

Impact of different dosage of protamine on heparin reversal during off-pump coronary artery bypass: a clinical study

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ABSTRACT

Introduction: Currently, a dose of protamine equal to 1 mg for each 100 units of heparin given is used to reverse the residual heparin activity following off-pump coronary artery bypass. We hypothesized that a 1:1 ratio (ratio of protamine to heparin) could be higher than necessary inducing post-operative disturbance of hemostasis.

Methods: Between January and March 2014 in 9 patients undergoing off-pump coronary artery bypass, we evaluated the effect of a dose of protamine equal to 1 mg per 100 units of heparin (Total Calculated Dose) on hemostasis as evaluated by means of thromboelastometry. Two data analyses were performed: the first after the administration of 2/3 of the Total Calculated Dose of protamine and the second after the administration of the Total Calculated Dose of protamine.

Results: We found that the administration of 2/3 of Total Calculated Dose of protamine was always able to reverse the anticoagulant effect of heparin and that a significant clotting time elongation was induced by the infusion of the second part of the Total Calculated Dose of protamine. No modification in clot firmness was observed.

Conclusions: The present study seems to suggest that the commonly applied ratio equal to 1:1 (ratio of protamine to heparin) could be higher than needed with potential and hazardous impacts on the efficacy of the coagulation system.

Keywords: *protamine, heparin, off-pump coronary artery bypass (OPCAB), thromboelastography.*

INTRODUCTION

Protamine is used in patients undergoing Off-pump Coronary Artery Bypass (OPCAB) surgery to reverse the anticoagulant effects of heparin and restore coagulation. However, protamine administration may produce a paradoxical anticoagulation (1)

as recently confirmed following cardiopulmonary bypass (CPB) surgery (2).

There are currently several schemes described for anticoagulation with heparin and its reversal with protamine during OPCAB. The most used in European countries and North America is a fixed dose scheme, with a bolus dose of heparin established in IU/kg of body weight, given at the beginning of the procedure, and a dose of protamine calculated based on the dose of heparin administered in a 1:1 ratio (1 mg of protamine for every 100 UI of heparin

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given) (3, 4) given to reverse the residual heparin activity after the termination of anastomosis. But this scheme does not take into account neither the inter-patient variability nor the physiological elimination of the heparin and it can result in overdose or sub-doses of one or both drugs.

In the present study, we evaluated the modification of hemostasis induced by the administration of the first 2/3 of the standard programmed dose of protamine to evaluate whether the full dose was really needed to reverse the residual heparin activity or rather this would result in an overdose.

METHODS

The study was carried out between January 2014 and March 2014 at the Sassari cardio-surgery department.

Eligibility. Inclusion criteria were an age of 18 to 75 years; New York Heart Association class I, II, or III or an LV ejection fraction of at least 35 percent; ≤ 5 planned Coronary Artery Bypass Grafts; body-mass index (the weight in kilograms divided by the square of the height in meters) lower than 40 and a weight higher than 55 kg. Exclusion criteria were preoperative congenital or acquired disorders of hemostasis, acute coronary syndrome, or renal impairment.

OPCAB were performed by three fully trained cardiac surgeons who had already performed a minimum of 100 off-pump operations.

Anesthesia. One hour before surgery, all patients were treated with standard pre-anesthesia. Anaesthesia was then induced with fentanyl, thiopental sodium and maintained with continuous infusion of propofol. Muscle relaxation was induced by cisatracurium (0.2 mg/kg). All patients received a bolus of tranexamic acid (total dose 30 mg/kg) following the induction of anesthesia. In all patients a radial artery

line, a central venous catheter and a pulmonary artery catheter were inserted with pulse oximetry, oesophageal temperature, urine output and capnometry continuously monitored. A cell saver collection device (Cell Saver, Hemonetics Corporation, Braintree, MA) was always used and the salvaged blood was processed and re-transfused following the last study-related measurements. According to specific guidelines (5), 1 L of 0.9% saline containing 30,000 units of heparin was mixed with aspirated blood at a ratio of 15 ml per 100 ml of collected blood.

Heparin and Protamine Management. All patients were anticoagulated with 150 IU/kg of mucosal heparin aiming at an Activated Clotting Time (ACT) of more than 300 s. Further doses of heparin (2500-5000 IU) were administered intra-operatively to maintain an ACT value ≥ 300 s as required.

Anticoagulation was programmed to be reversed with a calculated dose of protamine sulfate equal to 1 mg per 100 units of total heparin administered, diluted in 100 ml of saline solution and divided in two parts.

The first two thirds dose was administered directly after termination of anastomoses while the remaining dose was infused ten minutes after the initial dose. Blood samples were obtained 10 min after the first and the second part of the programmed dose of protamine and data regarding ACT, hematocrit, calcium ions, and thromboelastometry values were collected according to the scheme reported in *Figure 1*.

ACT was measured using the kaolin containing Activated Clotting Time Plus Medtronic®. Blood gas analyses were performed using Radiometer® ABL 800 Flex while thromboelastometry was evaluated by means of ROTEM® (Tem International GmbH, Munich, Germany).

Thromboelastometry. Different variables and tests were taken into account to evalu-

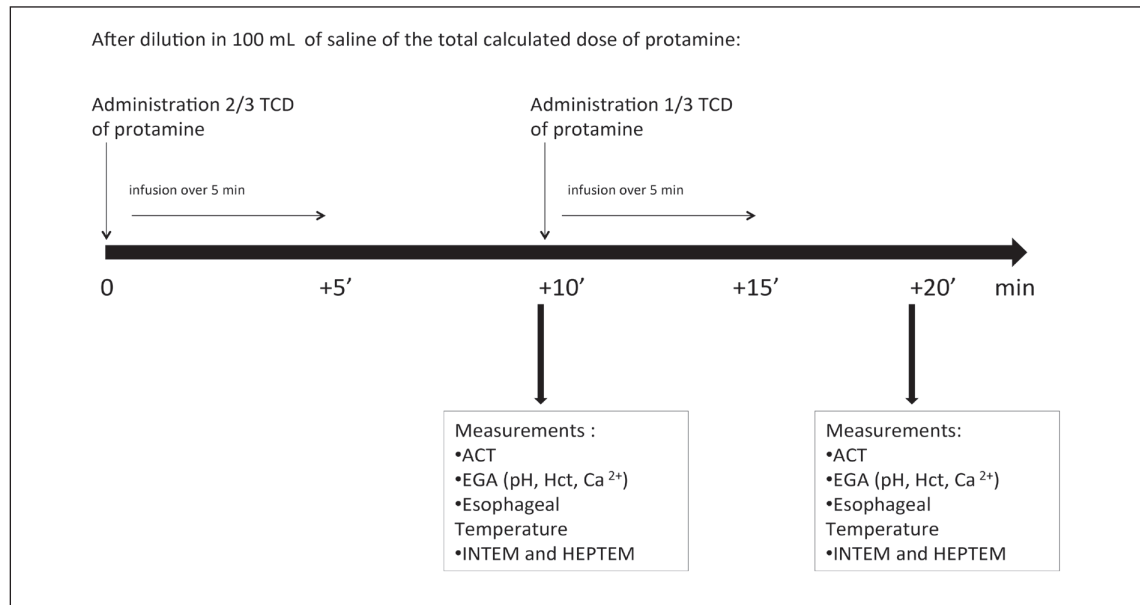


Figure 1 - Schematic description of study procedures. TCD = Total Calculated Dose.

ate the entire coagulation and clot formation processes:

- CT (clotting time) = time after administration of the reagent to the blood until the start of clot formation. Could be affected either by coagulation factors deficiencies or to heparin or protamine excess.
- CFT (Clot formation time) = time from CT to a clot firmness of 20 mm. Primarily influenced by platelet function.
- MCF (Maximum clot firmness) = the greatest vertical amplitude of the trace. It reflects the absolute strength of the fibrin and platelet clot.
- A10/A20 = the clot firmness (or amplitude) obtained 10 or 20 minutes after CT. Provides a forecast on the expected MCF value at an earlier stage.
- INTEM = this test activates the contact phase of hemostasis. The result is influenced by coagulation factors, platelets, fibrinogen and heparin.
- HEPTM = it represents an INTEM assay performed in the presence of hep-

arinase, a heparin degrading enzyme. It allows for the evaluation of the INTEM test without heparin or heparin like anticoagulants interferences. A difference in CT-value between HEPTM and INTEM confirms the presence of heparin.

Primary and Secondary End Points. The primary endpoint of the study was the evaluation of the effectiveness of two thirds the dose of protamine on heparin reversal and its effects on the hemostatic system.

The secondary endpoint was the evaluation of the accuracy of ACT for the detection of heparin reversal.

Statistical analysis. Continuous variables are presented as means \pm SD and categorical variables as proportion. Continuous data were analyzed using paired t-tests to evaluate significant changes between the two time points. A *P* value less than 0.05 was considered significant.

Informed consent. The study protocol has been approved by the Ethical Committee

of the Santissima Annunziata Hospital in Sassari. All patients gave written consent to participate in the study protocol before enrollment.

RESULTS

Nine patients were studied. The patient characteristics and operative data of the study population are reported in *Table 1*. Anticoagulation regimens are reported in *Table 2*. Neither vasoactive nor inotropic drugs in the intraoperative course were

Table 1 - Patients characteristics.

Age (yrs)	68.7 ± 8.4
Gender (M/F)	9/0
Weight (Kg)	72.7 ± 9.3
Height (cm)	168.5 ± 7.9
Preoperative clopidogrel	0
Preoperative Acetyl Salicylic Acid	9
Preoperative platelet count x 103/μL	291 ± 53
Patient with 2/3/4/5 distal anastomoses	3/5/0/1
Operation time (Skin to Skin) (min)	247.0 ± 76.4
Heparin to protamine time (min)	112.0 ± 21.2

Table 2 - Effect of protamine administration on thromboelastomeric parameters.

Patinet N°	Weight (Kg)	Heparin Inital bolus (UI)*	Cumulative heparin supplemental boluses (UI)*	Total heparin administered (UI)	Heparin to protamine time (minutes)	Total protamine dose programmed (mg total)
1	63	9500	7500	17000	101	170
2	74	11100	7500	17600	110	176
3	60	9000	5000	14000	90	140
4	80	12000	5000	17000	110	170
5	88	13200	5000	18200	106	182
6	80	12000	5000	17000	87	170
7	64	9600	10000	19600	145	196
8	70	10500	5000	15500	146	155
9	75	11300	5000	16300	1120	163

Table 3 - Anticoagulation regimen. *As required during the procedure to maintain the Activated Clotting Time (ACT) > 300 s. All values are reported in seconds.

Paitent N°	Basal ACT	Higer ACT	ACT 2/3 TCD	ACT TCD	CT Intem:Heptem 2/3 TCD	CT Intem:Heptem TCD
1	165	348	164	170	194:198	208:232
2	192	492	168	164	192:208	240:236
3	166	378	158	146	199:203	220:250
4	169	401	194	163	204:227	213:228
5	135	339	135	137	16.186	171:191
6	141	344	133	140	191:208	237:236
7	155	301	126	130	190:195	227:245
8	154	338	136	128	172:191	178:202
9	149	298	168	168	184:202	235:232

ACT = Activated Clotting Time; TCD = Total Calculated Dose of protamine.

Table 4 - Thromboelastomeric parameters at different stages of protamine administration.

	INTEM 2/3 TCD	INTEM TCD	t-test p vaule	HEPTEM 2/3 TCD	HEPTEM TCD	t-test p vaule
CT (s)	188.1 ± 12.0	214.0 ± 25.0	0.0035	202.0 ± 11.9	228.0 ± 19.3	0.0020
CFT(s)	71.9 ± 19.1	69.0 ± 12.0	0.4048	64.4 ± 15.4	66.1 ± 9.7	0.8934
Alfa°	75.8 ± 3.9	76.8 ± 1.6	0.3402	76.5 ± 3.0	76.6 ± 1.6	0.8417
A10(mm)	57.0 ± 5.9	57.2 ± 5.1	0.7458	57.9 ± 5,8	57,8 ± 4.9	08695
A20 (mm)	62.9 ± 5.4	63.0 ± 4.9	0.8548	63.7 ± 5.5	63.1 ± 4.9	0.4008
MCF(mm)	64.8 ± 5.9	63.7 ± 4.0	0.3566	64.5 ± 5.4	64.0 ± 5.1	0.3016

CT = Clotting Time; CFT = Clot Formation Time; α° = Alfa Angle; A10 = clot amplitude at 10 min after clotting time; A20 = clot amplitude at 20 min after clotting time; MCF = maximum clot firmness; TCD = Total Calculated Dose of protamine; s = seconds; mm = millimeters.

used, and patients were managed in order to maintain normo-thermic conditions as to reduce biases. No patient needed red blood cell, platelet concentrates, fresh frozen plasma or administration of coagulation factors for intraoperative or postoperative bleeding. Also, no change in hematocrit, temperature, pH and calcium ions levels were observed. According to protocol, the total calculated dose of protamine (TCD) has been divided in two doses: the first, equal to 2/3 of the TCD, directly administered after the termination of anastomoses and the remaining 1/3 infused ten minutes after. The effects on ACT and thromboelastomeric parameters are showed in *Table 3* and *4*. As shown in all cases, we found that the administration of 2/3 of the TCD was able to completely reverse the anticoagulant effect of heparin supported by the evidence that all CT-HEPTEM were always found to be longer than the relative CT-INTEM. In the great majority of cases, this is even confirmed by the ACT, which returned to a value equal to +10% with respect to baseline. This is not true in case #4, in which the return to a value equal to baseline +10% was obtained only after the second part of the TCD, and in case #9, in which not even the second part of the TCD was able to restore a value equal to baseline

+10%. Overall, we found a significant CT prolongation either in the INTEM or HEPTEM test ($p < 0.01$) after the infusion of the second part of the TCD compared to the data obtained after the first part of the TCD with an elongation of CT-INTEM equal to 26.2 ± 19.27 seconds and CT-HEPTEM 26.0 ± 17.3 seconds. No change in clot firmness was observed.

DISCUSSION

In this observational study designed to evaluate whether a dose of protamine equal to the dose of heparin administered would induce anticoagulation during OPCAB, we found that a complete reversal of heparin could be effectively achieved with 2/3 of the dose usually proposed by the literature and that the current dose could be higher than necessary inducing post-operative disturbances in hemostasis (3, 4). Moreover, we found that the additional administration of protamine seems to induce an elongation of the clotting time with no impact on clot firmness.

Our findings are in line with the results obtained by Gertler et al. (6) who observed that platelet function worsens with a protamine to heparin ratio higher than 1:1 in blood samples taken from ten healthy vol-

unteers. Similar impairment of platelet aggregation and function with the use of a protamine to heparin ratio above 2.6:1, was demonstrated by Carr et al. (7).

The impact of a high protamine to heparin ratio on coagulation time was studied by Mittermayr et al. either in-vitro (8) or in the clinical setting (9).

In blood samples from 26 healthy volunteers they found that increasing concentrations of protamine to a protamine to heparin ratio exceeding 1.6:1 lead to an elongation of either the CT-INTEM or CT-HEPTEM (8). In a clinical setting, they studied a group of 22 patients with coronary artery surgery and cardiopulmonary bypass (CABG) (9). After an initial dose of protamine based on the total amount of heparin initially administered, additional protamine (70 µg/kg) was administered to patients with an ACT above baseline and with clinical signs of diffuse bleeding. In the 16 patients receiving additional protamine, coagulation times significantly increased. This was not observed in the 6 patients receiving a single protamine dose.

The impact of a high protamine to heparin ratio was even studied by Kahn et al. (10). They studied blood samples of 46 patients undergoing cardiac surgery using CABG and found a significant worsening of the coagulation parameters using protamine to heparin ratio of more than 2:1.

In the clinical setting, even in absence of strict guidelines or recommendations, intraoperative anticoagulation and heparin reversal, is usually performed with 150 UI/Kg initial heparin dose and 1 mg of protamine per 100 units of total heparin administered respectively.

This lead to a protamine to heparin ratio that is far from the ratio studied in experimental setting. But, heparin levels are expected to decrease during cardiac surgery as the elimination half-life of heparin depends either on initial dose or body tem-

perature. It could hence be expected that, during OPCAB a protamine regimen based on a fixed dose might lead to a higher than expected protamine to heparin ratio with a consequent impairment of the coagulation system. It has been shown that doses of 100, 200, and 400 U/kg of intravenous heparin at 37° show an approximate half-life of 60, 100, and 150 min, respectively (11), and that decreases in the body temperature increase the elimination half-life (12).

A decrease of heparin concentration equal to 40% during CPB has recently been shown in a randomized double-blind controlled pilot study by Koster et al. (2). They compared the effects of two different protamine management regimens on thromboelastomeric parameters: heparin dose-based vs heparin concentration-based (assessed using the Hepcon MHS Plus device) protamine administration. A protamine overdose with the inhibition of the coagulation process was found in the heparin dose-related group due to the reduction of the concentration of heparin during CPB.

It could be expected that the expected reduction in heparin concentration in OPCAB could be higher than during CABG because a faster heparin turnover occurs in normo-thermic conditions in respect to hypothermia (12).

It is known that overdose of protamine has anticoagulant effects which might lead to bleeding complications. It causes platelet dysfunction (13) inhibits glycoprotein Ib-vWF activity (14) and serine proteases involved in coagulation (15) and enhances fibrinolysis (16). However, whether protamine excess can be clinically significant when used to determine bleeding is an open question.

Despotis et al. (17) demonstrated that a reduction in the protamine to heparin ratio reduced postoperative bleeding and the need for fresh frozen plasma and platelet administration after CPB. In a prospective

study of 250 patients, they found a reduction of post-operative blood, platelet, plasma and cryoprecipitate units administration when protamine dose was calculated considering residual heparin concentration at the end of CPB rather than the initial dose administered before CPB.

This conclusions were also confirmed in the study of DeLaria et al. (18). They demonstrated, in a non-randomized retrospective review of 150 patients undergoing open heart surgery during a 9-month period, that a reduction in protamine significantly reduces postoperative bleeding and blood product administration.

In contrast, Svenarud et al. (19) found in a retrospective cohort study of 300 patients undergoing CABG, that a single bolus dose of 1.3 mg protamine to 1 mg heparin is safe and efficient for neutralizing heparin.

They investigated 3 different protamine to heparin ratios: 1.3 to 1 mg vs 0.75 to 1 mg vs. a fractionated infusion 1 + 0.15 + 0.15 to 1 mg. The rate of red cell transfusion was significantly higher in the group that received a protamine to heparin ratio of 0.75 to 1 mg even in absence of differences in postoperative hemoglobin loss, incidence of re sternotomy for postoperative bleeding and losses from mediastinal drainage.

In our study we also evaluated the accuracy of ACT for the detection of heparin reversal. The ACT test is a global or functional test that measures the effect of many variables including medications, temperature, dilution, coagulation factors deficiency, heparin anticoagulation and also protamine excess.

An ACT value higher than baseline after protamine administration is not always a sign of incomplete reversal and even an ACT value that has returned to baseline can hide residual heparin. This has been shown by Despotis et al. (20) who reported that ACT correlates poorly with plasma levels of heparin during cardiac surgery.

This seems to be confirmed in our study by the two patients who maintained an ACT over 10 % of basal level after first dose of protamine. The hypothesis that this could be attributed to the need of additional doses of protamine could be ruled out considering the ROTEM assay CT-INTEM: CT-HEPTEM ratio was already almost 1 after the first dose of protamine administered.

In absence of specific measurements, we cannot rule out the hypothesis that the CT prolongation we observed with the administration of the second part of the total calculated dose of heparin could be due to different causes such as the presence of anticoagulants or fibrin-fibrinogen degrading products and/or a deficit of coagulation factors. But, the absence of difference in the clot firmness seems to contradict this hypothesis.

CONCLUSION

In the present study we found that a complete reversal of heparin administered in patients undergoing off-pump coronary artery bypass could be effectively achieved with 2/3 of the dose usually proposed by the literature and that the additional administration of protamine seems to induce an elongation of the clotting time with no impact on clot firmness.

Future studies, specifically designed to test the effectiveness of different regimes in reversing heparin activity after OPCAB are needed to confirm the results of the present observational study.

Moreover, the clinical impact of the CT thromboelastomeric test elongation and the duration in time of coagulation disturbance observed are yet to be clarified.

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