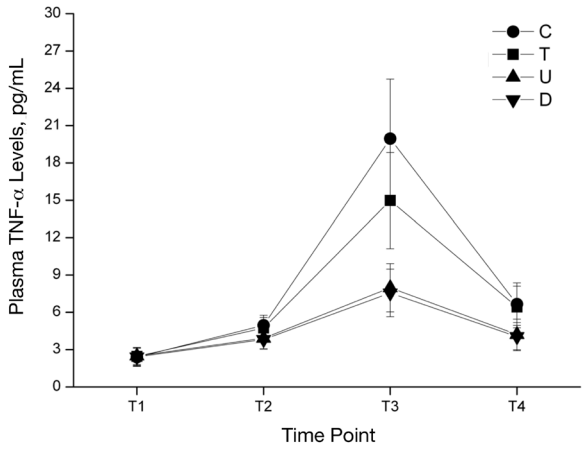

Figure 1. Plasma tumor necrosis factor (TNF)-α levels. The TNF-α levels at time points T2, T3, and T4 were significantly higher than those at T1 in all 4 groups (P < .05), and the levels at T3 were significantly higher than those at T2 and T4 (P < .05). There was no significant difference observed at T1 among groups, the TNF-α levels in Groups U and D were all significantly lower than those in Group C at T2, T3, and T4 (P < .05), and no significant difference was detected between Groups U and D (P > .05). A significantly lower TNF-α level was detected in Group T compared with that in Group C at T3 (P < .05), but no significant differences were found between Groups T and C at T2 and T4 (P > .05)

Changes of Levels of Inflammatory Cytokines and Comparison among Groups

Changing Trend of TNF-α Levels and Comparison among Groups

The TNF-α levels at the time points of T2, T3, and T4 were significantly higher than those at T1 in all 4 groups (P < .05), and the levels at T3 were significantly higher than those at T2 and T4 (P < .05). There was no significant difference observed at T1 among groups, the TNF-α levels in Groups U and D were all significantly lower than those in Group C at T2, T3, and T4 (P < .05), and no significant difference was detected between Groups U and D (P > .05). A significantly lower TNF-α level was detected in Group T compared with that in Group C at T3 (P < .05), and no significant differences were found between groups T and C at T2 and T4 (P > .05) (Table 2, Figure 1).

Changing Trend of IL-6 Levels and Comparison among Groups

The IL-6 levels at T2, T3, and T4 were significantly higher than those at T1 in all 4 groups (P < .05), and the levels at T3 were significantly higher than those at T2 and T4 (P < .05). There was no significant difference observed at T1 among groups, the IL-6 levels in Groups U and D were all significantly lower than those in Group C at T2, T3, and T4 (P < .05), and no significant difference was detected between Groups U and D (P > .05). Significantly lower IL-6 levels were detected in Group T compared with those in Group C at T3 (P < .05), but no significant differences were found between Groups T and C at T2 and T4 (P > .05) (Table 3, Figure 2).

Table 3. Plasma Interleukin (IL)-6 Levels in Patients from the 4 Groups at Different Time Points (pg/mL)

Group	T1 (before induction of anesthesia)	T2 (unclamping the ascending aorta)	T3 (1 hour after cardiopulmonary bypass)	T4 (24 hours after cardiopulmonary bypass)
C	1.71 ± 0.52	8.56 ± 1.98*	50.73 ± 13.42*	7.92 ± 1.89*
T	1.58 ± 0.54	8.28 ± 1.86*	39.64 ± 9.08*†	7.05 ± 1.78*
U	1.63 ± 0.65	6.62 ± 1.77*†	34.40 ± 12.46*†	5.92 ± 1.18*†
D	1.65 ± 0.59	6.29 ± 1.89*†	32.50 ± 11.32*†	5.59 ± 1.37*†

P < .05 vs. T1; †P < .05 vs. Group C.

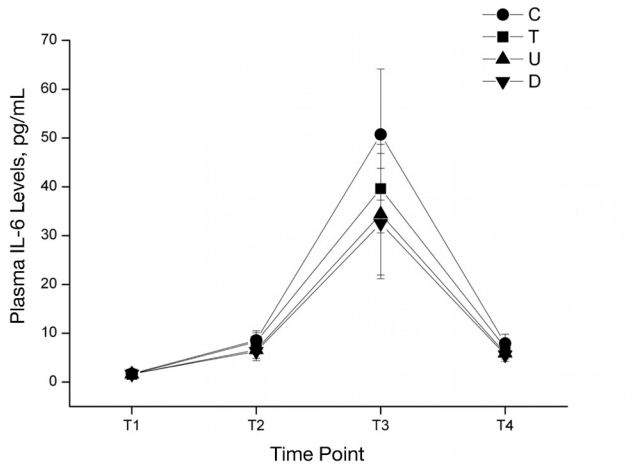

Figure 2. Plasma interleukin (IL)-6 levels in patients from the 4 groups at different time points. The IL-6 levels at T2, T3, and T4 were significantly higher than those at T1 in all 4 groups ($P < .05$), and the levels at T3 were significantly higher than those at T2 and T4 ($P < .05$). There was no significant difference observed at T1 among groups, the IL-6 levels in Groups U and D were all significantly lower than those in Group C at T2, T3, and T4 ($P < .05$), and no significant difference was detected between Groups U and D ($P > .05$). A significantly lower IL-6 level was detected in Group T compared with that in Group C at T3 ($P < .05$), and no significant differences were found between Groups T and C at T2 and T4 ($P > .05$).

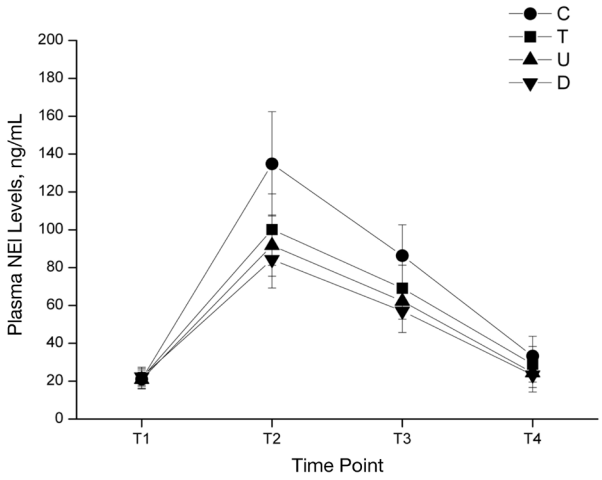

Figure 3. Plasma neutrophil elastase (NE) levels. The NE levels at T2 and T3 were significantly higher than those at T1 in all 4 groups ($P < .05$) and reduced greatly at T4 compared to the level at T1, but the levels at T2 were significantly higher than those at T3 ($P < .05$). There was no significant difference observed at T1 among groups, and the NE levels in Groups U and D were all significantly lower than those in Group C at T2, T3, and T4 ($P < .05$), but no significant difference was detected between Groups U and D ($P > .05$). Significantly lower NE levels were detected in Group T compared with those in Group C at T2 and T3 ($P < .05$), but no significant difference was found between Groups T and C at T1 and T4 ($P > .05$)

Table 4. Plasma Neutrophil Elastase Levels in Patients from the 4 Groups at Different Time Points (ng/mL)

Group	T1 (before induction of anesthesia)	T2 (unclamping the ascending aorta)	T3 (1 hour after cardiopulmonary bypass)	T4 (24 hours after cardiopulmonary bypass)
C	21.07 ± 4.88	134.83 ± 27.55*	86.31 ± 16.32*	33.31 ± 10.44
T	21.65 ± 5.72	100.11 ± 18.87*	69.13 ± 12.22*†	28.94 ± 9.37
U	20.95 ± 3.85	91.67 ± 16.12*	62.27 ± 9.51*†	24.47 ± 7.77†
D	22.28 ± 4.53	84.28 ± 15.08*	57.05 ± 11.30*†	23.26 ± 8.94†

$P < .05$ vs. T1; †$P < .05$ vs. Group C.

Table 5. Tissue Plasminogen Activator Levels in Patients from the 4 Groups at Different Time Points (ng/mL)

Group	T1 (before induction of anesthesia)	T2 (unclamping the ascending aorta)	T3 (1 hour after cardiopulmonary bypass)	T4 (24 hours after cardiopulmonary bypass)
C	5.79 ± 1.60	57.15 ± 11.40*	27.70 ± 5.46*	6.38 ± 1.18
T	5.76 ± 1.63	41.35 ± 10.71*†	20.72 ± 4.53*†	6.20 ± 0.97
U	6.18 ± 1.91	59.79 ± 9.66*	28.04 ± 4.46*	6.41 ± 0.84
D	6.16 ± 1.99	42.76 ± 7.36*†	21.04 ± 3.22*†	6.36 ± 1.13

$P < .05$ vs. T1; †$P < .05$ vs. Group C.

Changing Trend of NE Levels and Comparison among Groups

The NE levels at T2 and T3 were significantly higher than those at T1 in all 4 groups ($P < .05$) and reduced greatly at T4 compared to the level at T1, and the levels at T2 were significantly higher than those at T3 ($P < .05$). There was no significant difference observed at T1 among groups, and the NE levels in Groups U and D were all significantly lower than those in Group C at T2, T3, and T4 ($P < .05$), and no significant difference was detected between Groups U and D ($P > .05$). Significantly lower NE levels were detected in Group T compared with those in Group C at T2 and T3 ($P < .05$), and no significant difference was found between Groups T and C at T1 and T4 ($P > .05$) (Table 4, Figure 3).

Table 6. α2-Antiplasmin Levels in Patients from the 4 Groups at Different Time Points (μg/mL)

Group	T1 (before induction of anesthesia)	T2 (unclamping the ascending aorta)	T3 (1 hour after cardiopulmonary bypass)	T4 (24 hours after cardiopulmonary bypass)
C	63.46 ± 8.02	70.85 ± 12.39*	69.59 ± 11.57	64.16 ± 10.51
T	62.86 ± 8.74	71.86 ± 12.34*	81.89 ± 12.51*†	62.88 ± 10.15
U	62.03 ± 8.92	70.95 ± 13.77*	74.58 ± 13.41*	63.59 ± 8.27
D	63.35 ± 8.10	71.95 ± 14.11*	82.32 ± 13.31*†	64.85 ± 9.10

P < .05 vs. T1; †*P* < .05 vs. Group C.

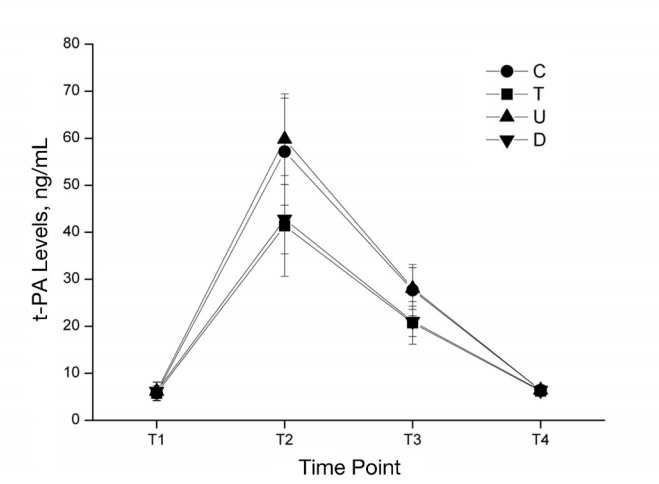

Figure 4. Tissue plasminogen activator (t-PA) levels. The t-PA concentrations at T2 and T3 were significantly higher than those at T1 in all 4 groups (*P* < .05) and reduced greatly at T4 compared to the level at T1. There were no significant differences observed at T1 at T4 among groups, and significantly lower t-PA concentrations were detected in Groups T and D at T2 and T3 compared with those in Group C (*P* < .05); no significant differences were detected between Groups U and C (*P* > .05).

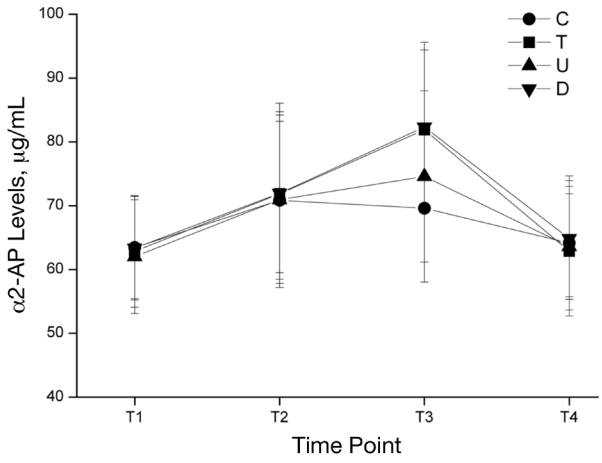

Figure 5. α2-antiplasmin (α2-AP) levels. The α2-AP concentrations at T2 were significantly higher than those at T1 in all 4 groups (*P* < .05), and significantly higher levels were detected in Groups T, U, and D at T3 compared with those at T1 (*P* < .05), but reduced greatly at T4 compared to the level at T1. There were no significant differences observed at T1 among groups, and there were no significant differences between Groups T, U, and D and the control group at T2 and T4 (*P* > .05). At T3, the α2-AP concentrations were significantly higher in Groups T and D than those in Group C (*P* < .05); however, no significant difference was detected between Groups U and C (*P* > .05).

Table 7. Plasma D-Dimer Concentrations in Patients from the 4 Groups at Different Time Points (mg/L)

Group	T1 (before induction of anesthesia)	T2 (unclamping the ascending aorta)	T3 (1 hour after cardiopulmonary bypass)	T4 (24 hours after cardiopulmonary bypass)
C	0.39 ± 0.11	3.54 ± 1.02*	2.42 ± 0.82*	0.51 ± 0.12
T	0.44 ± 0.05	1.56 ± 0.45*†	0.75 ± 0.28*†	0.50 ± 0.10
U	0.42 ± 0.13	3.39 ± 0.98*	2.48 ± 0.79*	0.53 ± 0.08
D	0.38 ± 0.09	1.54 ± 0.47*†	0.71 ± 0. 19*†	0.48 ± 0.09

P < .05 vs. T1; †*P* < .05 vs. Group C.

Table 8. Comparison of the Ratio of Volume of Pericardial Mediastinal Drainage to the Body Surface Area in Patients from the 4 Different Groups (mL/m^2)

Time	Group C	Group T	Group U	Group D
6 hours after surgery	243.1 ± 69.2	143.8 ± 40.2*	298.7 ± 89.0	153.0 ± 59.8*
12 hours after surgery	327.8 ± 82.9	207.8 ± 55.0*	312.0 ± 112.4	224.2 ± 65.9*
24 hours after surgery	393.9 ± 96.9	298.2 ± 67.2*	343.6 ± 98.0	287.8 ± 68.0*

*$P < .05$ vs. Group C.

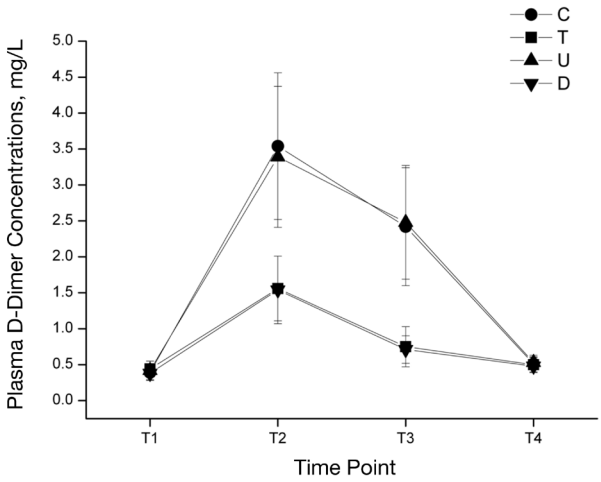

Figure 6. Plasma D-dimer concentrations. The D-dimer concentrations at T2 and T3 were significantly higher than those at T1 in all 4 groups ($P < .05$) and reduced greatly at T4 compared to the level at T1. There were no significant differences observed at T1 and T4 among groups, and significantly lower D-dimer concentrations were detected in Groups T and D at T2 and T3 compared with those in Group C ($P < .05$), but no significant differences were detected between Groups U and C ($P > .05$).

Changes in Levels of Indexes of Fibrinolytic System and Comparison among Groups

Changing Trend of t-PA Levels and Comparison among Groups

The t-PA concentrations at T2 and T3 were significantly higher than those at T1 in all 4 groups ($P < .05$) and reduced greatly at T4 compared to the level at T1. There were no significant differences observed at T1 at T4 among groups, and significantly lower t-PA concentrations were detected in Groups T and D at T2 and T3 compared with those in Group C ($P < .05$), but no significant differences were detected between Groups U and C ($P > .05$) (Table 5, Figure 4).

Changing Trend of α2-AP Levels and Comparison among Groups

The α2-AP concentrations at T2 were significantly higher than those at T1 in all 4 groups ($P < .05$), and significantly higher levels were detected in Groups T, U, and D at T3 compared with those at T1 ($P < .05$), but reduced greatly at T4 compared to the level at T1. There were no significant differences observed at T1 among groups, and there were no significant differences between Groups T, U, and D and the control group at T2 and T4 ($P > .05$). At T3, the α2-AP concentrations were significantly higher in Groups T and

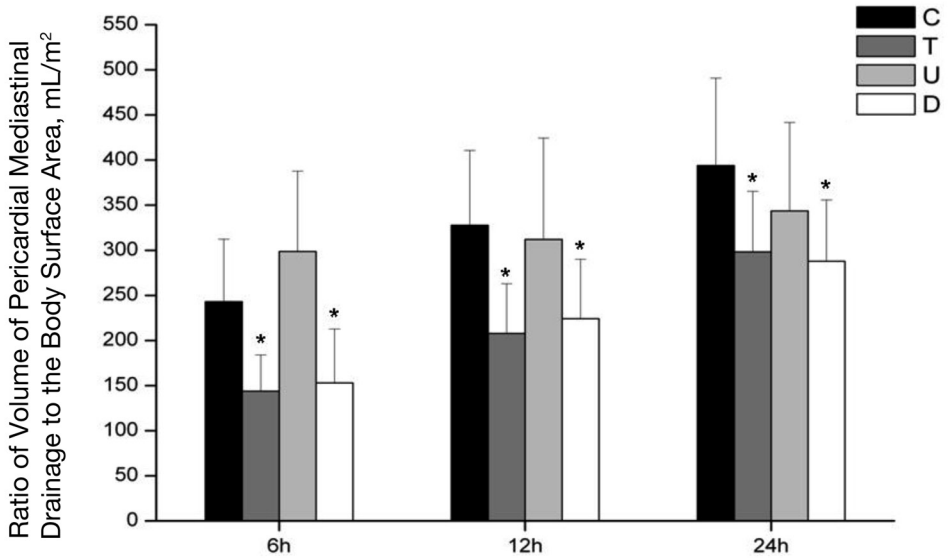

Figure 7. Comparison of the ratio of volume of pericardial mediastinal drainage to the body surface area. After 6 hours of surgery, the volumes of pericardial mediastinal drainage per body surface area in Groups T and D were significantly lower than those in Group C ($P < .05$), but no significant differences were observed between Groups U and C, as well as between Groups T and D ($P > .05$).

D than those in Group C ($P < .05$); however, no significant difference was detected between Groups U and C ($P > .05$) (Table 6, Figure 5).

Changing Trend of D-Dimer Levels and Comparison among Groups

The D-dimer concentrations at T2 and T3 were significantly higher than those at T1 in all 4 groups ($P < .05$) and reduced greatly at T4 compared to the level at T1. There were no significant differences observed at T1 and T4 among groups, and significantly lower D-dimer concentrations were detected in groups T and D at T2 and T3 compared with those in Group C ($P < .05$); no significant differences were detected between Groups U and C ($P > .05$) (Table 7, Figure 6).

Comparison of Other Monitoring Results

After 6 hours of surgery, the volumes of pericardial mediastinal drainage per body surface area in Groups T and D were significantly lower than those in Group C ($P < .05$), and no significant differences were observed between Groups U and C, as well as between groups T and D ($P > .05$) (Table 8, Figure 7).

The mean arterial pressure, heart rate, central venous pressure, and SpO2 during the perisurgery stage all remained stable, and no significant differences were observed. The ventilators were all successfully terminated in patients from all groups after CPB, and no significant differences were found following administration of vasoactive drugs. There were no severe complications such as low cardiac output syndrome, cardiac tamponade, and severe arrhythmia detected in any patients postsurgery.

DISCUSSION

Treatment with tranexamic acid inhibited both fibrinolytic hyperfunction and inflammatory response during CPB. Ulinastatin exhibited no inhibitory effect on fibrinolysis but showed a strong inhibitory effect on inflammatory response. Combined treatment of tranexamic acid and ulinastatin could effectively reduce postsurgical bleeding and the length of stay in ICU in valve replacement surgery.

The present study demonstrated that SIRS caused by CPB was a dynamic changing process, and the plasma TNF-α, IL-6, and NE levels significantly increased after unclamping the ascending aorta and continued to increase for a long time after CPB. However, the peak concentrations of various inflammatory cytokines were different. The NE peak concentration was seen at an early stage, which was present while unclamping the ascending aorta in CPB, and remained significantly higher than the baseline level 1 hour after CPB but decreased to approximately the normal level 24 hours after CPB. The plasma TNF-α and IL-6 levels reached the peak 1 hour after CPB and remained higher than the normal level 24 hours after CPB. Therefore, 1 hour after CPB may be considered the most severe stage of systemic inflammatory response, which is in agreement with a study described previously [Chandler 1995]. TNF-α is the earliest medium released during systemic inflammatory response, which induces the cascade-like release of other cytokines such as IL-6 and IL-8. In valve replacement surgery, after unclamping the ascending aorta, neutrophils enter the ischemic myocardium and lung, adhere to the activated endothelia, and release large amounts of NE, which further promotes the release of inflammatory cytokines. Therefore, the peak levels of TNF-α and IL-6 were observed after the presence of peak NE concentration.

The plasma TNF-α, IL-6, and NE levels significantly increased in Groups U and D during and after CPB; however, the levels at all time points were lower than those in the control group ($P < .05$), demonstrating that ulinastatin could effectively inhibit the release of proinflammatory cytokines and NE during CPB, down-regulate the acute response during CPB, and effectively reduce the systemic inflammatory response caused by CPB, which is consistent with previous studies [Kluft 1992; Greilich 2003]. The plasma NE level decreased in Group T after unclamping the ascending aorta and 1 hour after CPB, and the plasma TNF-α and IL-6 levels were significantly lower than those in the control group 1 hour after CPB ($P < .05$), indicating that tranexamic acid inhibited the release of proinflammatory cytokines including TNF-α and IL-6 to certain degree and reduced their peak levels during CPB.

It is thought that the inhibitory effect of ulinastatin on the release of inflammatory cytokines may be related to the inhibition of activity of the widely distributed serine protease, inhibition of activation of white blood cells, leukocyte transendothelial migration, reduction in inflammatory cell infiltration, and release of tissue toxic substances [Slaughter 1997; Despotis 2001]. In addition, ulinastatin treatment functions include stabilization of the lysosomal membrane, inhibition of presence of myocardial depressant factors, oxygen free radical scavenging during CPB, and improvement in circulation during low perfusion, which protects the important organs of the body during heart surgery [Munos 1999; Lu 2003; Chandler 2004]. Though tranexamic acid inhibits release of proinflammatory cytokines and may contribute to the reduction in activation of plasminogen, the inhibition of fibrinolytic hyperfunction results in alleviation of tissue injury [Karkouti 2004].

CPB-induced fibrinolysis hyperfunction is an extremely complex pathological process. Previous studies have demonstrated that the major mechanisms of enhanced fibrinolytic activity were increased in t-PA release and activity, contact-activated fibrinolysis, and α2-AP consumption [Hall 2002; Chandler 2004; Taylor 2005; Koch 2006]. The postoperative bleeding volume increases with the degree of fibrinolysis activation [Hunt 1998; Jimenez 2007]. D-dimer, a type of protein fragment dissolved by fibrin in plasma, is a specific degradation product hydrolyzed of cross-linked fibrin by plasmin, which represents the fibrinolytic state and changes of blood, and the elevated fibrinolytic level represents the increase in plasmin activity [Massoudy 2001]. D-dimer is proved to be a specific indicator for the presence of secondary fibrinolysis and is more sensitive than detection of the fibrin degradation products and more reliable [Sablotzki 2002], which can be considered as an index for measurement of fibrinolytic activity in CPB heart surgery.

In our study, the maximum level of the activated t-PA is present shortly after CPB. The t-PA concentration rose

greatly and remained higher than the normal level 1 hour after the termination of CPB, recovering to almost the normal level 24 hours after the surgery, indicating that the changes of t-PA concentration were associated with the use of CPB. During and after a period of CPB, t-PA was dominant, which led to the activation of fibrinolytic system, an increase in consumption of plasminogen and plasmin, a significant rise in fibrin degradation products and D-dimers, and more postoperative bleeding volumes. The current study observed that the prophylactic administration of tranexamic acid caused a significantly reduced t-PA activity and amount of D-dimers during CPB compared with those in the control group, and thus led to significant decrease in postoperative bleeding volume. The major role of tranexamic acid lies in effective occupation of the lysine binding site between plasminogen and plasmin, the key substances in the fibrinolytic system [Kluft 1992], competitive inhibition of t-PA, and reduction in speed of activation of prothrombin into thrombin by t-PA. In general, t-PA has low affinity with its substrate plasminogen, and its effect reduces the production of plasmin through inhibiting the activation of plasminogen, protecting the blood from clot dissolution, which is useful in patients to reduce postoperative bleeding.

In addition, sometimes high concentration of tranexamic acid has direct anti-plasmin effect. In the present study, the plasma α2-AP levels were significantly higher in patients from Groups T and D 1 hour after CPB than those in the control group, but no significant difference was observed between Groups U and C, suggesting that prophylactic treatment with tranexamic acid increased the plasma α2-AP level, which inhibited the plasmin activity and reduced the activation of fibrinolytic system.

In the current study, the D-dimer concentrations of patients in all groups were all at normal levels before the bypass and significantly increased 1 hour after unclamping the ascending aorta, demonstrating that plasmin was activated after CPB. As a result, fibrin hydrolysis increased, the fibrinolytic system became strongly activated, and fibrinolytic hyperfunction occurred. At T2 and T3, the plasma D-dimer concentrations in Groups T and D were significantly lower than those in the Groups C and U, and there was no significant difference observed between Group U and the control group, indicating that tranexamic acid could effectively inhibit the CPB-induced fibrinolytic hyperfunction, and thus reduce postoperative bleeding. In contrast, no significant effect on CPB-induced fibrinolytic hyperfunction was observed with ulinastatin.

Furthermore, the volumes of pericardial mediastinal drainage per body surface area in patients in Groups T and D at 6, 12, and 24 hours postsurgery were significantly lower than those in Groups C and U, but no significant differences were observed between Groups T and D and between Groups C and U, demonstrating that administration of tranexamic acid could inhibit the activation of the fibrinolytic system and significantly reduce the bleed volume after heart valve replacement surgery, but ulinastatin had no effect on postoperative bleeding. As for the length of stay in the ICU, the duration of stay in Group D was less than that in other groups.

CONCLUSIONS

In conclusion, it is evident that combined treatment with ulinastatin and tranexamic acid inhibits the release of both NE and proinflammatory cytokines, including TNF-α and IL-6 caused by CPB, and the fibrinolytic hyperfunction induced by CPB; decreases the plasma D-dimer concentration; reduces the postoperative bleeding; shortens the length of stay in the ICU; and may be considered as a beneficial therapeutic option in patients undergoing CPB.

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