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ARTIGO ORIGINAL

Lignocaine Preconditioning for Protamine-Induced Pulmonary Vasoconstriction in Pediatric Cardiac Surgery

Pré-Condicionamento com Lidocaína para Vasoconstrição Pulmonar Induzida por Protamina em Cirurgia Cardíaca Pediátrica

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ABSTRACT

Introduction: Protamine, a highly alkaline peptide, neutralizes heparin's anticoagulant effect but poses risks such as pulmonary vasoconstriction. This study evaluates lignocaine preconditioning as a method to mitigate these effects in pediatric cardiac surgeries.

Methods: In a prospective, randomized, double-blind study of 88 patients, lignocaine preconditioning reduced adverse pulmonary effects compared to saline placebo. Patients were divided into four groups: two with pulmonary hypertension and two without. Lignocaine preconditioning was administered in half the groups to assess its effect on pulmonary function post-protamine administration. Group I—non-pulmonary hypertension with lignocaine preconditioning; Group II—non-pulmonary hypertension with normal saline (as a placebo); Group III—pulmonary hypertension with lignocaine preconditioning; and group IV—pulmonary hypertension with normal saline (as a placebo). At various points during the surgery, hemodynamic parameters, pulmonary functions, and inflammatory markers were monitored. A p-value <0.05 was considered statistically significant.

Results: Pulmonary vasoconstriction occurred in 9.09% of cases after protamine administration. The II and IV Groups exhibited an increase in mean airway pressure (Paw), respiratory index (RI), alveolar-arterial oxygen difference (A-aDO_2), pulmonary artery pressure (PAP), and decreased dynamic pulmonary compliance (Cydn) and oxygen index (OI) after protamine administration. However, these changes were not observed in I and III Groups with lignocaine preconditioning. Plasma levels of TXB_2 in the II and IV Groups were higher than in the I and III Groups, but 6-keto-PGF1 alpha levels were lower in the II and IV Groups than in the I and III Groups.

Conclusion: Lignocaine preconditioning reverses protamine-induced pulmonary vasoconstriction and improves pulmonary function.

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RESUMO

Introdução: A protamina, um peptídeo altamente alcalino, neutraliza o efeito anticoagulante da heparina, mas apresenta riscos como vasoconstrição pulmonar. Este estudo avalia o pré-condicionamento com lignocaína como um método para mitigar esses efeitos em cirurgias cardíacas pediátricas.

Métodos: Em um estudo prospectivo, randomizado e duplo-cego de 88 pacientes, o pré-condicionamento com lignocaína reduziu os efeitos pulmonares adversos em comparação ao placebo salino. Os pacientes foram divididos em quatro grupos: dois com hipertensão pulmonar e dois sem. O pré-condicionamento com lignocaína foi administrado em metade dos grupos para avaliar seu efeito na função pulmonar após a administração de protamina. Grupo I — hipertensão não pulmonar com pré-condicionamento com lignocaína; Grupo II — hipertensão não pulmonar com solução salina normal (como placebo); Grupo III — hipertensão pulmonar com pré-condicionamento com lignocaína; e grupo IV — hipertensão pulmonar com solução salina normal (como placebo). Em vários pontos durante a cirurgia, parâmetros hemodinâmicos, funções pulmonares e marcadores inflamatórios foram monitorados. Um valor de p<0,05 foi considerado estatisticamente significativo.

Resultados: Vasoconstrição pulmonar ocorreu em 9,09% dos casos após administração de protamina. Os Grupos II e IV exibiram aumento na pressão média das vias aéreas (Paw), índice respiratório (RI), diferença alvéolo-arterial de oxigênio (A-aDO_2), pressão da artéria pulmonar (PAP) e diminuição da complacência pulmonar dinâmica (Cydn) e índice de oxigênio (OI) após administração de protamina. No entanto, essas alterações não foram observadas nos Grupos I e III com pré-condicionamento com lignocaína. Os níveis plasmáticos de TXB_2 nos Grupos II e IV foram maiores do que nos Grupos I e III, mas os níveis de 6-ceto-PGF1 alfa foram menores nos Grupos II e IV do que nos Grupos I e III.

Conclusão: O pré-condicionamento com lignocaína reverte a vasoconstrição pulmonar induzida por protamina e melhora a função pulmonar.

INTRODUCTION

Protamine is a polypeptide composed primarily of arginine. It has a molecular weight of approximately 4300 daltons and is highly positively charged. The primary commercial source of protamine is salmon sperm. Protamine is used to neutralize heparin's anticoagulant effect. Positively charged arginine moieties on protamine form ionic bonds with negatively charged sulfate groups on heparin and neutralize heparin's effect. In parallel, the binding of protamine to heparin dissociates the heparin-anti-thrombin III (AT III) complex, leading to the recovery of the original anti-thrombin activity. Therefore, protamine has been used in cardiac surgeries to neutralize anticoagulant heparin.[1] The adverse effects associated with protamine administration, including systemic hypotension, pulmonary vasoconstriction, allergic reactions, pulmonary hypertension, bronchoconstriction, and bradycardia, range from 0.06% to 10.6%.[2] In addition, its toxicology is also due to its interaction with the peptides on the surfaces of the vasculature and blood cells, which involves membrane receptors and ion channels targeted by different vasoactive compounds.[3] Even though the detailed mechanism of the toxicology of protamine remains unclear. Protamine's interaction with immunoglobulins and the activation of the complement system, which triggers the release of a wide variety of inflammatory mediators,[4,5] are associated with the development of the vast majority of adverse effects, ranging from minor hemodynamic instability to life-threatening anaphylactic incidents.[6] Despite its risks, including a poor therapeutic index and life-threatening complications, protamine remains the standard drug for neutralizing unfractionated heparin during cardiac surgery. The Guan *et al*, study between 2012 and 2016 involved 4160 cases of cardiac surgery; from that, only five cases experienced catastrophic pulmonary vasoconstriction.[7] This indicates the incidence rate is 1 in 832 (4160/5). Protamine is a double-edged sword. Without it, on-pump congenital cardiac repairs are not possible.

Lignocaine is the most commonly used local anesthetic in clinical practice. In addition to blocking the inhibitory effects of the nerve signal, lignocaine has a regulatory function in the immune system, which has been proven to be beneficial in multilinks of the inflammatory response.[8] Due to its significant anti-inflammatory characteristics, lignocaine can alleviate acute lung injuries caused by protamine and cardiopulmonary bypass (CPB).

There is a paucity of literature on lignocaine's dose, timing of administration, and effectiveness in treating catastrophic pulmonary vasoconstriction induced by protamine administration after separation from CPB in pediatric congenital cardiac repairs. Lignocaine's anti-inflammatory properties may offer a protective mechanism, yet its specific effects on protamine-induced pulmonary vasoconstriction remain under-researched.

MATERIAL AND METHODS

The study was conducted from August 2022 to November 2023 after obtaining ethical approval from the Committee on Human Research Ethics, SAMSRI, nº. CAU/306/Q/2022, as per the Helsinki Declaration and revised guidelines of 2013. The nature of the study was explained to the participants and the parents included in the study. A written informed consent was obtained from the parents or guardians. The study population consisted of pediatric patients of either sex at the age of 1 to 10 years with physical status II and III of the American Society of Anaesthesiologists (ASA) with acyanotic congenital heart disease, scheduled for elective open-heart surgery that requires CPB. They all received unfractionated heparin as an anticoagulant on CPB and reversed it with protamine while coming off the pump.

I. Study Design

This study was a prospective, randomized, and double-blind study. The sample size of the study was calculated using an online sample size calculator. Eighty-eight participants meeting the study criteria were randomly divided into four groups using a roll of the die. The two with pulmonary hypertension and two without. Lignocaine preconditioning was administered in half the groups to assess its effect on patients' pulmonary functions and hemodynamics. Group-I (nonpulmonary hypertension with lignocaine preconditioning), Group-II (nonpulmonary hypertension with normal saline as a placebo), Group-III (pulmonary hypertension with lignocaine preconditioning), and Group-IV (pulmonary hypertension with normal saline as a placebo). The volume of the preconditioning study drug or placebo (normal saline, NS) was kept at 5 mL in patients. A pulmonary/systemic circulatory pressure ratio of ≤0.3 was considered normal, while a ratio >0.3 was considered pulmonary hypertension.[9-13] Group 'A' prepared the study drugs and managed the patient, and Group 'B' was responsible for the patient's records in the cardiac care unit (CCU), while Group 'C' participated in the randomization process. People from Groups 'B', 'C' and the patient were kept unaware of the study drug to enable double blinding.

II. Inclusion Criteria and Exclusion Criteria

In this study, patients of both sexes, between the ages of 1 and 10 years, with ASA grades II and III, who underwent elective acyanotic congenital cardiac repair on CPB under general anesthesia, were included. Exclusion criteria from this study were patients with cyanotic heart disease, less than one year or more than 10 years, refusal to consent or participate in the study, history of protamine allergy, infection, preoperative hemodynamic instability, preoperative respiratory disease, emergency surgery, and abnormalities found in the lung, liver, kidney, or coagulation function.

III. Anaesthetic Protocol

All patients underwent a pre-anesthetic evaluation a day before surgery, with particular consideration to elicit any new complications and review previous anaesthetic history and drug sensitivity. All routine investigations were re-checked, and procedures were explained to the guardians. The patients fasted according to hospital protocol before elective surgery. Identification of the patient in the operating room (OR), a short preoperative history was taken along with the clinical examination, and routine investigations were re-checked. Oxygen saturation in all four limbs was checked without oxygen and with oxygen. Children under five years were premedicated by oral route with midazolam 0.5 mg/kg and ketamine 5 mg/kg along with glycopyrrolate in OR. Non-cooperative children or those over five years of age received inj. midazolam 0.03 mg/kg and inj. ketamine 0.5 mg/kg by intravenous (IV) route. The patient's vital signs were monitored, including blood pressure, electrocardiogram (ECG), and oxygen saturation. Induction of anesthesia was performed with IV opioids (inj. fentanyl 10 µg/kg) and benzodiazepines (inj. midazolam 0.1 mg/kg). For muscle relaxation, inj. pancuronium (0.1 mg/kg) was used. Intubation was performed after adequate muscle relaxation. Additional monitoring, including invasive blood pressure, central venous pressure, and rectal and nasal temperature, was performed after intubation. Anaesthesia was maintained with an infusion of inj. midazolam 0.02 mg/kg/h and inj. fentanyl 2 µg/kg/h as per patient requirement.

IV. CPB and Surgical Protocol

All patients received a standard CPB and surgical protocol. After sternotomy, the patient's vena cava and ascending aorta were cannulated for venous drainage and arterial perfusion. The patients were heparinated with 3 mg/kg and taken on the pump after ACT > 480 seconds. The CPB technique was standardized for all patients. Supplemental heparin was administered into the CPB circuit from time to time to maintain ACT > 480 seconds. The CPB flow was maintained at 2 L/minute/m^2 and the pressure was >30 mmHg. Core cooling was used in all patients; rectal and esophageal probes monitored temperature. The cardiac surgeon directly measured the PAP in the PA.

V. Diagnostic Criteria

After successfully weaning from the CPB, adequate volume loading from the pump, and stable hemodynamics. The I and III Groups received lignocaine (2 mg/kg in a 5 mL syringe), and the II and IV Groups received NS as a placebo (5 mL in a 5 mL syringe) one minute before neutralization of heparin with protamine. After CPB, the patients reversed with protamine sulfate (1.3 mg/1 mg of heparin) slowly in five minutes. Protamine-mediated PAC is considered when constriction occurs within 30 minutes of the protamine administration and meets one or more of the criteria[6,8,14,15] from the following:

- PAP increases by at least 25%, requiring inotropic drugs or reinstitution of CPB after administration of protamine, a ≥25% decrease from the baseline, or a ≥10% decrease in systemic arterial pressure;
- PO_2 decreased, requiring ventilatory support, indicating non-cardiogenic pulmonary edema;
- Peak inspiratory airway pressure elevation greater than 5 mmHg indicates bronchospasm.

These events lead to pulmonary hypertension, which may be clinically insignificant if hemodynamic instability does not occur.

PAP is continuously measured through a needle of 22G placed into the PA by a surgeon at (Min0) baseline, 1 minute before CPB, (Min1) 1 minute before the protamine start, (Min2) 1 minute after the protamine start, (Min3) 3 minutes after the protamine start, (Min5) 5minutes after the protamine start. Heart rate (HR), blood pressure (BP), mean arterial pressure (MAP), airway pressure (Paw), and dynamic lung compliance (Cdyn) were recorded at 6 points: Min1, Min2, Min3, Min5, (10 minutes after the protamine ends), and Min15 (15 minutes after the protamine ends).

In the analysis of arterial blood gas (ABG), the alveolar-arterial oxygen gradient (A-aDO_2), the respiratory index (RI), which is the relationship between P (A-a) DO_2 and the PaO_2, and the oxygenation index [(OI) = (mean airway pressure × FiO_2 × 100)/PaO_2)] were documented at 3-time points: Min0, Min1, and Min15.

The sample collection and cryopreservation of radial artery blood and right ventricular blood were performed at the Min1 and Min15 time points. Thromboxane B2 (TXB_2) and 6-keto-prostaglandin-1 alpha (6-keto-F1a) in plasma were detected by enzyme-linked immunosorbent assay (ELISA). In addition, the data on protamine adverse reactions was recorded. Routine perioperative data were collected, including age, weight, height, sex, types of operation, preoperative EF value, ACT value after protamine neutralization, CPB time, aortic cross-clamp time, and operation time.

VI. Parameters and Statistical Analysis

A structured data collection tool sought to ascertain 41 variables covering demographic and anthropometric characteristics, anesthesia, and surgery information. To measure laboratory parameters, blood and urine samples were collected and analyzed at different intervals. The Shapiro-Wilk test was used for normally distributed data. Continuous variables were expressed as mean±SD and compared across groups using one-way analysis of variance (ANOVA). Categorical variables were expressed as the

number and percentage of the total group and analyzed using the chi-square tests or Fisher's exact test. Hemodynamic indicators, pulmonary inflammatory factors, and pulmonary function indexes were assessed, and changes over time across the groups were performed using repeated-measures ANOVA in all the groups. Spearman's correlation analysis was performed to evaluate the relationship between pulmonary hemodynamic indicators and inflammatory factors. The SPSS 20.0 statistical package was used, and a *p*-value <0.05 was considered statistically significant.

RESULTS

Eighty-eight patients with acyanotic congenital cardiac disease were recruited and scheduled for elective on-pump cardiac surgery under general anesthesia. The patients were randomly assigned to each group (n=22). As shown in Table 1, there was no significant difference in the demographic profiles of the four groups (p>0.05).

As shown in Table 2, the clinical profiles such as diagnosis, ejection fraction, cardiopulmonary bypass time, aortic cross-clamp time, and activated clotting time of the four groups were also comparable (p>0.05).

As shown in Table 3, protamine-induced pulmonary vasoconstriction occurred in 8 cases of 88 study participants, representing 9.09%. In Groups II (cases 1-3) and IV (cases 4–7), protamine-induced catastrophic pulmonary vasoconstriction occurred within 3 minutes after the protamine infusion. The symptoms were bronchospasm, hypotension, bradycardia, and pulmonary hypertension. In Groups II and IV, three patients became asymptomatic within 30 seconds without any intervention, while the other four patients received calcium chloride for mild/moderate hypotension or adrenaline for severe hypotension and hyperventilated with 100% oxygen for hypoxia. In Group III, a patient had pulmonary vasoconstriction that occurred at 2 minutes of protamine infusion. That caused an increase in pulmonary artery and airway pressures. In addition, a sharp decrease in systemic blood pressure caused right ventricular distension and bradycardia. The patients received multiple doses of adrenaline, dopamine, and milrinone and were hyperventilated with 100% oxygen.

There was no pulmonary vasoconstriction in Group I. In Groups II and IV, an increase in protamine-induced adverse reactions was observed. This shows X^2=5.184 with Fisher's exact test (p=0.027). The intraoperative hemodynamic data during protamine administration are presented in Table 4. The heart rate and mean arterial pressure before protamine administration and 10 minutes after protamine showed no significant difference in all four groups. However, three minutes after protamine, all four groups showed a reduced heart rate, and at five minutes, all groups showed a reduced mean arterial pressure, as shown in Table 4. Compared to Group I, the MAP in Group IV at the Min3 point was significantly lower. The PAP in Groups II and IV at the Min3 point was significantly higher than the Min0 point, while the PAP in Group II at the Min5 point was significantly higher than Group I. As shown in Table 4 and Figure 1, Paw in Group II at the Min5 point was significantly higher than Min0 and the Group I, while Paw in Group IV at the Min5 point was significantly higher than the Group III.

Cdyn in Group II at the Min5 points decreased significantly more than the Min0 point. However, Cydn in the IV Group at the Min3 and Min5 points were significantly lower than the Groups I and III, as shown in Table 4 and Fig. 2.

Table 1. Comparison of the demographic profile of patients

Variables	Group I	Group II	Group III	Group IV	*p*-value
Age (yrs)	4.31±2.92	4.62±2.75	4.46±2.51	4.61±2.83	0.685
Weight (kg)	15.64±5.79	15.98±7.31	15.83±8.73	15.58±8.17	0.742
Height (cm)	101.21±16.57	102.43±17.27	102.12±18.41	103.83±16.38	0.531
M&F % ratio	(35%:65%) 1:1.85	(40%:60%) 1:1.5	(30%:70%) 1:2.33	(45%:55%) 1:1.22	0.274

Data are presented as means ± standard deviation (SD), percentages, and ratios. Group I- non-pulmonary hypertension with lignocaine preconditioning; Group II-non-pulmonary hypertension with normal saline; Group III- pulmonary hypertension with lignocaine preconditioning; Group IV- pulmonary hypertension with normal saline. Not significant p>0.05. F-female, , M-male, Yrs-years.

Table 2. Comparison of the clinical profiles of patients

Variables	Group I	Group II	Group III	Group IV	*p*-value
ASD: VSD %	40%: 60%	30%:70%	40% :60%	35%: 65%	0.578
EF %	68.52±6.31	69.45±6.89	69.38±6.75	71.53±5.24	0.692
CPB in min	49.57±19.06	53.06±21.35	48.10±14.54	46.32±20.68	0.385
Aortic cross-clamp in min	29.55±13.85	33.32±15.82	31.90±17.35	35.70±16.53	0.495
Post-protamine ACT in sec	123.35±13.47	125.35±11.86	124.50±16.39	128.80±17.63	0.417

Data are presented as means ± standard deviation (SD), ratios, and percentages. ASD- atrial septal defect, ACT-activated clotting time, CPB-cardiopulmonary bypass, EF- ejection fraction, min-minutes, VSD- ventricular septal defect.

Table 3. Comparison of the clinical manifestation of catastrophic pulmonary vasoconstriction

Variables	PAP baseline -> max (mmHg)	Paw baseline -> max (mmHg)	Cydn baseline -> min (mL/cmH_2O)	BP baseline -> mini (mmHg)	HR baseline -> mini (beats/min)
Group II Cases-1	16 -> 43	9 -> 29	10 -> 8	131/75(98) -> 88/45(65)	115 -> 111
Group II Cases-2	17 -> 31	13 -> 27	15 -> 6	104/57(82) -> 81/39(57)	117 -> 96
Group II Cases-3	13 -> 36	14 -> 29	27 -> 8	95/58(69) -> 65/34(47)	93 -> 81
Group IV Cases-4	23 -> 35	14 -> 18	18 -> 12	79/47(56) -> 47/34(40)	113 -> 86
Group IV Cases-5	22 -> 38	16 -> 22	15 -> 3	71/38(52) -> 67/38(48)	122 -> 117
Group IV Cases-6	15 -> 23	15 -> 17	16 -> 10	101/65(76) -> 92/51(64)	141 -> 132
Group IV Cases-7	36 -> 49	18 -> 25	12 -> 5	81/52(67) -> 65/41(52)	137 -> 134
Group III Case-8	138 -> 86	12 -> 18	10 -> 9	113/68(84) -> 51/33(46)	116 -> 102

Group I- non-pulmonary hypertension with lignocaine preconditioning; Group II-non-pulmonary hypertension with normal saline; Group III- pulmonary hypertension with lignocaine preconditioning; Group IV- pulmonary hypertension with normal saline. Mini-minimum, PAP- pulmonary artery pressure, Paw-airway pressure, Cydn- dynamic pulmonary compliance, BP-blood pressure, HR-heart rate.

Table 4. Comparison of Intraoperative clinical variables

Variables	Min0	Min1	Min3	Min5	Min10
Heart Rate (HR) (beats/min)					
I	113.59±28.13	115.04±15.68	113.71±17.06	111.49±15.67	114.58±17.11
II	120.51±14.65	118.76±14.27	116.68±15.57	118.38±9.94	117.10±11.72
III	116.03±17.31	115.07±15.52	114.09±15.19	114.58±17.78	115.23±14.16
IV	122.24±15.77	119.32±16.14	120.41±16.23	118.15±19.54	119.07±12.53
Mean Arterial Pressure (MAP) (mmHg)					
I	73.52±11.78	76.78±13.72	81.13±14.19	81.88±13.14	75.39±7.72
II	73.95±13.22	74.84±15.37	74.67±16.15	80.72±13.48	73.68±10.21
III	73.62±14.38	75.57±13.41	75.58±15.53	80.51±16.23	74.10±14.59
IV	71.82±16.23	74.15±15.56	68.76±15.92$^{\alpha}$	76.95±15.39	71.33±13.58
Pulmonary Artery Pressure (PAP) (mmHg)					
I	17.28±5.17	17.39±4.89	20.56±5.17	19.52±4.36	N/A
II	19.62±4.89	19.88±5.73	23.98±8.72$^{\alpha*}$	21.38±5.47	N/A
III	28.15±7.31	27.47±6.68	31.47±13.52	30.71±8.71	N/A
IV	27.39±8.47	27.89±6.75	32.62±11.28*	31.03±9.32	N/A
Mean Airway Pressure (Paw) (mmHg)					
I	13.79±2.35	14.27±4.62	15.82±3.18	15.57±2.38	13.68±2.44
II	13.63±3.27	14.05±3.11	16.69±7.55	18.26±6.53$^{\alpha*}$	13.71±3.87
III	14.39±2.15	14.79±2.68	15.73±3.92	15.11±3.08	14.36±2.61
IV	14.68±2.98	14.62±3.09	16.11±5.38	18.69±3.47$^{\beta}$	14.39±3.26
Dynamic Pulmonary Compliance (Cdyn)(mL/cmH_20)					
I	14.29±4.05	14.27±3.68	12.68±4.33	12.09±4.39	14.87±4.16
II	13.57±5.58	13.30±5.39	11.37±4.41	10.38±4.78*	14.49±5.26
III	13.18±4.83	13.05±4.75	12.93±4.68	12.15±5.37	12.83±4.12
IV	13.56±3.61	13.00±3.49	11.19±4.17$^{\alpha}$	9.49±4.11$^{\beta}$	13.33±5.84

Data are presented as mean ± SD. Significant differences are expressed as follows: Data compared with I- Group - $^{\alpha}$, with III- Group- $^{\beta}$ and with Min0 time point- * if $p<0.05$. N/A-not applicable time points-Min0-1 min before protamine administration, Min1- 1 min after protamine administration, Min3- 3 min after protamine administration, Min5- 5 min after protamine administration, Min10- 10 min after protamine administration end.

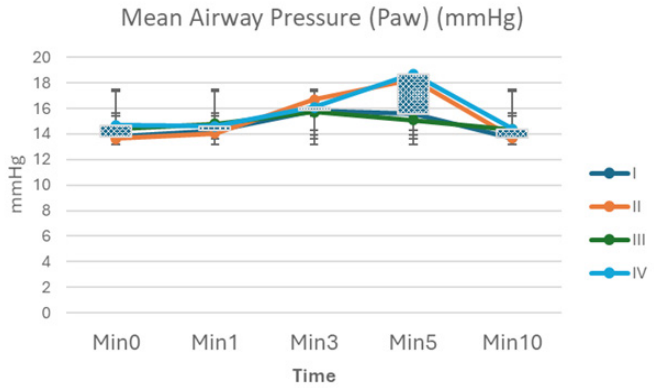

Figure 1. Intraoperative Pulmonary Artery Pressure
Pulmonary artery pressure at time points: Min0: 1 min before protamine administration, Min1: 1 min after protamine administration, Min3: 3 min after protamine administration, Min5: 5 min after protamine administration, Min10: 10 min after protamine administration end.

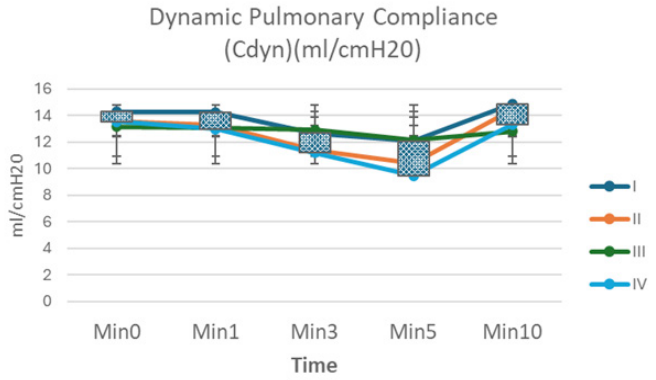

Figure 2. Intraoperative Dynamic Pulmonary Compliance (Cdyn) (mL/cmH$_2$0)
Dynamic pulmonary compliance at time points: Min0: 1 min before protamine administration, Min1: 1 min after protamine administration, Min3: 3 min after protamine administration, Min5: 5 min after protamine administration, Min10: 10 min after protamine administration.

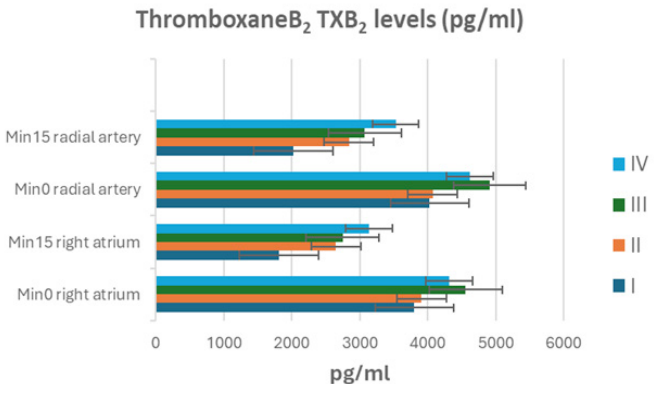

Figure 3. Intraoperative ThromboxaneB2 (TXB2) Levels in (pg/mL)
Min0-1 min pre protamine administration, Min15-15 min after protamine administration.

The plasma TXB_2 levels and plasma 6-keto-PGF-1alpha levels in the radial artery and the right atrium before administration of protamine and 15 minutes after administration are shown in Figs. 3 and 4. They are compared in all four groups at different time points.

As shown in Table 5, Spearman's correlation analysis was performed to assess the relationship between the plasma TXB_2 level in the radial artery at the Min0 point and the PAP value at the Min3 point. The Paw value at the Min5

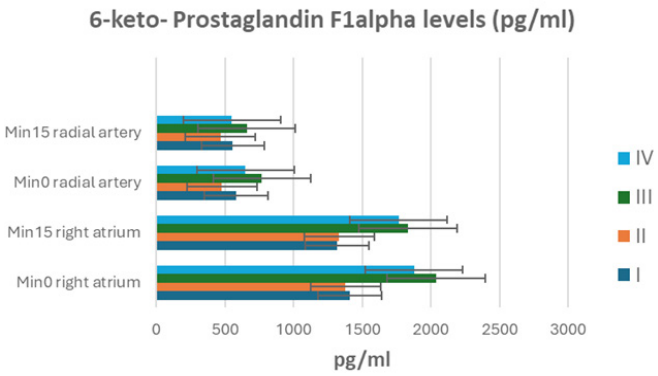

Figure 4. Intraoperative 6-keto- Prostaglandin F-1 alpha Levels in (pg/mL)
Min0- 1 min pre protamine administration, Min15-15 min after protamine administration.

point revealed a weak correlation (correlation coefficients of 0.42, p=0.001, and 0.24, p=0.026, respectively). Furthermore, the plasma level of TXB_2 in the right atrium at the Min15 point, the PAP value at the Min3 point, and the Paw value at the Min5 point revealed a weak correlation (correlation coefficients of 0.39, p=0.001, and 0.31, p=0.008, respectively). There was no correlation between the plasma 6-keto-PGF-1alpha level in the radial artery and the right atrium at the Min6 point. The PAP value at the Min3 point correlation coefficient was 0.07, p=0.405, and 0.3, p=0.72, respectively. The Paw value at the Min5 point correlation coefficient was -0.18, p=0.069, and -0.11, p=0.203, respectively.

As shown in Table 6, the oxygen index in Group IV at the Min15 point was significantly decreased than the min0 point, while the levels of RI and A-aDO_2 in the II and IV Groups at the Min1 point and Min15 point were significantly higher than the min0 point. Our results confirm that lignocaine preconditioning reduces the risk of protamine-induced pulmonary vasoconstriction and improves respiratory function.

DISCUSSION

Protamine, first isolated from salmon fish sperm and now produced through recombinant biotechnology, is available in a sulphate or chloride formulation. It neutralizes the anticoagulant properties of heparin on CPB. The incidence of protamine-related adverse reactions reported as varying from 0.06% to 10.6%.[2] Severe protamine-mediated pulmonary hypertension is more likely associated with the anaphylactoid reaction. The protamine binds to heparin in a charger-dependent manner, which is also immunogenic. This activates the complement system through two cascades. First, the protamine-heparin complexes can directly activate the complement system in the lung through the classical way of protamine administration. Second, contact with the oxygenator surface can activate the complement system through alternate pathways.[9] Studies showed activation of the complement system causes the generation of complements

Table 5. Comparison of Inflammatory Compounds ThromboxaneB_2 (TXB_2) & 6-keto-Prostaglandin F-1 alpha Levels

Group	Min0 right atrium	Min15 right atrium	Min0 radial artery	Min15 radial artery
ThromboxaneB_2 (TXB_2) levels in (pg/mL)				
I	3806.58±1359.52	1811.37±659.18*	4031.09±1343.19	2022.89±1129.68*
II	3911.73±1062.08	2652.94±1721.01$^{\alpha*}$	4074.25±1228.13	2842.01±1528.35$^{\alpha\text{¥}}$
III	4558.11±1234.37	2751.53±1402.28*	4912.01±1459.86	3074.51±2086.21*
IV	4316.62±1138.11	3138.29±1437.35$^{\beta*}$	4622.35±617.13	3529.37±1532.07$^{\beta*}$
6-keto- Prostaglandin F-1 alpha levels in (pg/mL)				
I	1411.48±347.58	1316.71±336.64	581.48±139.36*	558.83±129.53*
II	1379.87±331.52	1331.79±437.19	478.43±151.67$^{\alpha*}$	469.28±151.72$^{\alpha*}$
III	2039.47±379.71	1832.54±319.83	769.51±157.11*	658.42±161.94*
IV	1878.31±312.57	1766.89±521.68	649.53±162.78$^{\beta*}$	551.58±141.56$^{\beta*}$

Data are presented as mean ± standard deviation (SD). In addition, data compared to the I-Group- $^{\alpha}$, III- Group- $^{\beta}$ and with the Min0 time point- * and (p<0.05) significant differences are expressed. Min0- 1 min pre protamine administration, Min15-15 min after protamine administration.

Table 6. Comparison of intraoperative pulmonary function

Group	Min0	Min1	Min15
Oxygen index (OI) (mmHg)			
I	412.84±128.90	358.02±114.21	402.59±125.52
II	411.41±107.68	348.94±122.28	377.65±127.43
III	415.92±101.07	352.86±147.85	379.60±136.67
IV	414.67±114.64	339.84±120.21	359.11±139.06*
Respiratory index (RI)			
I	0.58±0.46	0.93±0.76	0.77±0.71
II	0.39±0.30	1.02±0.93*	0.83±0.68*
III	0.48±0.45	1.03±0.97	0.79±0.56
IV	0.52±0.39	1.16±1.10*	1.07±0.92*
Alveolar-arterial oxygen difference (A-aDO2) (mmHg)			
I	87.14±47.11	142.11±76.53	108.89±62.49
II	75.39±41.63	174.40±83.37*	132.11±63.72*
III	96.24±91.05	162.66±72.05	127.51±34.36
IV	101.14±73.87	185.46±113.12*	144.45±96.41*

Data are presented as mean ± SD. Significant differences are expressed as follows: Compared with M0 group - * (p<0.05). Min0 -baseline (1 min before CPB), Min1 -1 min before protamine administration, Min15 -15 min after protamine administration end, Oxygen index is calculated as (mean airway pressure × FiO_2 × 100) / PaO_2, a respiratory index is the ratio of P (A-a) DO_2 and PaO_2.

C3a and C5a.[10] This leads to smooth muscle contraction, platelet accumulation, and leukocyte activation in the lungs. Subsequently, this induces the release of many proteolytic enzymes and causes lung injury. Complement C5a-induces TXB_2 generation, leading to pulmonary vasoconstriction and lung injury.[11,12] The plasma TXB_2 levels in the II and IV Groups were higher than those in the I and III Groups with lignocaine preconditioning 15 minutes after protamine administration. This shows that lignocaine preconditioning inhibits the inflammatory response and reduces the generation of TXB_2. Our results are consistent with Morel and Zapol, who showed TXB_2 generation leads to pulmonary vasoconstriction.[9] In this study, protamine-induced pulmonary vasoconstriction occurred in 15.9% of patients administered with NS as a placebo instead of preconditioned with lignocaine. The adverse symptoms of protamine administration without lignocaine preconditioning were fatal bronchospasm, hypotension, bradycardia, and pulmonary hypertension, as reported in other similar studies.[5,9,13,14]

This study indicates lignocaine preconditioning can effectively increase the 6-keto-PG-F1a generation to inhibit the inflammatory response. The low levels of 6-keto-PGF1a in the II and IV Groups in the absence of lignocaine are a possible explanation for the increased PAP, hypotension, and hypoxia as shown by Petidis *et al*, which concur with our findings.[15]

Regarding hemodynamic function, the present study indicated that lignocaine preconditioning before the neutralization of heparin can effectively reduce pulmonary vasoconstriction response and enhance the resistance of

the blood vessel, reducing the stress responses and spasm reactions of the pulmonary vascular smooth muscles caused by the direct stimulation of protamine. At the time point of Min3 compared to Min0 (baseline), the values of PAP were significantly higher in Group II than Group I, while the values of PAP at the time point of Min3 compared to Min0 (baseline) were significantly higher in Group IV. The values of Paw at the time point of Min5 compared to Min0 (baseline) were significantly higher in Group II than in Group I, while the values of Paw in Group IV at the time point of Min5 were significantly higher than in Group III. The values of Cydn were significantly lower in Groups II and IV at the time point of Min5 compared to Min0 and the Groups I and III, respectively. Our results indicate that the precondition of lignocaine prior to heparin neutralization effectively prevents the protamine-induced pulmonary vascular reaction. PAP, Paw, and Cydn in patients who received lignocaine preconditioning before heparin neutralization experienced fewer fluctuations than patients who received NS placebo before heparin neutralization.

Pulmonary functions (OI, RI, and A-aDO_2) were unchanged when compared with baseline (Min0) in patients receiving lignocaine preconditioning, but this worsened in patients receiving NS placebo. RI is the ratio of P(A-a) DO_2 and PaO_2, calculated as mean airway pressure×FiO_2×100)/PaO_2, and it reflects the effects of respirator pressure on oxygenation, and it can reflect the function of pulmonary ventilation and oxygen exchange. OI is calculated as (mean airway pressure×FiO_2×100)/PaO_2, and it reflects the effects of respirator pressure on oxygenation. Groups II and IV exhibited an increased respiratory index (RI) at Min1 and Min15 (1.02 ± 0.93 and 0.83 ± 0.68) and (1.16 ± 1.10 and 1.07 ± 0.92), respectively, compared to Min0.

Conversely, from baseline (Min0) decreased alveolar-arterial oxygen difference (A-aDO_2) in the Groups II and IV at Min0, Min1 and Min15 (411.41 ± 107.68; 348.94 ± 122.28 and 377.65 ± 127.43 mmHg) and (414.67 ± 114.64; 339.84 ± 120.21 and 359.11 ± 139.06 mmHg), respectively. The protamine adverse reactions in groups I and III were lower than in Groups II and IV, respectively. RI is the ratio of P(A-a) DO_2 and PaO_2, reflecting the function of pulmonary ventilation and oxygen exchange. OI reflects the effects of respirator pressure on oxygenation.[16] Wood et al, in their study, confirmed a relationship between different pulmonary functions such as OI, RI, and A-aDO_2 and ventilation.[17] Post-protamine pulmonary function was unchanged compared to baseline in patients receiving lignocaine preconditioning, but this worsened in patients receiving NS placebo.

The anti-inflammatory effect of lignocaine is well-known in the literature. It inhibits neutrophil function, including chemotaxis and superoxide anion release, and inhibits granulocyte adhesion.[6,9] In addition, the inhibition of the release of proteolytic enzymes and cytokines had a protective effect on vascular endothelial cells.[6,10] Furthermore, lignocaine was known to inhibit platelet activation and aggregation and decrease TXB_2 concentration.[18]

Hamp et al. administered 1.5 mg/kg bolus of IV lignocaine, and their trials reported no hemodynamic effects or cardiovascular changes with lignocaine.[19] Singh et al compared bolus dosing of lignocaine on hemodynamics, varying between 1 mg/kg and 3 mg/kg, and reported that 2 mg/kg IV lignocaine has a significant effect on hemodynamics and cardiovascular.[20] This study was designed to investigate the potential cardiopulmonary benefits of lignocaine preconditioning before the neutralization of protamine. As per available clinical literature, lignocaine 2 mg/kg was considered hemodynamically safe and effective, so we decided on this dose of lignocaine.[21] Regarding hemodynamic function, the present study indicated that lignocaine preconditioning before protamine neutralization effectively reduces pulmonary vasoconstriction. The anti-inflammatory effect of lignocaine reduces harmful stimulation of protamine by reducing stress responses and spasm reactions of pulmonary vascular and tracheal smooth muscle caused by direct stimulation of protamine by a reduced generation of TXB_2 or increased production of 6-keto-PGF-1alpha.[3,5,15]

Limitations: As a single-center study, the generalization of study findings will be finite. To generalize the result, we need to conduct a multicentric trial with a large sample size that involves wider patient demographics, surgical techniques, or institutional practices that might differ across centers and could provide a more balanced interpretation of the results. Measurement of lignocaine plasma level was not possible in our institution. However, further studies with various doses of lignocaine and laboratory studies in animals need to be done to compare doses and elucidate the mechanism.

CONCLUSION

Severe pulmonary vasoconstriction induced by protamine in cardiac surgery is very commonly associated with hypotension shortly after protamine administration. It interacts with peptides on the surfaces of vascular and blood cells, triggering the release of a wide variety of vasoactive compounds and inflammatory mediators. This leads to inflammatory responses in the lung and pulmonary vasoconstriction. The use of lignocaine before heparin exerts its effect through inhibition of TXB_2 release and the generation of 6-keto-PG-F1alpha. Lignocaine preconditioning before protamine administration reduces pulmonary vasoconstriction in pediatric cardiac surgery, enhancing pulmonary function. These findings suggest lignocaine could be a useful protective agent in pediatric cardiac surgeries requiring cardiopulmonary bypass.

CONTRIBUTORSHIP STATEMENT / *DECLARAÇÃO DE CONTRIBUIÇÃO*

SS: Performed anesthesia in most cases, obtained ethics approval, data collection, analysis, protocol implementation, wrote the manuscript, and critical review of the manuscript.
MMAR: Study design, protocol implementation, and critical review of the manuscript.
DEM: Performed most surgeries and did a critical review of the manuscript.
AS: Reviewed of the literature and provided critical revision to the manuscript.
All the authors have approved the final version to be published.
SS: Realizou a anestesia na maioria dos casos, obteve aprovação ética, recolha de dados, análise, implementação do protocolo, escreveu o manuscrito e fez a revisão crítica do manuscrito.
MMAR: Conceção do estudo, implementação do protocolo e revisão crítica do manuscrito.
DEM: Realizou a maioria das cirurgias e fez a revisão crítica do manuscrito.
AS: Revisão da literatura e fez a revisão crítica do manuscrito.
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REFERENCES

1. Holzmacher JL, Sarani B. Indications and methods of anticoagulation reversal. Surg Clin North Am. 2017;97:1291-305.
2. Applefield D, Krishnan S. Protamine. Statpearls. Treasure Island: StatPearls Publishing; 2020. [2024 July 20]Available from: https://www.ncbi.nlm. nih.gov/books/NBK547753.
3. Kimmel SE, Sekeres M, Berlin JA, Ellison N. Mortality and adverse events after protamine administration in patients undergoing cardiopulmonary bypass. Anesth Analg. 2002;94:1402-8.
4. Sokolowska E, Kalaska B, Miklosz J, Mogielnicki A. The toxicology of heparin reversal with protamine: past, present and future. Expert Opin Drug Metab Toxicol. 2016;12:897-909. doi: 10.1080/17425255.2016.1194395.
5. Chooriyil N, Jom D, Panattil P, Sreedharan S. Protamine induced hypotension and pulmonary arterial hypertension. J Pharmacol Pharmacother. 2021; 12:23-5.
6. Nybo M, Madsen JS. Serious anaphylactic reactions due to protamine sulfate: a systematic literature review. Basic Clin Pharmacol Toxicol. 2008;103:192-6.
7. Guan Z, Shen X, Zhang YJ, Li XG, Gao YF, Tan J, et al. use of epoprostenol to treat severe pulmonary vasoconstriction induced by protamine in cardiac surgery. Medicine. 2018; 97: e10908.
8. Singh S, Laing EF, Owiredu WK, Singh A. Comparison of esmolol and lidocaine for attenuation of cardiovascular stress response to laryngoscopy and endotracheal intubation in a Ghanaian population. Anesth Essays Res. 2013;7:83-8. doi: 10.4103/0259-1162.114008.
9. Morel DR, Zapol WM, Thomas SJ, Kitain EM, Robinson DR, Moss J, et al. C5a and thromboxane generation associated with pulmonary vaso-and broncho-constriction during protamine reversal of heparin. Anesthesiol. 1987; 66: 597-604.
10. Bardhan M, Kaushik R. Physiology, Complement Cascade. [Updated 2023 Feb 14]. In: StatPearls [Internet]. Treasure Island: StatPearls Publishing; 2024. [accessed on 2024 July 20] Available from: https://www.ncbi.nlm.nih.gov/books/NBK551511/
11. Strainic MG, Shevach EM, An F, Lin F, Medof ME. Absence of signaling into CD4+ cells via C3aR and C5aR enables autoinductive TGF-β1 signaling and induction of Foxp3+ regulatory T cells. Nat Immunol. 2013;14:162-71.
12. Singh S, Singh A, Shaifulla P. The effect of BIS usage on Anaesthetic Agent Consumption in high-risk patients for coronary artery-bypass grafting off-pump surgery. J Applied Sci Nanotechnol. 2023;3:41-50.
13. Hogg K, Weitz JL. Blood coagulation and anticoagulant, fibrinolytic and antiplatelet drugs. In: Goodman & Gilman's the Pharmacological Basis of Therapeutics. Vol. 13.Chicago: McgGraw Hill; 2018. p. 591
14. Singh S, Singh A, Rahman MM, Mahrous DE, Singh LC. Is the combination of conventional ultrafiltration and modified ultrafiltration superior to modified ultrafiltration in pediatric open-heart surgery? J Med Soc. 2023; 37:159-66.
15. Liu L, Feng A, Du C, Qi C. Relationship between the changes in thromboxane B2, 6-keto-prostaglandin Fla, and blood glucose levels and progressive ischemic stroke. Ann Palliative Med. 2021; 10. doi: 10.21037/apm-21-774
16. Powers KA, Dhamoon AS. Physiology, Pulmonary Ventilation and Perfusion. [Updated 2023 Jan 23]. In: StatPearls [Internet]. Treasure Island: StatPearls Publishing; 2024-. [accessed on 2024 July 20]Available from: https://www.ncbi.nlm.nih.gov/books/NBK539907/
17. Woods BD, Sladen RN. Perioperative considerations for patients with asthma and bronchospasm. Br J Anaesth. 2009; 103: i57-i65.
18. Whitman GJ, Martel D, Weiss M, Pochanapring A, See WM, Hopeman A, et al. Reversal of protamine-induced catastrophic pulmonary vasoconstriction by prostaglandin E1. Ann Thorac Surg. 1990;50:303-5. doi: 10.1016/0003-4975(90)90758-x.
19. Hamp T, Krammel M, Weber U, Schmid R, Graf A, Plöchl W. The effect of a bolus dose of intravenous lidocaine on the minimum alveolar concentration of sevoflurane: a prospective, randomised, double-blinded, placebo-controlled trial. Anesth Analg. 2013; 117:323–8.
20. Singh S, Mahrous DE, Singh B, Mohammad Ateequr Rahman M, Bandoh I, Singh A. A Prospective Randomised Observational Study of Obstructed Total Anamalous Pulmonary Venous Connection (TAPVC) Repair Patients with Milrinone Versus Milrinone and Inhaled Nitric Oxide. Perspect Med Res. 2024;12:61-9.
21. Kulsum, Singh S, Shroff H, Annamalai A, Mahrous DE. Can sodium channel blocker lidocaine attenuate haemodynamic responses to endotracheal intubation in patients with coronary artery disease effectively? J Anesthesiol. 2013;1: 27-35.