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ORIGINAL ARTICLE

The effect of low molecular weight heparin (enoxaparin) on enhanced coagulation induced by crystalloid haemodilution*

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Summary

The purpose of this study was to establish whether a low molecular weight heparin (enoxaparin) attenuated or abolished the enhanced coagulation induced by crystalloid fluid therapy. Twenty young, healthy male volunteers were injected subcutaneously with either enoxaparin 40 mg or saline on two separate occasions one week apart, in a randomised, blinded study. Twelve hours later, a blood sample was taken for thrombelastography analysis and haematocrit. Saline 14 $ml.kg^{-1}$ was then infused over thirty minutes and thrombelastography and haematocrit measurements repeated. There was a significant post-dilutional difference in the alpha angle ($p = 0.002$) and *k*-time ($p = 0.001$) between the two groups. There was a trend towards reduced shortening of *r*-time in the enoxaparin group compared to the saline control ($p = 0.18$). The findings suggest that enoxaparin diminished acceleration of clot formation due to haemodilution.

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It has been previously demonstrated, in vitro [1] and in vivo [2], that haemodilution with 0.9% saline and other crystalloid solutions [3] causes enhanced coagulation as measured by the thrombelastograph [4–7]. These studies have demonstrated that moderate dilution of whole blood (20–40%) by crystalloid infusions results in enhanced coagulation both in vitro and in vivo. This enhancement is exemplified by accelerated coagulation (as demonstrated by a shortening of the *r*-time and *k*-time) and increased fibrin polymerisation and clot strength (as demonstrated by increased alpha angle and maximum amplitude). In a clinical study, Ng et al. [8] described an increase in coagulation after 1000 ml saline was infused over 30 min into patients undergoing anaesthesia for abdominal surgery. A study in vascular patients showed that haemodilution with saline, but not with colloids, resulted in enhanced coagulation [9]. Holte et al. [10] showed enhanced coagulation for up to 48 h postoperatively in patients undergoing knee arthroplasties who had received a median of 4.25 l crystalloids during the operation. The possible reasons for this effect may be a decreased concentration of the plasma anti coagulants, thus decreasing the threshold for positive feedback of thrombin into the intrinsic pathway and platelet activation [9, 11].

Another fluid study in orthopaedic patients undergoing lower limb arthroplasties, a group of patients known to be at high risk for venous thromboembolism, did not show enhanced coagulation after crystalloid haemodilution [12], but this study did not use thrombelastography to evaluate coagulation. In this study [12], the difference between the two groups of patients, those undergoing peripheral vascular surgery or those undergoing orthopaedic surgery, was that the latter group had received prophylactic low molecular weight heparin (LMWH). Unpublished data from our own laboratory confirmed the lack of crystalloid-enhanced coagulation using thrombelastography in patients undergoing orthopaedic surgery in

whom pre-operative LMWH had been given. The question therefore arose whether LMWH abolished or attenuated the enhanced coagulation previously seen with crystalloid haemodilution. With this in mind, we undertook a study to investigate the effect of LMWH administration on the enhanced coagulation induced by crystalloid haemodilution in a group of volunteers on two occasions, one week apart.

Methods

Approval to undertake the study was obtained from the University of Cape Town's Ethical Committee. Twenty healthy male volunteers, aged 18–28, were recruited. None of these subjects had taken any medication that could affect coagulation in the previous seven days and none of them suffered from any chronic illnesses.

After written informed consent was obtained, the volunteers were injected subcutaneously with enoxaparin 40 mg (Sanofi-Aventis, Midrand, South Africa) or the equivalent volume of saline, on two separate occasions, one week apart. The order of injections was randomised using random numbers and the investigators and volunteers were blinded as to which injection had been given. The study was designed as a randomised, double-blind crossover study with all volunteers being allocated to both groups.

Twelve hours after the LMWH injection, an IV cannula was inserted into a forearm vein of each volunteer and a blood sample was withdrawn (sample 1 – baseline). The equivalent of 20% of circulating blood volume (14 ml.kg^{-1}) of 0.9% saline was infused over 20–30 min, after which a second sample of venous blood was withdrawn (sample 2) and the IV line was removed. The same cannula was used for fluid administration and sampling so as to avoid the possibility that repeated attempts at venous cannulation would enhance systemic coagulation. Blood samples were collected using a two-syringe technique to eliminate tissue factors or contamination from IV lines, with 3–5 ml blood drawn into a separate syringe and discarded before the study sample was taken. On the second occasion, the sample was obtained from a site distal to the previous venipuncture to avoid possible contamination.

Haematocrit was measured on all samples to assess the degree of haemodilution; blood coagulation was tested using TEG® (Haemoscope 500 analyser; Haemoscope®, Skokie, Illinois) analysis. All the recommended (Haemoscope Inc.) constant time intervals established for native whole blood analysis were adhered to and all blood samples were placed on the TEG analyser within 4 min of being drawn.

Statistical analysis was performed using a two-way ANOVA, with the injection type and timing of the sample (pre- or post-dilution) as the independent variables. Where this analysis showed significant differences, post-hoc analysis was performed using the least significant difference technique to identify significantly different individual groups. A value of $p < 0.05$ was taken to denote statistical significance.

Results

Results are shown in Table 1. All recruited subjects completed both parts of the protocol. Both groups showed a significant reduction in haematocrit following haemodilution, but there was no difference between the groups.

Both groups showed a significant shortening of the *r*-time following haemodilution but the difference between the groups was not statistically significant. However, following haemodilution, both groups showed a significant shortening of the *k*-time, with the effect being significantly greater in the control group than in the enoxaparin group ($p = 0.001$). There was no significant increase in the alpha angle following haemodilution in the enoxaparin group, but it was significantly increased in the saline group ($p = 0.002$); the post-dilution alpha angle was significantly different between the groups

Table 1 Results from thromboelastography before (pre-dilution) and after (post-dilution) dilution of whole blood with saline in 20 volunteers pre-treated with low molecular weight heparin and saline. Values are mean (SD).

		Enoxaparin		Saline	
	Normal range	Pre-dilution	Post-dilution	Pre-dilution	Post-dilution
r-time; min	15.5–23.0	21.1 (4.0)	16.4 (4.3)	19.8 (3.5)	14.7 (4.1)
k-time; min	5.5–10.5	12.1 (3.2)	9.8 (2.9)*	9.8 (1.5)	7.1 (2.6)
Alpha; °	22.0–38.0	18.7 (4.7)	23.1 (3.1)†	22.2 (9.1)	30.5 (10.6)
MA; mm	47.0–58.0	43.0 (6.7)	44.7 (6.8)	44.6 (5.6)	48.0 (6.5)
Hct; %	42–54	44.0 (2.5)	39.8 (2.1)	44.7 (2.5)	40.5 (1.8)

Hct, haematocrit as a percentage of blood sample volume; MA, maximum amplitude (as a measure of final clot strength).
*p = 0.001 for differences between pre-dilution and post-dilution specimens.
†p = 0.002 for differences between enoxaparin and saline post-dilution specimens.

($p = 0.001$). There was no difference in clot strength as measured by the maximum amplitude.

Discussion

Many factors have an impact on the delicate balance between enhancement and inhibition of coagulation. Thrombelastography provides a measurement of the complete coagulation process in whole blood. Therefore coagulation measured by TEG reflects subtle abnormalities better than testing the activity of coagulant factors in anti coagulated plasma, as is commonly done in conventional analysis of coagulation [9].

In coagulation, positive feedback plays a major role in the architecture of the system [13]. The central point of coagulation, thrombin, triggers both platelet aggregation and fibrin formation [14]. Even small quantities of thrombin formed early in haemostasis bind to platelet receptors to elicit aggregation [15]. Platelets then accelerate the intrinsic pathway reactions [16], thus enhancing enzymatic activity and resulting in increased complex formation. This, in turn, ensures rapid generation of factor Xa and more thrombin [15], thereby accelerating coagulation. Normal plasma does contain small amounts of *activated* thrombin [17, 18]; however the positive feedback loop is inhibited through the effect of anticoagulants such as antithrombin III (AT III). This modulates the enhancement of the coagulation cascades and so bestows threshold properties on the system [19–21]. As a result, when the active thrombin concentration exceeds the anticoagulant-induced threshold, an exponential increase of further thrombin formation occurs, ensuring a clot is formed. All anticoagulants, of which AT III makes up about 50%, are maintained in an activated form at a constant concentration in normal blood, preventing this positive feedback of thrombin [11].

This anticoagulant effectiveness is susceptible to a reduction in concentration through dilution [9]. However, the effect of the AT III is enhanced markedly through the administration of LMWH.

Both groups in this study demonstrated a similar, significant decrease in haematocrit due to haemodilution following the infusion of saline.

The onset of coagulation (*r*-time) was accelerated by haemodilution in both groups and there was no significant attenuation of this process following the administration of LMWH. This enhancement of coagulation has been shown to be associated with an increase in the incidence of deep vein thrombosis [22]. Although the values for these coagulation parameters remain within the broad quoted normal ranges provided by the manufacturer, they nevertheless indicate an enhancement of coagulation in the individual subjects. Whether or not these changes are sufficient to increase the risk of deep vein thrombosis has not been investigated in any studies other than the one quoted above.

However, while both groups showed a significant increase in the rate of fibrin polymerisation, this was significantly greater in the control group. The rate of aggregation of fibrin and platelets as measured by the alpha angle was significantly increased in the saline group, but there was no significant acceleration of this process in the LMWH group. Final clot strength (maximum amplitude) was not changed in either group.

Although the clinical relevance of enhanced coagulation brought about by crystalloid haemodilution remains to be established, given the number of factors which may contribute to the peri-operative risk of enhanced coagulation, diminishing this risk in patients at risk through the administration of LMWH appears logical.

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Competing interests

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