Dynamic changes and clinical significance of LXA_4 in the perioperative period of cardiopulmonary bypass

MING WU[1,2], ZONGTAI FENG[2], JIANYI LIAO[3], HUITING ZHOU[4] and JIAN WANG[2]

[1]Department of Pediatrics, Xuzhou Medical University, Xuzhou, Jiangsu 221004; Departments of [2]Pediatric Surgery and [3]Pediatric Cardiology; [4]Institute of Pediatric Research, Children's Hospital of Soochow University, Suzhou, Jiangsu 215025, P.R. China

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Abstract. Dynamic changes in lipoxin A_4 (LXA_4) in child patients with congenital heart disease (CHD), in the perioperative period of cardiopulmonary bypass (CPB) were studied. Peripheral blood was collected from 16 child patients (CPB group) before operation (Tc), after operation (T0), at 1 day after operation (T1), at 3 days after operation (T3), and at 7 days after operation (T7); and from 17 children with no CHD (control group). The level of LXA_4 in peripheral blood was detected via enzyme-linked immunosorbent assay (ELISA). Clinical data of the child patients were collected. The white blood cell (WBC) count, the proportion of neutrophils (N%) and high-sensitivity C-reactive protein (hs-CRP) levels were also detected, followed by statistical analysis. The plasma LXA_4 levels in CPB group at Tc were significantly lower compared to that in the control group ($P<0.01$). In CPB group, the level of LXA_4 showed an increasing trend at T0, WBC and hs-CRP were transiently increased at T0 and increased most significantly at T1. N% was obviously increased at T0 compared to that at Tc and was still significantly higher at T7 compared to that at Tc. The CPB time and aortic clamping time were positively correlated with the time in the Pediatric Intensive Care Unit (PICU), the application time of ventilator, and the hs-CRP level at T0. The LXA_4 level at each time-point had no correlation with other indexes. In conclusion, the inflammatory response after CPB increases the synthesis of LXA_4 with an anti-inflammatory effect, but LXA_4 cannot be used as a sensitive index for monitoring inflammation.

Correspondence to: Dr Jian Wang, Department of Pediatric Surgery, Children's Hospital of Soochow University, 92 Zhongnan Street, Suzhou, Jiangsu 215025, P.R. China
E-mail: w7t927@163.com; wj196312@vip.163.com

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Introduction

Congenital heart disease (CHD) is a common congenital developmental malformation in pediatrics. Cardiopulmonary bypass (CPB) is a representative technique of cardiac surgery, and it has been increasingly improved and widely popularized in recent years. However, CPB is an invasive treatment, whose postoperative complications, such as postoperative infection, low cardiac output syndrome, heart failure, arrhythmia and bleeding, should be paid enough attention to. The surgical trauma, low temperature, ischemia reperfusion and the contact with the surface of artificial materials, such as the artificial tube during CPB, can activate the immune system in the body, lead to activation of inflammatory cells, coagulation system, complement system, white blood cells (WBCs) and platelets, and release inflammatory factors (1-3). Lipoxin A_4 (LXA_4), as an important endogenous anti-inflammatory mediator, can reduce the production of inflammatory factors and inhibit the activation and chemotaxis of neutrophils (4). This study compared the LXA_4 levels between child patients with CHD and non-CHD children, observed the dynamic changes of LXA_4 in the perioperative period of CPB, and analyzed its correlation with blood routine indexes, high-sensitivity C-reactive protein (hs-CRP) and other clinical indexes, so as to find the correlation between LXA_4 and the inflammatory response of CHD.

Patients and methods

Clinical specimens. The clinical specimens used in this study were obtained from child patients with CHD receiving CPB in the Department of Cardiovascular Surgery of the Children's Hospital of Soochow University (Suzhou, China), from February 2017 to December 2017. The children were aged from 10 days to 3 years without genetic diseases and signs of acute infection. The study was approved by the Ethics Committee of Children's Hospital of Soochow University. Patients who participated in this research had complete clinical data. Signed written informed consents were obtained from the patients and/or their guardians.

All child patients received intravenous-inhalational anesthesia and tracheal intubation, and extracorporeal circulation was constructed via intubation in the aorta and

superior and inferior vena cava. Cold crystalloid cardioplegia (15-20 ml/kg), at 4˚C, was injected into the aortic root of most patients for myocardial protection, while HTK solution (50 ml/kg) was injected into 1 case with complete transposition of the great artery and into 1 case with left coronary anomaly. Conventional ultrafiltration and modified ultrafiltration were combined (CUF + MUF), and colloidal fluid was used as the priming fluid. There were 12 cases of low temperature (30-33˚C) extracorporeal circulation, 3 cases of low to medium temperature (25-28˚C) extracorporeal circulation and 1 case of deep hypothermic circulatory arrest (DHCA). All child patients had sinus cardiac re-beat, and extracorporeal circulation was withdrawn successfully after complete rewarming. Patients were routinely admitted into the Pediatric Intensive Care Unit (PICU) and returned to the Cardiothoracic Surgical Ward, after their condition became stable, where they received treatments, such as routine infection prevention, myocardial nutrition, diuretic therapy, and maintenance of water-electrolyte and acid-base balance. After operation, tracheal intubation was performed, and ventilator was connected for assisted ventilation which was withdrawn as soon as possible after hemodynamic stability. The ventilator was withdrawn in 9 patients on the day of operation, and it was used for 115 h in maximum. Specific clinical data are shown in Table I.

Table I. Clinical information and data of child patients receiving CPB.

Information/data	CPB group (n=16)
Age (months)	3.5 (2, 11)
Sex (male/female)	10/6
Weight (kg)	7.063±3.228
Atrial septal defect (n)	5
Ventricular septal defect (n)	11
Complete transposition of great artery (n)	1
Left coronary anomaly (n)	1
CPB time (min)	92.938±45.261
Aortic clamping time (min)	54.062±24.960
Time in the PICU (days)	4.188±2.834
Duration of postoperative fever (days)	2.875±1.857
Application time of ventilator (h)	5 (3.25, 52.5)

CPB, cardiopulmonary bypass; PICU, Pediatric Intensive Care Unit.

Experimental instruments and materials. LXA_4 enzyme-linked immunosorbent assay (ELISA) kit (USCN Life Sciences, Inc., Wuhan, China); microplate reader (Thermo Fisher Scientific, Inc., Waltham, MA, USA); Terumo Advanced Perfusion System 1 artificial heart-lung machine, Terumo membrane oxygenator (both from Terumo, Tokyo, Japan); Sorin ultrafilter (Sorin Biomedica Spa, Saluggia, Italy); and Sysmex XN-9000 blood analyzer (Sysmex Corp., Kobe, Japan).

Experimental methods. Peripheral blood (0.5 ml) was collected from child patients in CPB group before operation (Tc), after operation (T0), at 1 day after operation (T1), at 3 days after operation (T3), and at 7 days after operation (T7); and 0.5 ml peripheral blood was also collected from 17 child patients without a history of CHD (control group), who were scheduled to undergo hernia and urinary malformation repair. The peripheral blood was placed in an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube, centrifuged at 1,000 x g, at room temperature, for 5 min to collect the plasma and cryopreserved at -80˚C. Every specimen was thawed to be detected. The optical density (OD) value of the standard and clinical specimens was determined using the microplate reader (wavelength, 450 nm), according to the instructions of the ELISA kit, and the standard curve was drawn, followed by LXA_4 quantification using the ELISA Calc software. At the same time, blood routine indexes and hs-CRP levels were detected, at each time-point.

Statistical analysis. GraphPad 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA) and Statistical Product and Service Solutions (SPSS) 18.0 software (IBM Corp., Armonk, NY, USA) were used for statistical analysis and plotting. Normally distributed data were expressed as mean ± standard deviation (mean ± SD), and non-normal distribution data were expressed as median (M) and interquartile spacing (Q). For normal distribution data, t-test was used for the comparison between two groups. One-way ANOVA was used for the comparison among three or more groups. LSD test was used for equal variances, and Dunnett's test for unequal variances. Non-parametric tests were used for non-normal distribution data. Pearson's linear correlation analysis was adopted for the correlation analysis between two indexes. $P<0.05$ was considered to indicate a statistically significant difference.

Results

There were no significant differences in age and sex between CPB and control group, and the plasma LXA_4 level in CPB group was significantly lower at Tc compared to that in the control group ($P<0.01$) (Table II). LXA_4 showed an increasing trend after operation, which was significantly increased at T1 compared to that at Tc ($P<0.01$), continued to show an upward trend, and was still significantly increased at T3 and T7 compared to that at Tc ($P<0.01$) (Fig. 1A). WBC was increased significantly at T1 and T3 ($P<0.05$) for a short time, and dropped at T7 near the level at Tc (Fig. 1B). The proportion of neutrophils (N%) was obviously and rapidly increased at T0 compared to that at Tc ($P<0.01$), for a long time, and was still remarkably increased at T7 compared to that at Tc ($P<0.01$) (Fig. 1C). The dynamic changes in hs-CRP were similar to those of WBC. hs-CRP level was significantly increased at T1 and T3 compared to that at Tc ($P<0.01$) and dropped at T7 near the level at Tc (Fig. 1D).

According to Pearson's linear correlation analysis, the CPB time and aortic clamping time were positively correlated with the time in the PICU, application time of ventilator and hs-CRP level at T0 (Table III). LXA_4 had no correlations with age, weight, CPB time and aortic clamping time. No correlation was found between LXA_4 and WBC, N% and hs-CRP at the corresponding time-points.

Table II. Comparison of clinical data and LXA_4 expression levels between CPB and control group at Tc.

Category	CPB group (n=16)	Control group (n=17)	P-value
Age (months)	3.5 (2, 11)	15 (1, 22.5)	>0.05
Sex (male/female)	10/6	13/4	>0.05
LXA_4 (pg/ml)	2,552 (1,879, 4,586)	17,129 (8,297, 33,267)	<0.01

LXA_4, lipoxin A_4; CPB, cardiopulmonary bypass; Tc, before operation.

Table III. Correlation analysis between indexes in child patients receiving CPB.

Categories	Correlation coefficient	P-value
CPB time - time in the PICU	0.7195	0.002[b]
Aortic clamping time - time in the PICU	0.7181	0.002[b]
CPB time - application time of ventilator	0.6570	0.006[b]
Aortic clamping time - application time of ventilator	0.6152	0.011[a]
CPB time - hs-CRP (T0)	0.8922	0.042[a]
Aortic clamping time - hs-CRP (T0)	0.9084	0.033[a]

[a]P<0.05 and [b]P<0.01. CPB, cardiopulmonary bypass; PICU, Pediatric Intensive Care Unit; hs-CRP, high-sensitivity C-reactive protein.

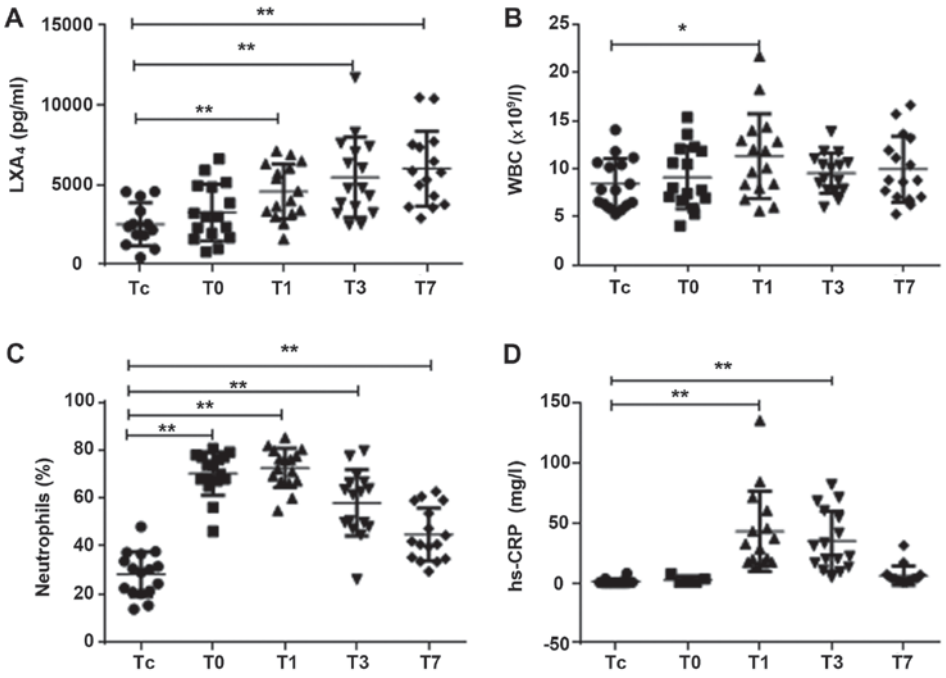

Figure 1. The dynamic variations of LXA_4, WBC, N%, and hs-CRP in patients of the CPB group, at 5 different time-points. Peripheral blood was collected from children with CPB at 5 time-points: before operation (Tc), after operation (T0), 1 day after operation (T1), 3 days after operation (T3), and 7 days after operation (T7). (A) LXA_4 was detected by ELISA, and an upward trend was observed after operation, compared to the LXA_4 levels before operation (**P<0.01). (B and D) WBC and hs-CRP increased significantly at 1 day after operation (*P<0.05, **P<0.01). (C) N% significantly and rapidly increased at 1 day after operation, and the high increase lasted longer (**P<0.01). LXA_4, lipoxin A_4; WBC, white blood cell; N%, proportion of neutrophils; hs-CRP, high-sensitivity C-reactive protein; ELISA, enzyme-linked immunosorbent assay.

Discussion

LXA_4, as the metabolite of arachidonic acid, is the first endogenous lipid mediator found with extensive functions of anti-inflammation and promotion of inflammatory resolution, and is hailed as the 'braking signal' of inflammatory response (5). LXA_4 is the metabolite produced by arachidonic acid through the lipoxygenase (LOX) pathway in the

inflammatory process, as well as an endogenous antagonist of leukotrienes, and its major function is to inhibit the chemotaxis of neutrophils and adhere to endothelial cells, which is related to the inflammatory resolution (6). LX can also stimulate endothelial cells to produce prostacyclin and nitric oxide, help dilate blood vessels and improve blood flow (7). Under the influence of inflammation or disease and the stimulus of inflammatory factors, different LOXs will sequentially catalyze arachidonic acid to promote LX synthesis, in which interactions between cells, such as that between leukocytes, between leukocytes and platelets and between leukocytes and epithelial cells, are needed. If something is wrong in these interactions, the LX synthesis will be affected (8,9). There have been no studies on the correlations of LXA_4 with CHD and CPB. Reina-Couto *et al* have found that the severity of chronic heart failure (CHF) is negatively related to the plasma LXA_4 level, and LXA_4 can be considered as a valuable marker for the risk stratification of CHF, which may be related to the defects in pro-inflammatory and anti-inflammatory mechanisms of CHF (10). Child patients with CHD are often accompanied with cardiac insufficiency in different degrees, and it has been found that the LXA_4 level declines in child patients with CHD compared to that in normal children, in consistency with the research results of Reina-Couto *et al*. Moreover, child patients with CHD are prone to concurrent infection, and some studies have found that they often suffer from immunodeficiency in different degrees, such as the lower chemotaxis and phagocytosis of neutrophils, complement function and secretion of IgA than normal subjects (11). The immunodeficiency in child patients with CHD may be related to the low content of LXA_4, which needs to be confirmed via further experiments.

In the present study, it was found that LXA_4 showed a gradually increasing trend after CPB, and its average level at T7 was still significantly different from that in control group. The contact with a large number of artificial materials, surgical trauma and pulmonary ischemia-reperfusion injury during CPB can lead to activation of the monocyte/macrophage and the release of a large number of inflammatory factors in the body in order to resist stress and promote repair (1). Serhan *et al* (12) were the first to find that inflammation resolution is a highly-ordered active process closely regulated by a large number of lipid mediators produced by unsaturated fatty acid via enzymatic catalysis. LXA_4 can bind to its coupled receptor to downregulate the expression of inflammatory factors in tissues through a variety of signaling pathways. Moreover, LXA_4 can inhibit the chemotaxis of leukocytes towards the inflammatory site and promote macrophages to phagocytose apoptotic granulocytes and other damaged cells, thereby inhibiting the inflammatory process, promoting the inflammation resolution, and exerting specific anti-infection and anti-inflammatory effects in inflammatory infection-related diseases (13). Therefore, it is speculated that the inflammatory response after CPB increases the synthesis of LXA_4 with a strong anti-inflammatory effect.

In this study, it was also found that WBC, N% and hs-CRP were transiently increased at T0 and were most significantly increased at T1, which is consistent with the results of a number of experiments (14,15). CRP is an acute-phase reactive protein and an important inflammatory marker, which will obviously increase in the case of acute inflammation and trauma in the body (16). With the application of anti-inflammatory and anti-infective drugs after operation and inflammation control, CRP gradually declines, and the body temperature also gradually drops to normal. We also found that the CPB time and aortic clamping time were positively correlated with the time in the PICU, application time of ventilator and hs-CRP level at T0. CPB time and aortic clamping time are often related to the complexity of operation and general condition of patients. The longer the surgical exposure is, the higher the probability of infection will be. Therefore, from the perspective of postoperative complications and recovery, the CPB time and aortic clamping time should be reduced as soon as possible during operation, so as to promote postoperative recovery.

In conclusion, the LXA_4 level in child patients with CHD is lower than that in normal children, but it has dynamic changes and gradually increases during CPB under the influence of inflammatory response. However, changes in LXA_4 have no correlation with inflammation and infection indexes, such as WBC, N% and hs-CRP, so it cannot be used as a sensitive index for monitoring inflammation, but can be used to evaluate the recovery of the disease.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MW, HZ and JW designed the study. MW, ZF and JL collected the samples and performed the experiments. MW, ZF and HZ were mainly devoted to the data analysis. MW, HZ and JW prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Children's Hospital of Soochow University (Suzhou, China). Patients who participated in this research had complete clinical data. Signed written informed consents were obtained from the patients and/or their guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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