

# The effects of protamine overdose on coagulation parameters as measured by the thrombelastograph

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**Background and objective** Protamine is routinely administered following cardiopulmonary bypass in order to neutralize the effects of heparin. An excess of protamine can contribute to coagulopathy, hence predisposing to bleeding with associated morbidity and mortality. Thromboelastography (TEG) is recognized as an invaluable bedside tool to detect coagulation parameters; however, the effects of protamine overdose on TEG parameters have not been fully established.

**Methods** Forty-six patients undergoing cardiac surgery using cardiopulmonary bypass were recruited in the study. Following heparinization, the patient's blood heparin level was measured using Hepcon HMS. Incremental doses of protamine [at a protamine-to-Hepcon-derived heparin ratio (PHR) of 1 : 1, 2 : 1 and 3 : 1] were added to patients' blood samples *in vitro* and four TEG coagulation parameters, including *R* (time to clot initiation), *K* (clot kinetics),  $\alpha$  (clot kinetics) and maximum amplitude

(ultimate clot strength), were monitored. Statistical analysis was performed using NCSS software.

**Results** Protamine caused dose-dependent worsening of coagulation parameters on TEG; *K* was significantly elevated, whereas  $\alpha$  and maximum amplitude showed significant reduction ( $P < 0.001$ ) compared with baseline at a PHR of 2 : 1 and 3 : 1, respectively. *R* was significantly prolonged compared with baseline ( $P < 0.001$ ) at a PHR of 3 : 1.

**Conclusion** Protamine adversely affects clot initiation time, clot kinetics and platelet function in a dose-dependent manner, which can predispose to bleeding.

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## Introduction

Cardiac surgery carries a risk of bleeding postoperatively, brought on by the surgery itself, or due to a reduction of coagulation factors or platelets, a consequence of residual heparin, or an excess of protamine given at the end of cardiopulmonary bypass (CPB) to neutralize heparin.<sup>1,2</sup> Most centres use a dose of protamine equal to or 1.2 times the initial dose of heparin (administered at the beginning of CPB), without measuring the actual quantity of systemic heparin.<sup>3</sup> It is known that heparin levels fall during CPB, so that the heparin level at the end of surgery may in fact be quite low.<sup>4</sup> Hence, a protamine dose equal to the initial heparin dose may in fact be an overdose.

Anticoagulation has traditionally been monitored throughout CPB using the activated clotting time (ACT).<sup>4,5</sup> Increasing evidence suggests that the ACT does not correlate with true heparin levels, and that it can be affected significantly by patient hypothermia and haemodilution.<sup>6,7</sup> Hepcon HMS (Hepcon) (Medtronic Hemo-Tec, Parker, Colorado, USA) is a whole-blood haemostasis system that determines the quantity of heparin per millilitre of blood via the protamine titration method and estimates the required protamine dose.<sup>8</sup> The use of Hepcon reduces the risk of protamine overdose by measuring the actual heparin levels, thus accounting for

the effects of haemodilution, hypothermia and heparin decay.<sup>9</sup>

The thrombelastograph (TEG) (Haemostasis System; Haemoscope, Illinois, USA) is a bedside monitor used to assess and predict postoperative coagulopathy.<sup>6,10,11</sup> TEG measures the clot's physical properties and kinetics and produces a haemostasis profile of the various stages of clotting and consequent dissolution of the clot. Hence, TEG can be used as an effective tool to measure the coagulopathic effects of excess protamine. The aim of our study was determine the effects of adding incremental doses of protamine to heparinized blood on coagulation parameters measured by TEG.

## Methods

Forty-six patients undergoing routine cardiac surgery using CPB and full systemic heparinization were recruited to the study. At the time of the research, aprotinin was used as a standard perioperative bleeding prophylaxis for cardiac surgery in our centre. The majority of patients (38) had coronary artery bypass grafting (CABG) only, two patients had an aortic valve replacement with CABG, two patients had a mitral valve replacement with CABG, one patient had an aortic valve replacement only, two patients had just mitral valve replacement and three patients had both aortic and mitral valves replaced. Exclusion criteria included patients with liver disease, known bleeding diathesis, a low platelet count of less than  $150 \times 10^9/L$ , those who had received antiplatelet or fibrinolytic agents within 2 days of surgery and those patients who were significantly anaemic preoperatively [haemoglobin (Hb)  $< 10 \text{ g dl}^{-1}$ ]. This research

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**Table 1 Protamine titration table**

Hepcon concentration (units ml <sup>-1</sup> )	Single-dose protamine (μg)	Double-dose protamine (μg)	Triple-dose protamine (μg)
2	200	400	600
2.7	270	540	810
3.4	340	680	1020
4.1	410	820	1230
4.8	480	960	1440
5.4	540	1080	1620

was approved by the local research ethics committee, and informed consent was obtained from each patient.

We intended to study the effects of protamine overdose in increments of 1:1, 2:1 and 3:1 protamine-to-Hepcon-derived heparin ratios (PHRs). At the start of surgery, the loading dose of heparin was measured using the Hepcon system. The blood sample was collected from the arterial line after removal of at least one dead space, and this was applied for all samples in the study. The CPB pump was primed according to local standard protocols, and ACT was maintained for greater than 480 s during CPB with unfractionated porcine heparin. Within 2–5 min of systemic heparinization, the blood heparin level was again determined using Hepcon. This heparin level was used to calculate the quantities of protamine needed to create an overdose. At the same time, another blood sample was collected to determine the baseline coagulation ability by using heparin-modified TEG.

With the range of titration levels of the Hepcon between 2 and 5.4 units ml<sup>-1</sup>, a total of 200–540 μg (0.02–0.054 ml) protamine is required to reverse heparin in a 1:1 PHR in 10 ml of blood. Hence, a double or triple overdose of protamine can be easily calculated (Table 1). It should be remembered that our calculated protamine doses did not necessarily resemble these figures, as our readings depended on the Hepcon values for heparin and protamine.

After measuring the quantity of heparin with Hepcon, three further blood samples were immediately taken for an in-vitro heparin neutralization. The appropriate dose of protamine (at 1:1, 2:1 or 3:1 PHR) was then added to each specimen pot using a pipette. Following gentle mixing, a TEG trace was obtained for each sample, and the following parameters were monitored: *R* (reaction time to clot initiation; represents the time until the TEG tracing amplitude reaches 2 mm); *K* (clot formation time; from the end of *R* until the TEG tracing amplitude

reaches 20 mm); alpha angle ( $\alpha^\circ$ ) (the angle formed by the slope of the TEG tracing from the *R* to the *K* value); and maximum amplitude (maximum amplitude of the TEG tracing).

All statistical analyses were performed using the NCSS 2004 software (NCSS, Kaysville, Utah, USA). Owing to nonparametric distribution of TEG results, intergroup comparisons were made using the Kruskal–Wallis test, with the Bonferroni correction applied to all results. A *P* value of less than 0.05 was considered statistically significant.

## Results

Table 2 presents the medians (range) for various TEG parameters. All the coagulation parameters were labelled according to the PHR; for example, *R1* for PHR 1:1, *R2* for PHR 2:1 and so on. Figures 1–4 present the changes in each individual parameter on a box plot (Table 2).

### Reaction time to clot initiation (*R* time)

*R* time showed a dose-dependent increase with protamine. Although there was no statistically significant difference between *R1* and *R2* compared with baseline, a PHR of 3:1 led to a significant rise in *R* time ( $P < 0.001$ ) (Fig. 1).

### Clot formation time (*K* value)

We found a dose-dependent increase in *K* value with protamine. The *K* values obtained with 2:1 and 3:1 PHR (*K2* and *K3*, respectively) were significantly higher than the baseline value ( $P < 0.001$ ) (Fig. 2).

### Alpha ( $\alpha$ )

Alpha decreased as the dose of protamine increased, and both  $\alpha_2$  and  $\alpha_3$  were significantly lower than the baseline value ( $P < 0.001$ ) (Fig. 3).

### Maximum amplitude

The maximum amplitude showed a decline at higher protamine doses. Once again, MA2 and MA3 were significantly lower than the baseline maximum amplitude ( $P < 0.001$ ) (Fig. 4).

## Discussion

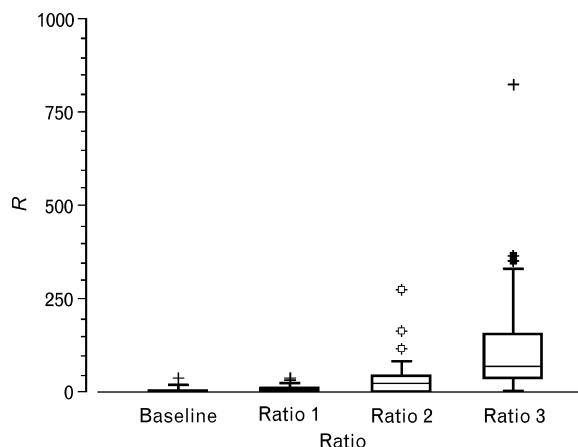
At the end of CPB in cardiac surgery, it is usual practice to deliver a fixed dose of protamine equal to 1–1.2 times the initial dose of heparin administered at the beginning of CPB.<sup>12,13</sup> However, this takes no account of the heparin

**Table 2 Thromboelastography parameters at baseline and different protamine-to-Hepcon-measured heparin ratios**

	<i>R</i> (min)	<i>K</i> (min)	Alpha (°)	MA (mm)
Baseline	9 (3–38)	2 (1–13)	61.9 (17.7–74.8)	58.5 (34.4–89.7)
PHR, 1:1	12.95 (5.2–37.8)	2.55 (0.9–15.4)	56.5 (10.2–75.3)	58.3 (42.5–70.4)
PHR, 2:1	32 (2.8–275.0)	*4.5 (1.1–45)	*31.6 (0.9–73.5)	*47.0 (4.3–74.3)
PHR, 3:1	*78.5 (6.3–822.0)	*8.4 (1.3–59.2)	*11.8 (0.3–69.1)	*37.5 (2.0–82.8)

All data are presented as median (range). MA, maximum amplitude; PHR, protamine-to-Hepcon-measured heparin ratio. \* $P < 0.001$  compared with baseline.

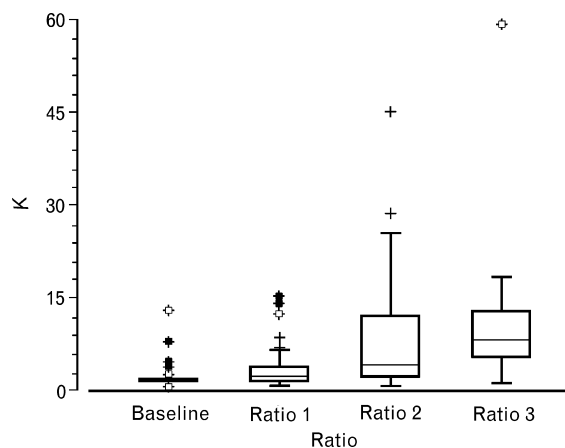
Fig. 1



Box plot showing  $R$  times at baseline and various protamine-to-heparin ratios.

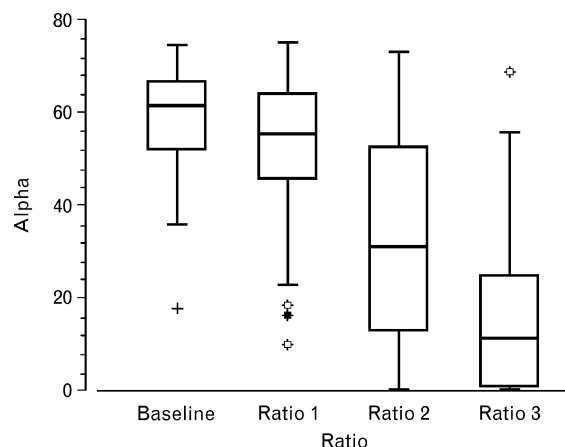
decay during CPB, as well as the effects of haemodilution and hypothermia on heparin levels.<sup>14</sup> Hence, clinically excessive doses of protamine can be given inadvertently, which may compromise coagulation cascade. Indeed, use of low-dose protamine has been associated with less postoperative bleeding.<sup>3</sup> The use of Hepcon, by measuring the actual concentration of heparin, can demonstrate the effects of heparin decay, haemodilution and hypothermia. Several studies have demonstrated the clinical utility of Hepcon in reducing blood product utilization.<sup>15</sup> In their study comparing the fixed-dose heparin approach and the Hepcon approach, Despotis *et al.*<sup>15</sup> found that 33% of the fixed-dose controls received blood product transfusions compared with just 17% in the Hepcon group ( $P < 0.05$ ). Somewhat similar observations were made by Shigeta *et al.*,<sup>16</sup> who identified that the

Fig. 2



Box plot showing  $K$  times at baseline and various protamine-to-heparin ratios.

Fig. 3

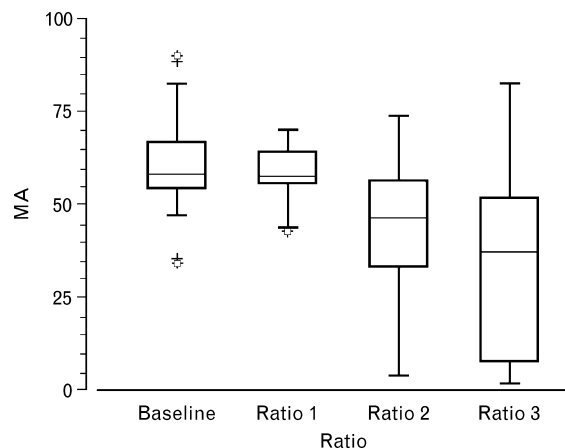


Box plot showing the angle  $\alpha$  at baseline and various protamine-to-heparin ratios.

Hepcon titration group received a lower protamine dose and demonstrated greater recovery of platelet aggregation ( $55 \pm 18$  vs.  $20 \pm 20\%$ ,  $P = 0.0009$ ). This suggests superior restoration of coagulation in patients who received protamine according to the Hepcon titration methods.<sup>16</sup>

It is also well documented that excessive protamine leads to prolonged ACT, weakened clot structure, altered clot kinetics and ADP-induced platelet aggregation in a dose-dependent manner.<sup>17,18</sup> TEG is a rapid and convenient bedside monitor of coagulation, providing invaluable information regarding platelet function, fibrinolysis and coagulability.<sup>6</sup> We looked at the effects of excess protamine on the TEG parameters and, in doing so, found that protamine has an effect on all aspects of coagulation. We

Fig. 4



Box plot showing the maximum amplitude at baseline and various protamine-to-heparin ratios. MA, maximum amplitude.

demonstrated that protamine adversely affects clot initiation and clot kinetics, as well as platelet function. A decline in  $\alpha$  and maximum amplitude values with higher protamine doses suggests less rapid fibrin build-up and cross-linking.

Our results are unable to explain the precise mechanism by which protamine achieves its anticoagulant actions. Previous studies<sup>9,19,20</sup> have shown that the heparin–protamine complex may alter coagulation by affecting platelet aggregation, reducing expression of P selectin or by reducing the  $\text{Ca}^{2+}$  influx into platelets. Shanberge *et al.*<sup>21</sup> proposed that an excess of protamine does not itself act as an anticoagulant but exerts its effects by producing large heparin–protamine complexes that activate antithrombin III. However, Griffin *et al.*<sup>20</sup> showed that protamine alone can directly affect platelet function. Protamine has also been described more recently to significantly enhance fibrinolysis by inhibition of tissue factor-initiated thrombin generation.<sup>17</sup> Taken together, these observations suggest that protamine overdose impairs platelet function<sup>18</sup> and weakens clot structure.<sup>18</sup>

One limitation of our study is that it was conducted *in vitro*, so may not represent *in-vivo* effects of excess protamine. Although  $K$  time,  $\alpha$  and maximum amplitude were affected at a PHR of 2:1,  $R$  time showed a significant difference at a PHR of 3:1 only. We suppose that this arbitrary result was due to the influence of aprotinin, which was administered to most patients before initiation of CPB, and aprotinin is known to delay clot initiation.<sup>22</sup> Further studies are required to demonstrate the effect of protamine on  $R$  time independent of aprotinin.

Another limitation of our work was that some patients who were on antiplatelet agents, such as aspirin or clopidogrel, within 2–8 days preoperatively may have been included. These data are unavailable and could potentially affect the results, as these agents are known to affect clot strength.<sup>23</sup> Again, future studies shall determine the effects of protamine on TEG tracing in patients not taking antiplatelet agents.

## Conclusion

It is clear that the conventional approach of administering protamine based on the total quantity of heparin used is likely to lead to protamine overdose and an increased risk of haemorrhage after cardiac surgery. The risk can be circumvented by using Hepcon to measure the required protamine dose. We demonstrated that TEG parameters were adversely affected by protamine in a dose-dependent manner, with most parameters altered at a PHR of 2:1. This suggests impaired platelet function and clot kinetics at higher protamine doses. Further studies are needed to find the exact PHR at which the toxicity of protamine first becomes evident.

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