Original Article

H_1 and H_2 antihistamines pretreatment for attenuation of protamine reactions after cardiopulmonary bypass: a randomized-controlled study

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Background: Protamine administration post-cardiopulmonary bypass (CPB) can potentially cause hemodynamic instability. Histamine released from mast cells is believed to be responsible for hypotension after protamine administration. The aim of this study was to examine the effects of pretreatment with H_1 and H_2 antihistamines on changes in systemic arterial pressure following protamine administration.

Methods: This study was a randomized, triple-blinded, placebo-controlled study, conducted at a university hospital. Forty adult patients undergoing elective coronary artery bypass grafting (CABG) or single valve surgery were included. The patients were randomly allocated (20 patients in each group) to receive a single dose of combined chlorpheniramine 10 mg and ranitidine 50 mg or normal saline intravenously immediately after separation from CPB prior to protamine administration. Trajectory changes in systolic blood pressure (SBP), mean arterial pressure (MAP), and vasoactive-inotropic score (VIS) from baseline until 35 minutes following protamine administration (24-time points) were compared between the two groups. Serial serum tryptase levels were also obtained at baseline, 30 and 60 minutes after protamine was given.

Results: Forty patients were included in the analysis. Demographic and baseline blood pressure were similar between the two groups. At 30 minutes after protamine administration, there were no significant differences in both crude SBP [mean difference: −7.1 mmHg, 95% confidence interval (CI), −1.1 to 15.3 mmHg, P=0.09] and SBP after adjustment for the European System for Cardiac Operative Risk Evaluation (EuroSCORE II), CPB time, and VIS (mean difference: −3.9 mmHg, 95% CI, −11.9 to 4.0 mmHg, P=0.33). There were also no significant differences in crude MAP (mean difference: −2.1 mmHg, 95% CI, −6.9 to 2.7 mmHg, P=0.39) and adjusted MAP (mean difference: −0.7 mmHg, −5.9 to 4.4 mmHg, P=0.78) between the two groups. None of the patients in both groups had a significant increase in serum tryptase from baseline. No differences in median serum tryptase levels at baseline, 30 and 60 minutes were demonstrated between the two groups.

Conclusions: Pretreatment with H_1 and H_2 antihistamines does not attenuate blood pressure responses to protamine administration in patients after CPB. Mechanisms other than histamine release from mast cells might be responsible for protamine-induced cardiovascular changes.

Trial Registration: ClinicalTrials.gov NCT03583567.

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Introduction

Protamine is a basic protein extracted from fish sperm heads, which is the only standardized drug for heparin neutralization after cardiopulmonary bypass (CPB). Potential protamine reactions were first described in 1949 in dogs by Jaques *et al.* (1). Since then, there have been a number of reported cases of adverse hemodynamic effects of protamine in different clinical scenarios (2-9). The severity of hemodynamic reactions to protamine varies greatly, ranging from transient hypotension, pulmonary hypertension, impaired ventricular functions (5), and profound hypotension to fatal cardiac arrest (7,9,10). In a systematic review of serious protamine reactions by Nybo *et al.*, which included a total of 27 different studies, they reported that the incidence of serious reactions ranged from 0.06% to 10.6% (11). Although severe adverse cardiovascular reactions are not common, protamine administration may induce systemic hypotension (drops in blood pressure >20%) in up to 52% of the patients (12).

Highlight box

Key findings

- This study suggests that pretreatment with H_1 and H_2 antihistamines does not attenuate blood pressure responses to protamine administration in patients after CPB.

What is known and what is new?

- The mechanisms of protamine-induced cardiovascular reactions are not clearly understood, but are believed to be related to anaphylactic reactions, complement activation, and histamine release from mast cells.
- Our findings suggest that clinical doses of protamine administration may not induce mast cell degranulation in patients without risks for protamine allergy. Other mechanisms might be responsible for protamine-induced cardiovascular changes.

What is the implication, and what should change now?

- The clinical benefit of pretreatment with H_1 and H_2 antihistamines for protamine-related hemodynamic instability is uncertain, therefore, should not be recommended.

Protamine-induced cardiovascular reactions can potentially lead to complications and greater risks of in-hospital mortality following cardiac surgery (12,13). Welsby *et al.* demonstrated that all degrees of systemic arterial hypotension and pulmonary artery hypertension during the first 30 minutes after protamine administration were independently associated with in-hospital mortality (12). Therefore, it is important to prevent hypotension after protamine administration. Although the mechanisms by which protamine produces adverse reactions are still not clearly understood, it is believed that intravascular administration of protamine exposes basophils and tissue mast cells to the compound resulting in mast cell degranulation and the release of histamines (14). Several studies have investigated the effects of pretreatment medications to minimize the cardiovascular effects of protamine (15,16). Antihistamines are one of the pretreatment drugs being examined (17-23). However, previous studies are outdated, published more than 20 years ago, and some are poorly designed. The results are heterogeneous and inconclusive because of the differences in the dosage of protamine administration, route of administration, and hemodynamic outcome measurements, which can be easily influenced by many factors.

Due to the inconsistency among the previously published studies (17-23), the primary aim of this study was to examine the effects of pretreatment H_1 (chlorpheniramine) and H_2 (ranitidine) antihistamines on changes in systemic arterial pressure following protamine administration in patients after CPB. We also evaluated systemic mast cell activity after protamine administration using serial serum tryptase levels. We present the following article in accordance with the CONSORT reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-22-714/rc).

Methods

This study was a parallel-group, randomized, triple-blind, placebo-controlled study, conducted at Siriraj Hospital, a 2,200-bed quaternary-care medical center located in

Bangkok, Thailand. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the Siriraj Institutional Review Board (No. Si 518/2018) and was registered at clinicaltrail.gov (No. NCT03583567). Informed consent was obtained from all participants before recruitment. Patients aged ≥18 years old scheduled for elective coronary artery bypass grafting (CABG) or single valve surgery were included. Exclusion criteria were patients with a history of exposure to protamine-containing insulin, previous heart surgery, and history of allergy to either salmon or any of the study drugs.

Between October 2018 and March 2019, all patients scheduled for elective CABG or single valve surgery were screened for eligibility criteria and enrolled in the study (PW, CK). Blocks of 4 randomization was done using a computer-generated method. The randomization sequence was placed in opaque sealed envelopes separately with running numbers from 1 to 40. The patients were randomly allocated into two groups (1:1 ratio): group antihistamines (chlorpheniramine and ranitidine) and group NSS (normal saline). The participants, the attending anesthesiologists, and the outcome assessors were all blinded to group allocation.

Upon arrival in the operating room, standard noninvasive monitors were placed and midazolam 1 mg and dexmedetomidine 50 mcg were infused into all patients before arterial line insertion. Anesthesia was induced with propofol 1–2 mg/kg, fentanyl 1–2 mcg/kg, and cis-atracurium 0.02 mg/kg to facilitate intubation. Central venous catheter, urinary catheter, and nasopharyngeal temperature probe were placed after induction. Anesthesia was maintained with 1–2% sevoflurane in a mixture of air/O_2, fentanyl 1 mcg/kg/hr, and cis-atracurium 0.03 mg/kg every 30 minutes throughout the operations. All medications that potentially trigger histamine release were avoided. Patients were ventilated to achieve normocarbia ($PaCO_2$ of 35–45 mmHg). Systolic blood pressure (SBP) and mean arterial pressure (MAP) were maintained within 20% of the patient's baseline before CPB was established. Heparin 3 mg/kg (300 U/kg) was given before aortic cannulation and activated clotting time (ACT) was maintained more than 480 seconds before CPB initiation and throughout CPB.

Management during CPB was based on our institutional standard. The bypass circuit was primed with acetate ringer's solution 1,800 mL, heparin 5,000 U, sodium bicarbonate 44.6 mEq, 20% mannitol 0.5 g/kg, and 20% albumin 100 mL. CPB was performed with a non-occlusive roller pump and non-pulsatile flow was maintained between 2.0–2.4 L/min/m^{-2} with a target perfusion pressure of 50–80 mmHg during the CPB period. Intermittent bolus of norepinephrine was used to maintain perfusion pressure as needed if the target non-pulsatile flow was achieved. Mild hypothermia (32–34 ℃) was instituted along with alpha-stat pH management. St. Thomas cardioplegia was used for myocardial protection in all patients. The blood cardioplegia was mixed at a 4:1 blood to crystalloid ratio and was delivered antegrade or retrograde until asystole was achieved.

During CPB, the enclosed envelopes with group allocation numbers were opened and the study drugs were prepared by one of the authors (PW, SS) who were not involved in patient care. Immediately after separation from CPB (the venous cannula was clamped), the study drugs were given according to group allocation. Each syringe was labeled as 'study drug'. In group antihistamines, chlorpheniramine 10 mg and ranitidine 50 mg in two separate syringes were administered intravenously, while in group NSS, the patients received 2 mL of normal saline in a 3-mL syringe and 1 mL of normal saline in another 3-mL syringe identical to the study drugs. Five minutes after the study drugs were given, 3 mg/kg of protamine was given in 5 minutes infusion via peripheral line. Types and doses of inotropic and vasopressor drugs for hemodynamic support after separation from CPB were selected based on the attending anesthesiologist's judgment to maintain MAP within 20% of baseline. Surgical manipulation of the heart was minimized after the starting of protamine infusion in order to avoid abrupt hemodynamic changes from surgical factors. Decannulation was performed once half of the dose of the protamine was given. After the procedures ended, all patients were transferred to the cardiac surgical intensive care unit intubated.

Outcomes

SBP, MAP, diastolic blood pressure (DBP), heart rate, and central venous pressure (CVP) were recorded every 1 minute for the first 20 minutes (T_1, T_2, T_3, …, T_{20}) and every 5 minutes until 35 minutes (T_{25}, T_{30}, T_{35}) after protamine was initiated (T_0). Baseline SBP, MAP, and DBP were recorded at the time of the beginning of protamine infusion. All changes in SBP and MAP after protamine administration were calculated as percent differences from baseline. The percent differences between blood pressures (SBP and MAP) before and after protamine administration

= [blood pressure at x minute (T_x) – blood pressure at baseline (T_0)/blood pressure at baseline (T_0)] ×100%. If the patients developed hypotension (SBP <90 mmHg or MAP <65 mmHg), either norepinephrine 4 mcg or 10% calcium carbonate 1 g bolus would be given intravenously. The doses of inotropes, vasopressors, and calcium administered were documented. We also calculated vasoactive-inotropic score (VIS) (24) at each time point as vital signs were recorded until 35 minutes after protamine. VIS = dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 × epinephrine dose (mcg/kg/min) + 50 × levosimendan dose (mcg/kg/min) + 10 × milrinone dose (mcg/kg/min) + 10,000 × vasopressin dose (units/kg/min) + 100 × norepinephrine dose (mcg/kg/min).

Serial serum tryptase levels were obtained at baseline (T_0), 30 (T_{30}), and 60 minutes (T_{60}) after the beginning of protamine infusion. All blood samples were drawn via arterial line (after discarding 10 mL of blood) into the tube containing EDTA. The samples were centrifuged and then stored at –20 ℃ until assayed. Serum tryptase was measured by an enzyme-linked immunosorbent assay (ELISA) with Anti-Tryptase immunoCAP® (Unicap-Tryptase, Pharmacia-Sweden). Differences between serum tryptase levels at T_{30} and T_{60} from baseline were also calculated. Mast cell degranulation was considered if there was a significant elevation above the individual's baseline tryptase level according to the formula: (baseline tryptase × 1.2) + 2 ng/mL (25).

Statistical analysis

From the previous study by Kambam *et al.* (18), the reported mean difference in MAP among the patients who received antihistamines and the control group was 15 mmHg. The primary outcome of this study was the differences in MAP between the two groups. Using a 2-sided type I error of 0.01 and 90% power, a sample size of 20 participants per group was estimated to test the difference in MAP of 15 and standard deviation (SD) of 12.

Study data were collected and managed using REDCap® electronic data capture tools hosted at Mahidol University. All data were processed using PASW Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp. Demographic and perioperative data were collected. Preoperative risk was accessed using the European System for Cardiac Operative Risk Evaluation (EuroSCORE II) (26) calculated using the online calculator (https://www.euroscore.org/index.php?id=17&lang=en). Continuous data such as age, weight, height, ejection fraction, EuroSCORE II, differences in blood pressure, and serum tryptase levels were reported as mean ± SD or median [interquartile range (IQR)]. Continuous data were compared using Student's *t*-test or Mann-Whiney U test as appropriate. Categorical data such as gender, underlying disease, and types of surgery are presented as number (%) and were compared using chi-square test. Generalized estimating equations (GEE) with exchangeable correlation structure was used to assess the effect of protamine administration on changes in SBP and MAP during the first 35 minutes (20-time points) after protamine administration. Time was treated as a categorical variable. GEE model included protamine, time, and protamine-by-time interaction. For each outcome variable (i.e., SBP, MAP), two GEE models were fitted i.e., without and with adjustment for EuroSCORE II, CPB time, and VIS at each time point. Effects of protamine were reported as crude and adjusted mean difference, 95% CI, and P value at baseline, $T_{10,}$ and T_{30}. A P value less than 0.05 was considered statistically significant.

Results

Between October 2018 and March 2019, 40 patients were enrolled (20 participants in each group) and completed the study (*Figure 1*). Demographic, clinical, and intraoperative characteristics are presented in *Table 1*. There were no significant differences in overall characteristics of the patients between the two groups, except for gender. More patients in group NSS were male (85%) compared to group antihistamines (50%) (P=0.02). There were also no differences in EuroSCORE II, types of surgery, duration of CPB and aortic cross-clamp time, and doses of heparin or protamine.

Figures 2 and *3* demonstrate trajectory SBP and MAP from the beginning until 35 minutes after protamine infusion, respectively. Within 5 minutes after the initiation of protamine infusion (T_1–T_5), both mean SBP and MAP increased from baseline in both groups. Then, after 5 minutes (completion of protamine infusion), SBP and MAP decreased to within 10% of baseline values in both groups. The mean and 95% confidence intervals (CI) of unadjusted and adjusted (after adjustment for EuroSCORE II, CPB time, and VIS) trajectory SBP and MAP over the first 35 minutes after protamine administration are shown in *Figure 2A,2B* and *Figure 3A,3B*, respectively. There were no significant differences in both crude SBP (mean difference: 3.3 mmHg, 95% CI, –8.9 to 15.4 mmHg, P=0.60 and mean

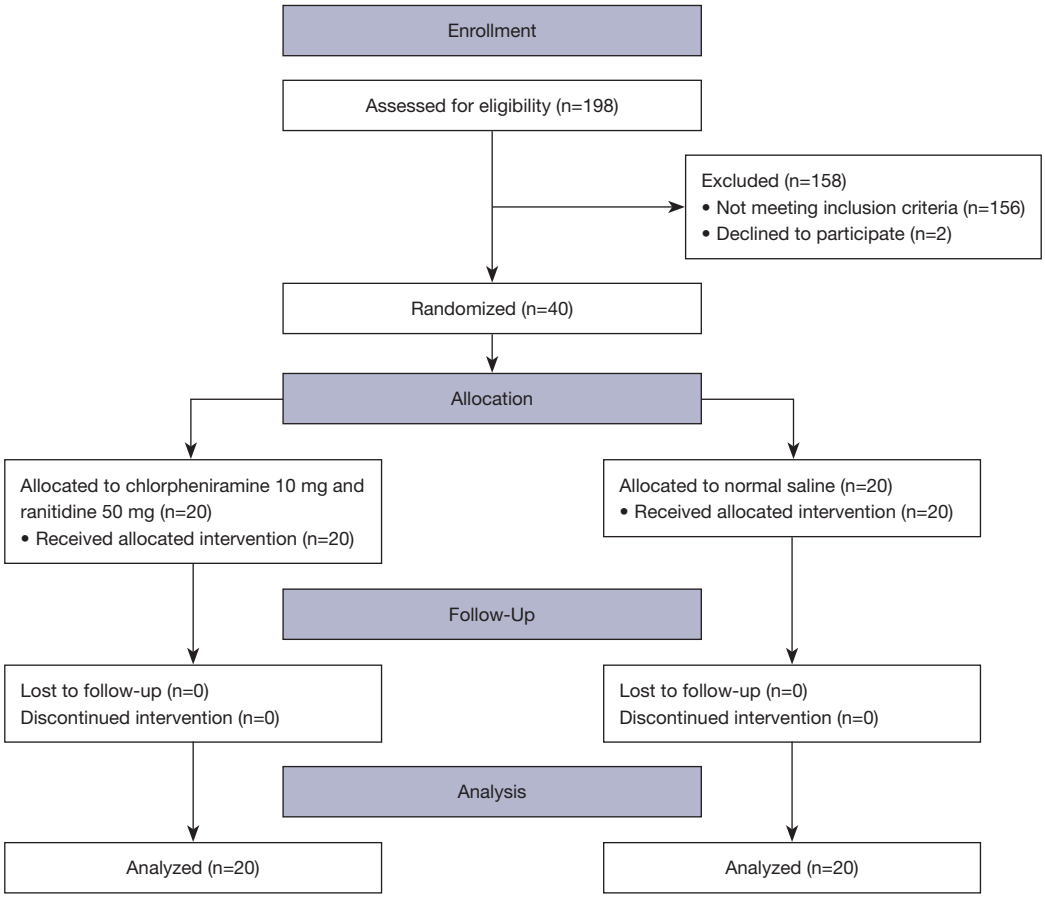

Figure 1 CONSORT flow diagram.

difference: −7.1 mmHg, 95% CI, −1.1 to 15.3 mmHg, P=0.09 at T_{10} and T_{30}, respectively), and adjusted SBP (mean difference: 5.8 mmHg, 95% CI, −5.4 to 17.1 mmHg, P=0.31 and mean difference: −3.9 mmHg, 95% CI, −11.9 to 4.0 mmHg, P=0.33 at T_{10} and T_{30}, respectively). There were also no significant differences in crude MAP (mean difference: 4.3 mmHg, 95% CI, −2.4 to 10.9 mmHg, P=0.21 and mean difference: −2.1 mmHg, 95% CI, −6.9 to 2.7 mmHg, P=0.39 at T_{10} and T_{30}, respectively) and adjusted MAP (mean difference: 5.3 mmHg, −1.1 to 11.7 mmHg, P=0.11 and mean difference: −0.7 mmHg, 95% CI, −5.9 to 4.4 mmHg, P=0.78 at T_{10} and T_{30}, respectively) between the two groups (*Table 2*).

Figure 4 demonstrates individual's serum tryptase levels at baseline, $T_{30,}$ and T_{60}. None of the patients in both groups had a significant increase in serum tryptase levels from baseline (>20% +2 ng/mL). The median (IQR) serum tryptase at baseline, T_{30} and T_{60} were 4.1 (2.8) ng/mL, 4.3 (2.9) ng/mL, and 5.0 (3.2) ng/mL in group antihistamines, and 3.4 (3.2) ng/mL, 3.9 (3.1) ng/mL, and 4.5 (3.4) ng/mL in group NSS, respectively. There were no differences in serum tryptase levels at baseline, T_{30} and T_{60} between the two groups (P=0.49, P=0.49 and P=0.55 respectively). There was also no significant difference in changes in serum tryptase level from baseline between the two groups (P=0.62).

Discussion

The findings from this study indicate that pretreatment with intravenous H_1 (chlorpheniramine) and H_2 (ranitidine) antihistamines before protamine administration does not attenuate systemic blood pressure responses to protamine after CPB. There were no differences in trajectory blood pressures, vasopressor/inotropic uses, and serum tryptase levels in patients receiving pre-treatment antihistamines compared to the placebo. None of the patients in both groups had significant serum tryptase elevation from

Table 1 Demographic and perioperative data

Variable	Group antihistamine (n=20)	Group NSS (n=20)	P value
Age (year)	63.6±18.5	62.8±16.5	0.89
Weight (kg)	61.0±13.1	62.3±11.9	0.74
Height (cm)	159.1±7.4	163.1±7.6	0.10
BMI (kg/m^2)	24.0±4.6	23.3±3.3	0.55
Male	10 (50%)	17 (85%)	0.02
ASA-PS classification III:IV	19:1	19:1	1.00
EuroSCORE II* (%)	2.25 (1.28, 3.26)	1.63 (1.01, 2.54)	0.11
Underlying disease			
Diabetes mellitus	9 (45%)	5 (25%)	0.19
Hypertension	13 (65%)	15 (75%)	0.49
Dyslipidemia	14 (70%)	12 (60%)	0.51
Serum creatinine (mg/dL)	1.2±1.0	1.1±0.4	0.78
Creatinine clearance† (mL/min)	74.3±27.7	73.7±23.7	0.95
Left ventricular ejection fraction (%)	61.1±12.3	57.4±15.1	0.40
Types of surgery			0.31
CABG	15 (75%)	12 (60%)	
Valve surgery	5 (25%)	8 (40%)	
Cardiopulmonary bypass time (min)	122.0±42.7	123.1±37.5	0.93
Aortic cross clamping time (min)	81.3±21.2	86.7±31.7	0.57
Protamine (mg)	247.5±131.7	260.0±102.2	0.74
Heparin (mg)	186.8±44.6	188.0±39.4	0.93
Calcium administration	9 (45%)	11 (55%)	0.53
Blood/blood products administered after cardiopulmonary bypass (mL)	974±603	1081±434	0.26
Baseline (T_0)			
Systolic blood pressure (mmHg)	104.3±14.9	100.8±15.2	0.46
Mean arterial pressure (mmHg)	71.2±10.9	72.5±10.1	0.70
VIS	12.4±10.8	9.1±5.3	0.23

Data are presented as mean ± standard deviation, median (P25, P75) or number (%). *, the European System for Cardiac Operative Risk Evaluation II was calculated using online calculator provided at https://www.euroscore.org/index.php?id=17&lang=en; †, creatinine clearance was estimated using the Cockcroft-Gault equation. NSS, normal saline; BMI, body mass index; ASA-PS, American Society of Anesthesiologists Physical Status; CABG, coronary artery bypass grafting; VIS, vasopressor inotropic score.

baseline suggesting that the clinical dose of protamine administration may not induce mast cell degranulation in patients without risks for protamine allergy. Hemodynamic changes following protamine administration might occur as a result of other mechanisms. Therefore, pretreatment with antihistamines did not modify the hemodynamic effects of protamine.

The mechanisms of protamine-induced hemodynamic reactions remain unclear. There are four types of adverse reactions being postulated (27,28). First, the classic anaphylactic reaction to the heparin-protamine complex via IgE-mediated, which rarely occurs but can cause

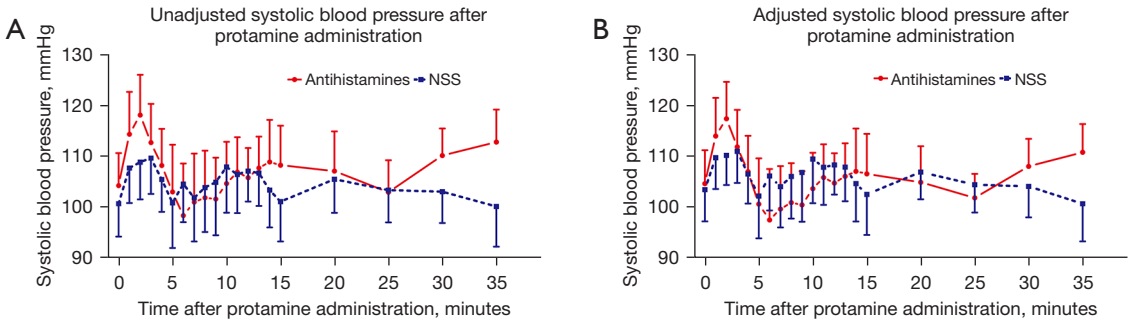

Figure 2 Mean and 95% confidence intervals of systolic blood pressure trajectory (A) unadjusted and (B) adjusted for the EuroSCORE II, cardiopulmonary bypass time, and VIS over 35 minutes following protamine administration. NSS, normal saline; EuroSCORE, European System for Cardiac Operative Risk Evaluation; VIS, vasopressor inotropic score.

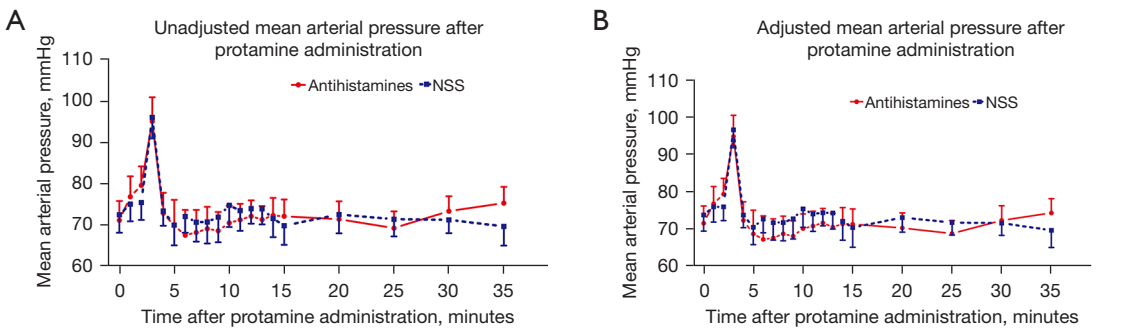

Figure 3 Mean and 95% confidence intervals of mean arterial pressure trajectory (A) unadjusted and (B) adjusted for the EuroSCORE II, cardiopulmonary bypass time, and VIS over 35 minutes following protamine administration. NSS, normal saline; EuroSCORE, European System for Cardiac Operative Risk Evaluation; VIS, vasopressor inotropic score.

Table 2 Differences in systolic and mean arterial pressure between the antihistamines and the NSS groups after protamine administration without and with adjustment for the EuroSCORE II, cardiopulmonary bypass time, and VIS at baseline (T_0), 10 minutes (T_{10}), and 30 minutes (T_{30}) after protamine administration

Variable	Crude		Adjusted[†]	
	Mean (95% CI)	P value	Mean (95% CI)	P value
Systolic blood pressure (mmHg)				
Baseline	−3.6 (−12.6, 5.6)	0.44	−1.2 (−10.4, 8.2)	0.81
10 minutes	3.3 (−8.9, 15.4)	0.60	5.8 (−5.4, 17.1)	0.31
30 minutes	−7.1 (−1.1, 15.3)	0.09	−3.9 (−11.9, 4.0)	0.33
Mean arterial pressure (mmHg)				
Baseline	1.3 (−5.0, 7.6)	0.69	2.2 (−4.2, 8.6)	0.50
10 minutes	4.3 (−2.4, 10.9)	0.21	5.3 (−1.1, 11.7)	0.11
30 minutes	−2.1 (−6.9, 2.7)	0.39	−0.7 (−5.9, 4.4)	0.78

[†], adjusted for EuroSCORE II, cardiopulmonary bypass time, and VIS. NSS, normal saline; EuroSCORE, European System for Cardiac Operative Risk Evaluation; VIS, vasopressor inotropic score; CI, confidence interval.

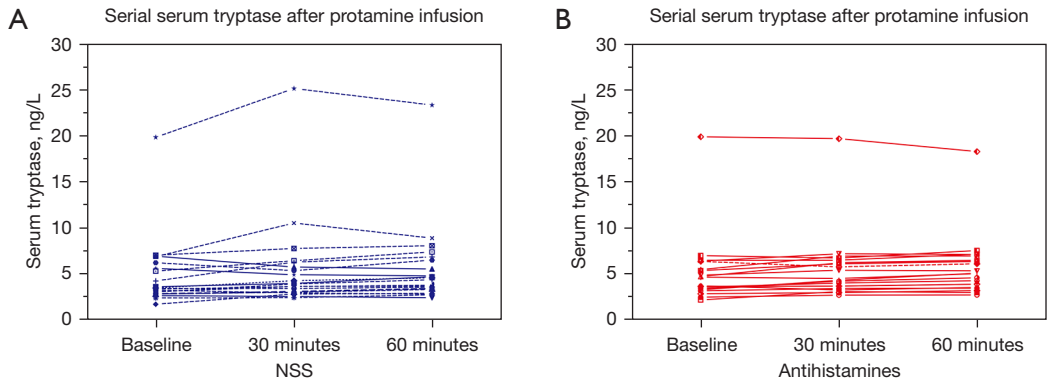

Figure 4 Serum tryptase levels at baseline, 30- and 60 minutes after protamine infusion of each patient in group NSS (A) and group antihistamines (B). Each of the different symbols represents each individual's serum tryptase levels at each time point. There were no differences in serum tryptase at any time point between the two groups (P=0.49, 0.49, and 0.55, respectively). NSS, normal saline.

severe abrupt onset cardiovascular collapse. Second, the anaphylactoid reaction involves activation of the complement system which results in systemic hypotension due to vasodilation. Third, complement-mediated C5a-induced thromboxane A_2 generation is another mechanism that may lead to pulmonary vasoconstriction and systemic hypotension but is also uncommon. Finally, the most commonly believed mechanism is transient hypotension due to the histamine release from mast cells, which triggers nitric oxide (NO) production, a potent vasodilator, resulting in decreased systemic vascular resistance. Thus, antihistamines are one of the most common pretreatment drugs being investigated because of their safety profiles and availability worldwide. Antihistamine was also recommended by the Task Force on Patient Blood Management for Adult Cardiac Surgery of the European Association for Cardio-Thoracic Surgery (EACTS) and the European Association of Cardiothoracic Anaesthesiology (EACTA) guideline on patient blood management for adult cardiac surgery as a prophylactic drug to prevent hemodynamic perturbation following protamine administration (29). However, the evidence on the efficacy of pretreatment with antihistamines on cardiovascular reactions to protamine is not strong.

Table 3 presents a summary of the previous randomized clinical studies (RCT) investigating the effects of pretreatment antihistamines on cardiovascular and hemodynamic responses to protamine administration (17-23). All of the studies were old, published more than 20 years ago, and were small RCTs. Some of the studies divided the participants into several groups, hence, making it challenging to compare the changes in hemodynamics between groups (19,21). The studies did not mention placebo-controlled in the methods, hence, may be subject to bias. Furthermore, most of the studies only examined clinical hemodynamics, such as blood pressure, heart rate, and CVP (17-19,22,23), which might be influenced by several confounders, including, patient's baseline conditions, volume status, surgical manipulation, and anesthetic agents. The study presented here was designed to examine the effects of combined H_1 & H_2 antihistamines pretreatment on systemic arterial pressure after protamine was administered. The authors also aimed to investigate whether the release of histamines from mast cells played a key role in hypotension after protamine. We chose serial serum tryptase levels as a surrogate for mast cell degranulation instead of serum histamine level because of the short half-life of histamine in the blood samples, which would make it difficult to properly handle the samples in the operating room before being transported to the laboratory.

In this study, we could not demonstrate the differences in trajectory SBP and MAP during the first 30 minutes after protamine infusion between pretreatment with antihistamines and the control groups. After adjusted for EuroSCORE II, CPB time, and vasopressors and inotropic uses, the results remained the same. The findings are consistent with that reported by some of the previous studies (20,21). Kanbak *et al.* (21) did an RCT in 40 cardiac patients and compared between patients receiving prophylactic H_1 (oral terfenadine), H_2 (oral ranitidine), and both H_1 & H_2 antihistamines before operation compared to the control group. They reported no significant differences in hemodynamic variables within the 5 minutes after protamine infusion between any of the groups and the control group. Another study by Behne *et al.* also reported

Table 3 Summary of the randomized-controlled studies investigating the effects of pretreatment antihistamines on cardiovascular responses to protamine administration

Study	Population	Interventions	Protamine administration	Outcomes & conclusions	Remarks
Parsons 1989 (17)	40 elective CABG, normotensive patients, good LVEF	2 groups: no drug, H_1 & H_2 (chlorpheniramine & cimetidine)	Ratio 1:1, 10 minutes after CPB, via central line, rate 2.5 mg/sec (150 mg/min)	Lower BP and slower recovery in the control group; hemodynamic responses only be partially mediated by histamines	Rapid protamine administration via central venous line; follow parameters only the 5 minutes; 2 patients excluded due to hemodynamic instability
Kambam 1990 (18)	30 elective CABG	2 groups: no drug, H_1 & H_2 (diphenhydramine & cimetidine)	Ratio 1:2 (8 mg/kg of protamine), 5–8 minutes, via peripheral vein	33% (control) *vs.* 0% (antihistamine) had decrease in BP >20% from baseline; prophylactic H_1 and H_2 antihistamines prevents hemodynamic side effects	High dose of protamine given; no placebo-control; compared maximum changes in hemodynamic parameters
Mayumi 1992 (19)	126 [103] open heart surgery	4 groups: no drug, H_1 (diphenhydramine), H_2 (famotidine), H_1 & H_2 (both)	N/A	Lower BP and slower recovery in the control group; only H_2 antihistamine was effective	No placebo-control at first; further divided another 23 patients into 2 groups (famotidine and NSS)
Behne 1994 (20)	20 elective CABG	2 groups: no drug, H_1 & H_2 (dimetindene & ranitidine)	350 u/kg protamine in 4 minutes, via peripheral vein	Comparable hemodynamics; no correlation between plasma histamine and hypotension; histamine may not play role in protamine reactions	Plasma histamine levels obtained until 10 minutes after protamine; small sample size; no placebo-control mentioned
Kanbak 1996 (21)	40 cardiac surgery, normal LVEF	4 groups: no drug H_1 (terfenadine), H_2 (ranitidine), H_1 & H_2 (both)	Based on ACT protamine dose assay worksheet, >3 minutes via peripheral vein	Plasma histamine-like activity (H-LA) increased after heparin not protamine; no difference in hemodynamic; histamine may not play role in protamine reactions	Small sample size; too many groups; excluded patients with sudden hemodynamic changes; follow parameters only 5 minutes
Lango 2000 (22)	58 elective CABG, 5 (4 drugs, 1 control) patients excluded	2 groups: no drug, H_1 (clemestine)	Ratio 1:1.5, within 7 minutes via peripheral vein	Faster increase in BP to normal in clemestine group; clemestine pretreatment normalized BP after protamine	Per-protocol analysis; no placebo-control; compared parameters at the same interval, not trajectory
Sikander 2008 (23)	60 open heart surgery (unspecified types)	2 groups: no drug, H_1 (chlorpheniramine)	Ratio 1:1.3 protamine >10 minutes i.v. (site not mentioned)	Higher BP and reduced inotropic requirement in the antihistamine group; antihistamine pretreatment helps maintain hemodynamic after protamine	Demographic data of the patients are not presented; no placebo-control; no statistical analysis explained

CABG, coronary artery bypass grafting; LVEF, left ventricular ejection fraction; CPB, cardiopulmonary bypass; BP, blood pressure; NSS, normal saline; ACT, activated clotting time.

no difference in hemodynamic profiles between the group with pretreatment H_1 & H_2 antihistamines and the control in 20 patients who underwent elective CABG (20). In their study, besides hemodynamic parameters, serial serum histamine levels until 10 minutes after protamine were also examined. A slight increase in serum histamine levels was observed in both groups, however, there was no association between the slightly elevated histamine levels and clinical

hypotension. The authors concluded that histamine release may not be the underlying mechanism of protamine-related hypotension.

In contrast to our findings, some of the previous studies had demonstrated the benefit of pretreatment with antihistamines in attenuating hemodynamic changes following protamine administration (17-19,22,23). Lango *et al.* reported a more rapid recovery in systemic blood pressure after drops following protamine in patients pretreated with H_1 antihistamine (clemastine) compared to the control group in 53 patients who underwent CABG (22). However, four patients in the antihistamine group and one patient in the control group were excluded from the analysis after randomization due to severe hemodynamic instability. Moreover, the differences in SBP, MAP, and DBP reported in this study were only about 5–10 mmHg, which may be considered insignificant clinically. Another study by Parsons *et al.* in 38 patients with good left ventricular ejection fraction who underwent elective CABG, demonstrated a more subtle decline in blood pressures from baseline in the antihistamine group (–25%) compared to the control group (–32%) (17). In their study, dramatic falls in blood pressure were observed in both groups following protamine administration since protamine was rapidly injected (2.5 mg/sec) via central venous catheter, while in our study, we slowly administered protamine in 5 minutes via peripheral line. A faster rate of administration of protamine resulted in a more severe hemodynamic perturbation than slow administration (6,30). Furthermore, the patients did not receive any vasopressor and inotropic support after separation from CPB, therefore, the magnitude of hemodynamic effects of protamine was more prominent than in our study which hypotension was partially treated with vasopressor and inotropic medications. In this study, an increase in blood pressure from baseline (T_0) was observed during the first few minutes after separation from CPB, which might be due to the restoration of preload and effects from inotropic/vasopressor titration, followed by a reduction of blood pressure to baseline level after the completion of protamine infusion in both groups. During the first few minutes of protamine infusion, the serum concentration of protamine had not reached its peak, hence, prominent hemodynamic effects from protamine were not observed. We used a fixed 1:1 ratio (1 mg protamine to every 100 IU of heparin) because this ratio was still commonly used in our institute when we started the recruitment and was a common recommendation (31). Despite the dose used in our study may have caused excess free drug compared to the lower dose (ratios of 0.6 or 0.8) as recommended by current evidence, we could not demonstrate any difference in systemic blood pressure after protamine was given. This suggests that pretreatment with antihistamines should not be useful in lower dose ranges of protamine as well. Unlike our study, the statistical differences in blood pressures between groups being reported in most of the positive studies were only minor (<10 mmHg), which might not be clinically meaningful and should be manageable with a slow rate of protamine administration and low-dose vasopressors. Hence, the clinical benefit of pretreatment with antihistamines for attenuating protamine-related hemodynamic instability is uncertain.

Regarding serial serum tryptase levels, none of the patients in both groups had significant elevation in serial serum tryptase within 60 minutes after protamine exposure, suggesting that protamine reactions might not be mediated through mast cell degranulation, in patients without anaphylaxis. An anaphylactic reaction is rare and we also excluded patients with potential risks for protamine allergy, such as a history of exposure to protamine-containing insulin, previous heart surgery, and history of allergy to salmon. An *in vitro* study demonstrated that protamine caused dose-related histamine release from human skin mast cells but only when much higher concentrations than that in clinical practice were used (32). A previous study by Kambam *et al.* demonstrated a significant decline in blood pressures (MAP –15 mmHg) after protamine in the control group compared to stable blood pressures in the group with antihistamines pretreatment (18). In their study, 8 mg/kg of protamine was used, which was much higher than the dose currently used nowadays. While in our study, only 3 mg/kg of protamine was used and slowly administered, therefore, the level of protamine might not reach intravascular concentrations which trigger the histamine release.

Mechanisms other than histamine release from mast cells have also been elucidated. First is the inflammatory response induced by C-reactive protein resulting in complement activation through the classical C4a pathway (33). In a prospective observational study examining the incidence of protamine reactions among 243 patients who underwent surgery using CPB, they reported an increase in C4a levels in all groups of patients after protamine administration and significantly higher levels in the group with reactions (34). Another possible mechanism for hypotension following protamine is vasoplegia, which has been demonstrated *in vitro* (14). Protamine activates endothelial NO synthase pathway resulting in vasorelaxation demonstrated in the isolated internal thoracic artery (35). NO is a potent

vasodilator and also inhibits platelet aggregation. Moreover, a study reported that protamine augmented intracellular endothelial Ca^{2+} release in response to mechanical stretch, causing vasodilatation in the isolated human umbilical vein cells (36). In a small study among 22 patients after CPB, protamine neutralization of heparin resulted in gradual drops in systemic vascular resistance and blood pressure eventually decreased once cardiac output was over-compensated (2). Lastly, a recent *in vitro* study using isolated mitochondria from rat hearts demonstrated that protamine caused an excessive increase in mitochondrial reactive oxygen species (ROS), resulting in mitochondrial dysfunction (37). The authors proposed that excessive formation of ROS might be the pathogenesis of the cardiovascular (both systemic hypotension and pulmonary hypertension) side effects of protamine. All in all, the findings from our study supported that mast cell degranulation might not play a major role in the unwanted cardiovascular side effects of protamine and other mechanisms should rather be taken into account and further investigated.

There are some limitations to this study. First, the small sample size may increase the likelihood of type II error. Secondly, the primary outcome of this study was the differences in MAP between the two groups. Although we tried to analyze the differences in blood pressures after adjusted for EuroSCORE II, CPB time, and VIS score at each time point, however, blood pressures can also be influenced by many factors, such as volume status, ventricular functions, vasoplegic state, surgical bleeding, and surgical manipulation. It can be challenging to control and compare these factors, hence, may be a confounder to our study. Although we tried to include only the patients who underwent similar procedures (elective CABGs and single valve surgery), it was not possible to control the differences in the complexity of the procedures and CPB time of each individual, which may influence hemodynamics during post-CPB. Lastly, we excluded patients with risks for protamine allergy, such as a history of exposure to protamine-containing insulin, previous heart surgery, and a history of allergy to salmon, therefore, the negative results of pretreatment antihistamines from our study might not be applicable and antihistamines may still be beneficial for these patients.

Conclusions

In conclusion, pretreatment with H_1 and H_2 antihistamines does not attenuate blood pressure responses to protamine administration in patients after CPB. Moreover, none of the patients in our study had significant elevation in serial serum tryptase levels, thus suggesting that it is not common for protamine administration to cause systemic mast cell degranulation and histamine release in patients without allergy. Other mechanisms might be responsible for protamine-induced cardiovascular changes after protamine administration.

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Footnote

Reporting Checklist: The authors have completed the CONSORT reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-22-714/rc

Trial Protocol: Available at https://apm.amegroups.com/article/view/10.21037/apm-22-714/tp

Data Sharing Statement: Available at https://apm.amegroups.com/article/view/10.21037/apm-22-714/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-22-714/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the Siriraj Institutional Review Board (No. Si 518/2018) and was registered at clinicaltrail.gov (No. NCT03583567). Informed consent was obtained from all participants before recruitment.

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