Original Article
Protective effects of ulinastatin combined with nitric oxide and prostaglandin E1 on lung injuries and coagulation in infants undergoing cardiopulmonary bypass

Shengxing Zheng*, Qian Wang*, Lixia Ji, Wenqing Lu, Qingquan Lian, Mingpin Hu

*Department of Anaesthesia and Critical Care, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China. *Equal contributors.*

Abstract: Cardiopulmonary bypass (CPB) may elicit a systemic inflammatory response leading to lung dysfunction, activating the coagulation system. This study investigated the effects of ulinastatin combined with nitric oxide and prostaglandin E1 (UPN) on pulmonary protection in infants undergoing CPB. Sixty Infants with congenital heart disease, undergoing CPB, were equally divided into five groups. Each group received, respectively, the following treatments: (1) 10000 U.kg^{-1} of ulinastatin; (2) Intravenous infusion of 10 ng.kg^{-1}.min^{-1} of PGE1; (3) Inhalation of 20 ppm nitric oxide; (4) Combination of all three agents (UPN); and (5) No treatment group during CPB. Plasma concentrations of sICAM-1, vWF, IL-6, and GMP-140 were measured at 5 time points: before CPB (T1), 30 minutes after the start of CPB (T2), at the end of CPB (T3), and 3 hours (T4) and 24 hours (T5) after termination of CPB. At the same time points, artery blood gas analysis was performed to calculate respiratory index (RI). Prothrombin time (PT), activated partial thromboplasin (APTT), and platelet number were also measured in the five groups at 3 hours and 24 hours after surgery, respectively. Postoperative blood loss and blood transfusions were recorded. It was found that the UPN group inhibited plasma levels of sICAM-1, vWF, IL-6, and GMP-140, while decreasing RI. At 3 hours and 24 hours after the operation, PT and APTT levels were significantly shortened and platelet numbers were obviously higher in the UPN group. Cumulative postoperative bleeding was significantly less in the UPN group. This study demonstrated that combination treatment of UPN protected patients from lung injury, decreased plasma vWF, sICAM-1, IL-6, and GMP-140 levels, while inhibiting platelet activation.

Keywords: Cardiopulmonary bypass, inflammatory response, coagulation cascade, lung injury

Introduction

Cardiopulmonary bypass (CPB) is a nonphysiologic procedure that may elicit a systemic inflammatory response leading to lung dysfunction [1, 2]. Clinically, lung injuries induced by CPB usually manifest excessive interstitial pulmonary edema and subsequent abnormal gas exchange [3], with a high incidence among infants and younger patients [4]. Cytokines, such as interleukin (IL)-6, IL-8, and tumor necrosis factor-alpha (TNF-a), and activation of cells, such as leukocytes, vascular endothelial cells, and platelets, play an important role in this systemic inflammatory response to cardiac operations with CPB [5, 6].

Although coating the surface of the CPB circuit and oxygenator with heparin or other compounds prevents clotting during CPB, the coagulation system is still activated [7, 8]. Inflammation and coagulation activation are intricately linked. Inflammation upregulates microvascular expression of tissue factor (TF), triggering coagulation activation. Inflammation also downregulates natural anticoagulant proteins [9, 10]. Degrees of inflammatory response and levels of coagulation activation are associated with postoperative complications, including bleeding, thrombosis, and lung injury [11]. Outcomes cannot be substantially improved by individual inhibition of inflammation or coagulation.

NO and PGE1 have been reported to inhibit neutrophils, reduce the release of IL-8, and inhibit activation of leukocytes, endothelial cells, and platelets [12-16]. Ulinastatin, a human uri-

Table 1. Patient characteristics and perioperative data

Group	Male/Female (n)	Age (month)	Weight (kg)	Aortic crossclamp time (min)	Total CPB time (min)
Ulinastatin (U)	7/5	17.4±5.2	10.8±2.9	28.8±6.9	51.3±8.6
PGE1 (P)	7/5	17.1±5.1	10.9±2.8	28.2±6.6	49.2±7.8
No (N)	8/4	17.6±5.8	11.1±3.5	29.2±6.2	49.7±7.2
UPN	7/5	17.2±4.9	11.2±3.3	28.9±6.9	49.6±8.4
Control	8/4	17.3±5.4	10.7±2.6	28.3±6.7	50.7±7.3

nary trypsin inhibitor, can be found in urine, plasma, and all organs [17]. In previous studies, ulinastatin has been shown to exert protective effects against pulmonary damage [18, 19]. Ulinastatin treatment can attenuate inflammatory response, reduce levels of cytokines [20], and inhibit activation of leukocytes, endothelial cells, and platelets. This study investigated the effects of combination of nitric oxide (NO), prostaglandin E1 (PGE1), and ulinastatin on lung injuries and coagulation in infants undergoing cardiopulmonary bypass.

Patients and methods

This prospective study was conducted at the 2nd Affiliated Hospital, Wenzhou Medical University. The protocol was approved by the Medical Ethics Committee of the 2nd Affiliated Hospital, Wenzhou Medical University. Informed consent was obtained from the guardians or legal representatives of the patients before enrollment. Eligible participants were American Society of Anesthesiologists I to III patients, aged from 6 to 36 months, having congenital heart disease with pulmonary artery pressures less than 30 mmHg (as assessed by preoperative echocardiogram), requiring CPB under general anesthesia. Exclusion criteria included patients younger than 6 months and older than 36 months, patients born prematurely, patients with body temperatures under 36.5 or higher than 37.5 (ear temperature), patients with abnormal liver, renal function, or major chromosomal abnormalities, patients showing pulmonary inflammation before surgery, patients with hemodynamic dysfunction, and patients refusing to participate in the study.

All patients were evaluated by standard echocardiography and/or cardiovascular angiography before surgery. Preoperative characteristics of patients are presented in **Table 1**. Patients were orally intubated in the operating room. Anesthesia was managed according to standard protocol, including induction with sevoflurane (2-5%) in oxygen, midazolam (0.10-0.20 mg/kg), fentanyl (5-10 ug/kg), vecuronium (0.1 mg/kg), and maintenance with propofol (2-3 mg/kg), fentanyl (5-10 ug/kg), and sevoflurane (1-3%) in oxygen. Neuromuscular blockade was achieved with vecuronium (0.1 mg/kg, once every 60 minutes). The CPB circuit, identical for all patients, included a microporous hollow fiber membrane oxygenator (OXIM-Plus, America) and a Stockert III roll pump (Sarns 8000, America). Before aortic cannulation, 3 mg/kg heparin was administered with the target kaolin-ACT value more than 450 seconds. The bypass circuit was primed with lactated Ringer's solution, colloid (20% albumin, plasma 150 mL), mannitol (0.5-1.5 ml/kg), packed red blood cells (1.5 U), heparin (3 mg/ml), and 5% sodium bicarbonate (5 mL/kg). Pump flow rates ranged from 3.0 to 2.0 L/min/m². Core temperature was controlled at 30 to 32°C using a heat exchanger in the bypass circuit. At the end of CPB, to maintain the fluid balance, modified ultrafiltration was used to remove excess fluid in the body, according to the hematocrit (maintenance of hematocrit > 30%). Also, blood pressure (aortic blood pressure: 75-110/50-78 mmHg; left atrial pressure: 5-12 mmHg; right atrial pressure: 5-14 mmHg according to patient age and weight) was monitored.

After CPB set-up, patients were randomly divided into five groups, with 12 patients in each group. In the ulinastatin group, each patient received 1000 U/kg of ulinastatin (Livzon Pharmaceutical Trading Co., Zhuhai, China) by adding into the primed solution. Patients in the NO group were given 20 ppm NO (Beijing Beiyang Lianhe Qiti Co., Ltd.) by inhalation from the bubble oxygenator (monitored by NOxBOX W+ from Bedfont Scientific Ltd.) throughout CPB. In the PGE1 group, 10 ng/kg minute of PGE1 (Pukang Pharmaceutical Co, Nanyang, China) was given to each patient by intravenous infusion during CPB. The three interventions were combined in the ulinastatin, PGE1, and NO (UPN) groups, while the Control group received no treatment during CPB. Anaesthesiologists, surgeons, and laboratory staff were blinded throughout the study.
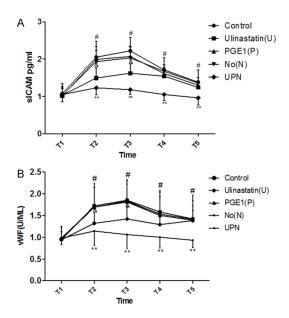
Figure 1. Effects of different treatments on plasma levels of sICAM-1 and vWF in five groups. Data are expressed by the mean ± standard deviation. **P < 0.01 versus other four groups. &P < 0.05 versus Control group. #P < 0.01 versus baseline values at T1 in group U, P, N, and Control group, respectively. There were no significant changes in plasma concentrations of sICAM-1 and vWF after CPB compared with baseline values at T1 in the combination treatment (P > 0.05). T1, prior to the initiation of CPB; T2, at 30 minutes after the institution of CPB; T3, at the end of CPB; T4, at 3 hours after the end of CPB; T5, 24 hours after the end of CPB.

Plasma von Willebrand factor (vWF), soluble intercellular adhesion molecule-1 (sICAM-1), IL-6, and Granule membrane protein-140 (GMP-140) measurement: For each patient, 1.8 mL of fresh blood from radial artry was drawn into a vacuum tube containing EDTA, prior to the initiation of CPB (T1), at 30 minutes after the institution of CPB (T2), at the end of CPB (T3), and at 3 hours (T4) and 24 hours (T5) after termination of CPB. After centrifugation at 3000 rpm for 10 minutes at 4°C, the plasma was divided into aliquots and frozen at 80°C until assay. Plasma vWF, sICAM-1, IL-6, and GMP-140 levels were measured via commercial vWF, sICAM-1, IL-6, and GMP-140 ELISA kits (Invitrogen, Camarillo, CA), according to manufacturer instructions.

Blood gas assay: For each patient, 0.5 mL of fresh blood from radial artry was drawn into a vacuum tube containing EDTA prior to the initiation of CPB (T1), at 30 minutes after the institution of CPB (T2), at the end of CPB (T3), and at 3 hours (T4) and 24 hours (T5) after the end of CPB. Blood gas analysis was done to calculate the respiratory index (RI) at each time point. Respiratory index (RI) RI = P (A-a) DO2/PaO$_2$ = (PAO$_2$-PaO$_2$)/PaO$_2$, PAO$_2$ = FiO$_2$ × (BP-PH$_2$O)-PaCO$_2$/RQ, BP (barometric pressure): 760 mmHg, PH$_2$O (water vapor pressure of pulmonary alveoli): 47 mmHg, RQ (respiration quotient): 0.8; FiO$_2$ according to the anesthesia machine or respirator setting parameters. Because body temperatures and blood dilution degrees change constantly during CPB, to eliminate its effects on the measured value, all measurements must be corrected (including blood gas analysis). Corrected value of HCT = (measured value of HCT * preoperative HCT)/measured value of HCT.

Prothrombin time (PT), activated partial thromboplasin (APTT), and platelet number were also measured in the five groups prior to surgery and at 3 hours and 24 hours after surgery, respectively. Postoperative blood loss and blood transfusions were recorded as well.

All demographic data are described as mean ± standard error of the mean. One-way analysis of variance (ANOVA) was used for comparison of variables among treatment groups using an SPSS software program (SPSS Inc, Chicago, IL, USA), with significance defined as P < 0.05. If analysis of variance revealed a significant interaction, pair-wise tests of individual group means were compared by means of multiple comparisons (Tukey's test), using levels of significance of P < 0.05, P < 0.01, and P < 0.001. P-values < 0.05 are considered statistically significant.

Results

Plasma vWF and sICAM-1 levels suppressed by combination treatment (UPN) of ulinastatin, nitric oxide, and prostaglandin E1

Plasma concentrations of sICAM-1 and vWF were significantly lower in the combination group than in the other four groups (P < 0.01) (**Figure 1A**, **1B**). Plasma concentrations of sICAM-1 and vWF after CPB were significantly increased in ulinastatin, PGE1, NO, and Control groups, compared with baseline values at T1 (P < 0.01) (**Figure 1A**, **1B**). Compared to the Control group, plasma concentrations of sICAM-1 and vWF were significantly lower with ulinastatin
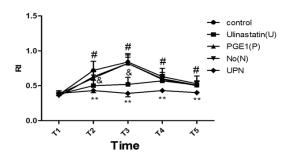
Figure 2. Effects of different treatments on RI in five groups. **P < 0.01 versus other four groups. &P < 0.05 versus Control, nitric oxide group, and prostaglandin E1 group. #P < 0.01 versus baseline values at T1 time point.
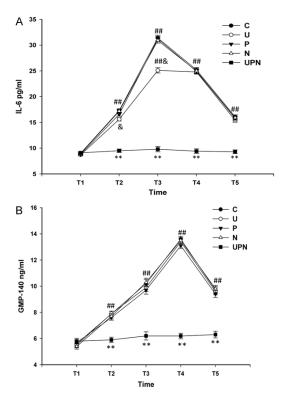

Figure 3. Effects of different treatments on plasma levels of IL-6 and GMP-140 in five groups. Data are expressed by the mean ± standard deviation. **P < 0.01 versus other four groups. &P < 0.05 versus Control group. #P < 0.01 versus baseline values at T1 in group U, P, N, and control group, respectively. There were no significant changes in plasma concentrations of IL-6 and GMP-140 after CPB compared with baseline values at T1 in the combination treatment (P > 0.05).

treatment at T2-T3 time points (P < 0.05) (**Figure 1A**, **1B**). Plasma concentrations of sICAM-1 and vWF after CPB were significantly increased, compared with baseline values at T1 in U,

P, N group, and Control group, respectively (P < 0.01) (**Figure 1A**, **1B**). There were no significant changes in plasma concentrations of sICAM-1 and vWF after CPB, compared with baseline values at T1 in the combination group (P > 0.05) (**Figure 1A**, **1B**).

Lung dysfunction lessened by combination treatment (UPN) of ulinastatin, nitric oxide, and prostaglandin E1

There were no significant changes in RI between the nitric oxide group and prostaglandin E1 group with the Control group at T2-T5 time points (P > 0.05). There were no significant changes in RI between the ulinastatin group and Control group at T4-T5 time points (P > 0.05). Comparing the Control group, nitric oxide, and prostaglandin E1 group, RI was significantly smaller in the ulinastatin group at T2-T3 time points (P < 0.05) (**Figure 2**). Compared with the other four groups, RI was significantly smaller in the combination treatment group (UPN) at T2-T5 time points (P < 0.01 versus other four groups after CPB) (**Figure 2**). There were no significant changes in RI after CPB, compared with baseline values at T1 in the combination treatment group (P > 0.05). RI was significantly raised at T2-T5 time points in Control, nitric oxide, and prostaglandin E1 groups, as well as at T4-T5 time points in the ulinastatin group, compared with baseline values at T1 time point (P < 0.01) (**Figure 2**).

Plasma IL-6 and GMP-140 levels suppressed by combination treatment of ulinastatin, nitric oxide, and prostaglandin E1

Plasma concentrations of IL-6 and GMP-140 after CPB were significantly lower in the combination treatment group than in the other four groups (P < 0.01) (**Figure 3A**, **3B**). Plasma concentrations of IL-6 and GMP-140 after CPB were significantly increased, compared with baseline values at T1 in group U, P, N, and Control group, respectively (P < 0.01) (**Figure 3A**, **3B**). Compared to the Control group, plasma concentrations of IL-6 were significantly lower in the ulinastatin treatment group at T2-T3 time points (P < 0.05) (**Figure 3A**). There were no significant changes in plasma concentrations of IL-6 and GMP-140 after CPB, compared with baseline values at T1 in the combination treatment group (P > 0.05).
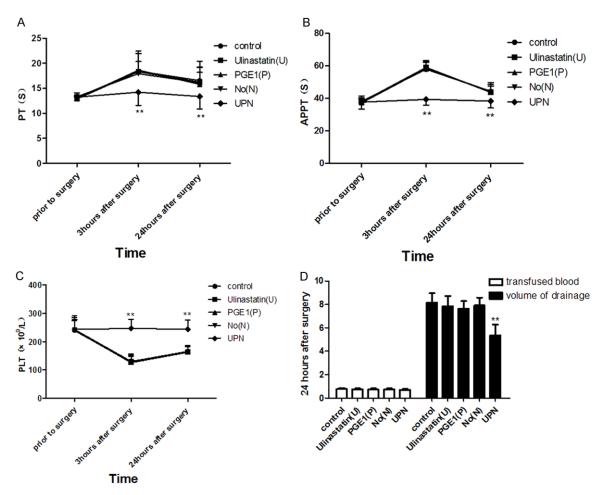
Figure 4. Effects of different treatments on PT, APTT and platelet counts in five groups. Data are expressed by mean ± standard deviation. **P < 0.01 versus other four groups.

Platelet activation inhibited by combination treatment of ulinastatin, nitric oxide, and prostaglandin E1

After 3 hours and 24 hours, PT and APTT were significantly shortened in the combination treatment of UPN group than the other four groups (P < 0.01) (**Figure 4A**, **4B**). Compared with the other four groups, increased platelet counts levels were observed with the combination treatment of UPN (P < 0.01) (**Figure 4C**). There were no significant changes in PT, APTT, and platelet counts, after the operation, compared with baseline values prior to surgery in the combination treatment of UPN (P > 0.05). Cumulative postoperative bleeding was significantly less with the combination treatment of UPN than in other groups (P < 0.01) (**Figure 4D**).

Discussion

The exact underlying pathophysiological mechanisms inducing post-CPB lung injuries remain unclear [21]. It has been traditionally considered that post-CPB lung injuries are caused by ischemia reperfusion injury (IRI) and systemic inflammatory response syndrome, induced by contact of blood with the foreign surface of the bypass circuit [22, 23]. Several methods have been proposed and used to inhibit lung dysfunction. The present study demonstrated that combination treatment of UPN protected patients from lung injury and decreased plasma vWF, sICAM-1, IL-6, and GMP-140 levels, while inhibiting platelet activation.

Intercellular adhesion molecule-1 (ICAM-1) is found on endothelium. Endothelial injury is part of the systemic inflammatory response induced by CPB [24]. Increased levels of sICAM-1 result either from increased expression in activated endothelial cells or increased proteolytic cleavage due to endothelial cell injuries [25]. Patients with postoperative respiratory dysfunction have significantly higher levels of sICAM-1 [26].

Endothelium cells are a source of von Willebrand factor (vWF), a potential marker of endothelial injury [27, 28]. This study also measured plasma sICAM-1 and vWF concentrations, perioperatively, in 60 patients undergoing cardiopulmonary bypass. This study demonstrated that, compared to preoperative values, levels of plasma sICAM-1 and vWF increased after CPB, arriving at their peak at the end of CPB, suggesting the impact of cardiac surgery with CPB on endothelial dysfunction. The present study also suggested no significant changes in plasma concentrations of sICAM-1 and vWF among nitric oxide (NO) treatment, prostaglandin E1 (PGE1) treatment, and the Control group. Hallstrom et al. and Zhou et al. also did not observe any anti-inflammatory effects at low concentrations of NO and PGE1 [29, 30]. The present study demonstrated that plasma concentrations of sICAM-1 and vWF increased after CPB but significantly decreased at T2-T3 time points by ulinastatin treatment, suggesting that ulinastatin can partly inhibit activation and injury of endothelial cells, in accord with Tanaka R et al. reports [31]. With combination treatment of UPN, plasma concentrations of sICAM-1 and vWF were significantly inhibited, compared to the other four groups, implying that UPN treatment inhibits activation and injury of endothelial cells and improves endothelial function, protecting patients from lung injuries after cardiac surgery.

Previously, Zhou Jing et al. reported that combination treatment of UPN inhibited release of inflammatory mediators [30]. However, whether this affects lung function after CPB is unclear. Pulmonary dysfunction, induced by CPB, mainly manifests by poor gas exchange. Respiratory index is an index of oxygenation, reflecting lung function in a variety of circumstances [32]. In the present study, RI began to rise at 30 minutes after the outset of CPB, arriving at a peak at the end of CPB. This study also demonstrated no significant changes in RI among nitric oxide group, prostaglandin E1 group, and Control group at T2-T5 time points. Compared with Control group, RI was significantly smaller in the ulinastatin group at T2-T3 time points, suggesting that ulinastatin improved oxygenation. Several studies have also reported that ulinastatin improved pulmonary function [19, 33, 34]. However, there were no significant changes in RI between the ulinastatin group and

Control group at T4-T5 time points, which may be due to its short half-life. The present study also demonstrated that RI was significantly smaller with combination treatment of UPN at T2-T5 time points. This suggests that their protective effects on pulmonary function may be due to suppression of levels of intercellular adhesion molecule-1 (ICAM-1) and von Willebrand factor (vWF) after CPB, inhibiting activation and injury of endothelial cells and improving endothelial function after CPB.

Inflammatory response and coagulation cascade are both activated after CPB. Inflammatory response is a complex network with increased cytokine production and activation of cells, such as leukocytes, vascular endothelial cells, and platelets [35]. Proinflammatory cytokine IL-6 is a good predictor of clinical outcomes [36, 37], thought to be associated with postoperative pulmonary dysfunction [38]. Behr et al. reported that IL-6 plasma levels increased during and after surgery and CPB [39]. The present study also found that IL-6 plasma levels began to increase at 30 minutes after the outset of CPB, arriving at a peak at the end of CPB. IL-6 plasma levels remained elevated until 24 hours after CPB. Thus, CPB evoked the release of IL-6, which is involved in modulating the acute phase response [40]. This study also demonstrated that combination treatment (UPN) significantly decreased plasma levels of IL-6 after CPB, compared to the other four groups, suggesting that UPN distinctly inhibits adverse systemic reactions and degree of active inflammation related to CPB. Compared to Control group, ulinastatin significantly decreased plasma levels of IL-6 at T2-T3 time points. Several other studies have also reported that ulinastatin attenuated inflammatory response [19, 33, 34]. However, there were no significant changes in plasma levels of IL-6 between ulinastatin group and Control group at T4-T5 time points, which may be due to its short half-life. In this study, Neither NO nor PGE1 was found to reduce plasma levels of IL-6. Present results were confirmed by Troncy [5].

Inflammation response and coagulation cascade are intricately linked during CPB. Inflammation, induced by CPB, will elevate fibrinogen synthesis. Moreover, platelet activation and consumption during CPB leading to an inadequate postoperative platelet number have be-

en recognized as major contributors to bleeding after the operation. Granule membrane protein 140 (GMP-140) has been identified as a platelet glycoprotein, mainly located on the membrane of blood platelet [41]. When platelets are activated, GMP-140 is not only expressed on the membrane of blood platelet but also secreted into the plasma. Plasma levels can directly reflect the activity of blood platelets [42]. Zilla P et al. reported that the amount of GMP-I40 increased during CPB [43]. The present study found that GMP-I40 plasma levels began to increase at 30 minutes after the outset of CPB, reaching a peak at 3 hours after termination of CPB. GMP-I40 plasma levels remained elevated until 24 hours after CPB. Thus, CPB evoked platelet activation. In addition, it was found that GMP-I40 plasma levels were not significantly inhibited by ulinastatin, nitric oxide, and prostaglandin E1, separately, which may be due to two possible reasons. First, doses of inhaled NO and infused PGE1 may have been insufficient. Second, many positive results have been reached from *in vitro* studies [13, 44]. However, the negative results observed by this study were from *in vivo* studies. The present study demonstrated that combination treatment of UPN significantly decreased plasma levels of GMP-140 after CPB, compared with the other four groups, suggesting that UPN distinctly inhibits platelet activation related to CPB. This study also demonstrated that, after 3 hours and 24 hours, PT and APTT were significantly prolonged, while platelet count levels were decreased in Control, ulinastatin, nitric oxide, and prostaglandin E1 groups. Taken together, results suggested that a large amount of coagulation factors was consumed and that the fibrinolytic system was overactivated during CPB. In addition, it was found that there were no significant changes in PT, APTT, and platelet counts after the operation compared with baseline values prior to surgery in the combination treatment of UPN. Cumulative postoperative bleeding was significantly less in the combination treatment of UPN than in other groups, Therefore, combination treatment of UPN can inhibit fibrinolytic system activation. However, there were no significant changes in blood transfusions among the five groups, which may be due to infantile transfusions requiring consideration of the comprehensive situation and inadequate sample size.

In conclusion, present results suggest that administration of combination treatment of NO, PGE1, and ulinastatin can effectively inhibit activation of endothelial cells and protect pulmonary function induced by CPB. Furthermore, it even distinctly inhibited adverse systemic reactions, activation of platelets, and fibrinolytic system activation related to CPB.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Mingpin Hu and Qingquan Lian, Department of Anaesthesia and Critical Care, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, 109 West Xueyuan Road, Wenzhou 325027, Zhejiang, China. Tel: +86-577-88002262; Fax: +86-577-88002286; E-mail: mingpinhu1958@163.com (MPH); Tel: 86-577-88002262; E-mail: lianqingquanmz@163.com (QQL)

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