Does prior administration of enoxaparin influence the effects of levobupivacaine on blood clotting? Assessment using the Thrombelastograph®

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The low molecular weight heparin, enoxaparin (by inhibition of factors Xa and IIa) and amide local anaesthetics (by altering platelet function) exert anti-clotting effects. Although these agents are often used in combination during the perioperative period, their potential interactive effect on clotting has not been defined. Blood from 10 ASA I–II patients who received enoxaparin 0.5 mg kg⁻¹ s.c. was studied using a Thrombelastograph (TEG®) either alone or in combination with levobupivacaine (2.5 mg ml⁻¹ or 2.5 µg ml⁻¹) or saline (50% dilution). In blood from patients who had received enoxaparin 0.5 mg kg⁻¹ s.c. 12 h previously, levobupivacaine 2.5 mg ml⁻¹ (but not 2.5 µg ml⁻¹) produced significant changes in TEG clotting parameters (mean (SD) 15.7 (4.8) mm, 29.6 (25.6) mm, 34.4 (14.6) mm, 34.3 (12.2)° compared with control values of 6.1 (1.3) mm, 2.5 (0.5) mm, 63.5 (6.4) mm and 74.1 (2.9)° for r, K, MA, and α angle respectively).

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Venous thromboembolism is a major complication of lower limb orthopaedic surgery and associated pulmonary embolism is an important cause of perioperative death.¹ Prophylactic subcutaneous heparin decreases the incidence of deep venous thrombosis (DVT) from 24 to 10% after hip surgery¹ and from 60 to 40% after knee surgery.² Low molecular weight heparins (LMWH) such as enoxaparin sodium (Lovenox, Cleoxane, Aventis™, Strasbourg, France) have been shown to be as effective and safe as low dose unfractionated heparin (UFH).³–⁵ Using the Thrombelastograph (TEG®), enoxaparin (at anti-Xa activity concentrations of 0.1–0.3 unit ml⁻¹) has been shown ex vivo to produce a dose dependent increase in reaction time (r), (a measure of the rate of fibrin formation) and coagulation time (K), and a decrease in α angle (the rate of clot formation).⁶ Neuraxial block provides excellent operating conditions for lower limb surgery, good postoperative analgesia and decreases blood loss and the incidence of DVT.²–⁴ ⁶–⁸ The safety of spinal or epidural techniques in patients who have received prophylactic enoxaparin is controversial.⁹ ¹⁰ The prescribing information for enoxaparin contains a warning of the risk of spinal haematoma when the drug is used in combination with neuraxial block. More than 60 cases of epidural haematoma associated with the administration of enoxaparin in conjunction with neuraxial block have been reported.¹¹ The risk is probably greater in elderly females, when epidural catheters are used and when a dose of 30 mg bd rather than 40 mg once daily is administered.¹²

Amide local anaesthetics such as levobupivacaine are potent inhibitors of platelet aggregation and release.¹³ ¹⁴ Levobupivacaine has recently been approved for clinical use and is likely to be commonly used for epidural anaesthesia. The anti-platelet effects of levobupivacaine are reflected on thromboelastography as a dose dependent decrease in maximum amplitude (a measure of clot formation).¹⁵

LMWHs, in combination with non-steroidal anti-inflammatory drugs, prolong bleeding time.¹⁶ ¹⁷ It is now recommended that this combination should not be given to patients undergoing surgery under central nerve block.¹¹ ¹² Non-steroidal anti-inflammatory drugs, like local anaesthetics, exert their anti-coagulant action, in
part, by inhibition of platelet aggregation. To date, the potential interactive effect of enoxaparin and amide local anaesthetic agents on blood clotting has not been examined.

The aim of this study was to assess, using thromboelastography, the effect of levobupivacaine on clotting of blood from patients who have received enoxaparin 12 h previously.

**Methods**

With institutional ethical approval, and having obtained written informed consent, 10 ASA I–II patients (aged 28–60 yr) undergoing elective lower limb orthopaedic surgery were studied. Only patients who required anticoagulant prophylaxis with enoxaparin for clinical reasons were recruited. Patients with a history of allergy to heparin or local anaesthetics, a disorder of platelet function, coagulation or fibrinolysis or those who were taking any drug known to affect these systems were excluded.

On the night before surgery, a venous cannula was sited in the patient’s non-dominant forearm. A two-syringe technique was used to withdraw 11 ml of blood. The initial 2 ml was discarded to decrease tissue thromboplastin contamination. The remainder was divided between two polypropylene tubes, each containing 0.5 ml of 3.2% sodium citrate. These were analysed for:

1. Factor Xa activity. Blood (4.5 ml) from the first citrated tube was centrifuged at 240 g at 4°C for 10 min. The plasma samples were stored at −70°C and the assay carried out within 1 month of collection.

2. TEG parameters. After 30 min 1000 ml of blood from the other citrated tube was transferred to a celite 1% vial of which 340 ml was transferred to a TEG® cup and analysed as below (sample S1 in Figs 1–4).

Enoxaparin 0.5 mg kg⁻¹ (Clexane®, Aventis™, Ireland) was administered s.c. after the control venous samples had been taken (this avoided the relative overdose that may occur in smaller patients).¹⁸

On the morning of surgery, 12 h after the administration of the enoxaparin, a further 11 ml of blood was taken using a two-syringe technique as described and the blood analysed for factor Xa activity. Blood was collected and stored as for the first sample. Coagulation analysis was carried out on whole blood, blood/saline as a control for dilution effects, and two blood/levobupivacaine solutions. Citrated whole blood, 1000 ml, was transferred to each of four celite™ 1% (Haemoscope Corp., Skokie, IL, USA) bottles to produce four solutions as follows:

S₂: Whole blood 340 ml was recalcified with 20 ml of RECAL™ (to assess the effect of enoxaparin alone).

S₃: Whole blood 160 ml and NaCl 0.9% 180 ml with 20 ml RECAL™ (to assess the effects of dilution).

S₄: Whole blood 160 ml and levobupivacaine 5 mg ml⁻¹ 180 ml with RECAL™ 20 ml (to produce an end concentration of 2.5 mg ml⁻¹) (to mimic the concentration if a mixture occurred of levobupivacaine 0.5% and whole blood in equal quantities in the epidural space).

S₅: Whole blood 331.7 ml and levobupivacaine 10 µg ml⁻¹ 8.3 ml with RECAL™ 20 ml (to produce an end
concentration of 2.5 μg ml⁻¹) (to mimic the concentration in the systemic circulation after bolus epidural administration of local anaesthetic).

Levobupivacaine solutions were prepared using levobupivacaine hydrochloride 5 mg ml⁻¹ (Abbott Laboratories Ltd, Ireland) in 0.9% saline.

Biochemical analysis
The samples were analysed for plasma anti-factor Xa activity in a single batch. An amidolytic assay using a commercially available kit (Chromostat™, Organon Teknika Corp., Boxtel, The Netherlands) and a spectrophotometer set at 405 nm were used. The assay has a minimum detection limit of 0.01 anti-factor Xa unit ml⁻¹.

Thromboelastography
A pre-warmed computerized TEG® machine (Haemoscope 2000D®, Haemoscope Corp., IL, USA) was used with standard calibrations for strain, alignment, balance and clearance. Each TEG® cup at 37°C was filled with a total of 340 μl of blood, blood/levobupivacaine solution or blood/saline and recalcified with RECAL™ 20 μl. Three drops of mineral oil was applied to the upper surface. Celite-activated thromboelastography profiles were recorded until Ly 30 was achieved. The morphology and data for four TEG® parameters were collected and stored.

Based on an α=0.05 and β=0.2 and previous work (66% decrease in MA by racemic bupivacaine 2.5 mg ml⁻¹19) power analysis indicated a minimum sample size of 10 was necessary to detect a 50% effect on MA with an SD for residuals of 30%.

Data were analysed using one-way ANOVA and a Student Newman–Keuls test using the Sigma Stat™ software (Jandel Scientific Corporation Version 1). P<0.05 was considered significant. All data are expressed as mean (SD).

Results
Ten ASA I patients (six female, four male, age 40.1 (12.8) yr, weight 77.6 (17.4) kg) undergoing elective orthopaedic surgery were studied. All the values for whole blood (S₁) were within normal limits (r values, although less than those quoted for celite-activated whole blood²⁰ were similar to those achieved after recalcification²¹) (Figs 1–4). Anti-Xa activity was not detectable in the pre-enoxaparin sample (S₁) and was 0.17 (0.08) unit ml⁻¹ 12 h later.

The blood of patients treated with enoxaparin 12 h previously (S₂) was similar in all parameters to the pre-enoxaparin controls (S₁) (Figs 1–4). Maximum amplitude and α angle were less in the saline controls (S₃, enoxaparin-treated blood/saline) than in whole blood controls (S₁) and enoxaparin-treated whole blood (S₂) (MA=41.3 (13.5) mm, for S₃ vs 62.5 (6.0) mm for S₁ and 60.6 (7.2) for S₂, α angle=51.5 (17.3)° for S₃ vs 73.2 (4.6)° for S₁ and 69.4 (6.3)° for S₂) (Figs 3 and 4). In blood of patients who had received enoxaparin 12 h previously the following results...
were found: (1) dilution (50%) with saline resulted in an increase in MA (41.3 (13.5) mm) and α angle (51.5 (17.3)°) (P<0.05), (2) levobupivacaine 2.5 mg ml⁻¹ (S₄) (but not 2.5 μg ml⁻¹) resulted in an increase in r (17.5 (17.5) mm) and K (29.6 (25.6) mm) and a decrease in MA (27.2 (16.8) mm) and α angle (29.8 (12.5)°) (all P<0.05) compared with the pre-enoxaparin controls (6.8 (1.8) mm, 2.6 (1.5) mm, 62.5 (6.0) mm, 73.2 (4.6)° for r, K, MA, and α angle, respectively), (3) the alterations in r, K, MA, and α angle produced by the levobupivacaine 2.5 mg ml⁻¹ (S₄) were also significantly greater than those produced by 50% dilution with saline (S₃) and (4) addition of levobupivacaine 2.5 mg ml⁻¹ (S₄) produced a significant alteration in r, K, MA, and α angle compared with the whole blood similarly treated with enoxaparin (S₂) (r, K, MA, and α angle for S₂=9.0 (3.4) mm, 3.1 (1.2) mm, 60.6 (7.2) mm and 69.4 (6.3)°, respectively) (Figs 1–4).

Discussion

The most important finding of this study is that levobupivacaine produces a dose dependent decrease in clotting of whole blood from patients previously treated with enoxaparin. In this preparation (levobupivacaine 2.5 mg ml⁻¹ in whole blood from patients who had received enoxaparin 12 h previously), the indices of clot formation (r, K, MA, α angle), measured with thromboelastography demonstrated impaired clotting. The results were also significantly different from the saline control (50% dilution of whole blood with saline) indicating that the impairment cannot be ascribed to dilution alone.

Enoxaparin is a LMWH, which exerts an anti-coagulant effect by inhibition of factor Xa and IIa (thrombin) in the ratio 2.7:1.22 This is reflected on TEG° by a dose dependent increase in reaction time (r) and a decrease in α angle.6 Maximum anti-Xa activity occurs 4 h after subcutaneous injection and at 12 h is still up to 50% of this.22 It produces effective prophylaxis for thromboembolism after lower limb joint replacement.2-5 Since 1993, there have been more than 60 reports of spinal haematoma occurring when neuraxial block is used concurrently with enoxaparin prophylaxis.11 Analysis of these cases indicates that insertion of an epidural catheter, extreme age, a dose of 30 mg bd and the concomitant use of anti-platelet medication increase the risk.10 12

We did not demonstrate a clotting defect on the TEG° in blood from patients treated with a single dose of enoxaparin 12 h previously. In this study, anti-Xa activity was 0.17 (0.08) unit ml⁻¹ with a range of 0.06–0.31 unit ml⁻¹. Previous workers have also demonstrated a wide variability in plasma anti-Xa activity achieved after a single dose of enoxaparin.23 24 Thrombosis is inhibited at anti-factor Xa concentrations of 0.2–0.3 unit ml⁻¹ in an animal model.25 Anti-factor Xa activity in four out of 10 patients was within this range at 12 h. Our results demonstrate that this effect in isolation is not sufficient to alter clotting to an extent that is detectable by TEG°. The significance of these contrasting results is unclear. Current guidelines recommend that neuraxial block can be safely carried out 12 h or more after administration of enoxaparin 40 mg s.c. but the large controlled studies that would be required to detect any difference in incidence of spinal haematoma with concurrent use of LMWH have not been carried out.18

The effects of a number of amide local anaesthetics on coagulation and fibrinolysis have previously been assessed using thromboelastography.15 16 27-36 Racemic bupivacaine, levobupivacaine, ropivacaine and lignocaine in vitro decrease MA in a concentration dependent manner.19 26-29 Lignocaine and ropivacaine increase K at high concentrations.28 29 A decrease in α angle is produced by lignocaine, racemic bupivacaine and ropivacaine.19 26 28 29 Levobupivacaine 2.5 mg ml⁻¹ alone (in blood of volunteers) produced a decrease in MA only (mean reduction in MA of 13.5% compared with 35.3% with the levobupivacaine 2.5 mg ml⁻¹ enoxaparin combination).17 Enoxaparin alone did not significantly alter clotting. However, the addition of levobupivacaine 2.5 mg ml⁻¹ to the blood of patients previously treated with enoxaparin produced a prolongation of r and K, and a decrease in α angle in addition to the augmented effect on MA. This result suggests that the presence of enoxaparin enhances the anti-clotting effects of levobupivacaine.

Platelet aggregation and coagulation are interdependent processes leading to thrombus formation. Cytoplasmic ionized calcium is the key ‘second messenger’ in platelet activation. Calcium dependent activities include aggregation, clot retraction and secretion of the contents of storage granules.31 It is also essential for the activation of the ‘lipid scramblase’.32 This induces surface expression of platelet membrane phospholipid, which promotes binding and catalytic activity of IXa and Xa leading to thrombin formation.32 In the resting platelet, ionized calcium is concentrated in the plasma membrane, the dense tubular system and the storage granules. Thrombin induces platelet activation by stimulating release of calcium from intracellular stores and an increase in cytosolic free calcium.33 Local anaesthetics inhibit platelet aggregation and secretion by block of calcium mobilization from intra-platelet storage pools and calcium influx across the platelet membrane.13 14 34 35 Heparins also decrease calcium mobilization within the platelets inhibiting thrombin-induced platelet activation.36 Both LMWHs and local anaesthetics may diminish the increase in platelet cytosolic ionized calcium normally associated with platelet activation. This is one potential explanation for the effect on MA seen in this study with the addition of levobupivacaine 2.5 mg ml⁻¹ to enoxaparin-treated blood. The effect of the levobupivacaine/enoxaparin combination on r and K parameters which are dependent on the coagulation cascade, may be because of a decrease in cytosolic ionized calcium and inhibition of lipid scramblase reducing factor Xa binding and activity.
No effect on TEG® parameters was demonstrated with the levobupivacaine 2.5 μg ml⁻¹ solution when compared with controls. This indicates that the effect is concentration dependent and that levobupivacaine at concentrations produced systemically after an epidural bolus are unlikely to increase the effect of enoxaparin on clotting.

A number of limitations apply to this study. We did not correct for the effect of 50% dilution on calcium or pH as previous studies have shown this to be minimal. Our samples were citrated and assessed after recalcification. This process can alter coagulation parameters as assessed with the Thrombelastograph®. Blood recalculated after 30 min demonstrates a decrease in r, K and MA and an increase in the α angle. The magnitude of these effects is small (~5%) relative to those demonstrated in our levobupivacaine (2.5 mg ml⁻¹)/enoxaparin combination (~55–250%). The mixing of levobupivacaine solutions with blood occurred ex vivo and so the effect of endothelial surface on clotting function was not assessed. The saline control had a significantly decreased MA and α angle when compared with whole blood control. This is consistent with the work of Tobias and colleagues who demonstrated that 50% dilution causes a decrease in α angle and MA. This is the degree of dilution which we produced in the saline control and in the levobupivacaine 2.5 mg ml⁻¹ preparation. The effect on clotting produced by this solution can, therefore, be attributed, in part, to dilution. All indices of clot formation (r, K, MA and α angle), however, were significantly impaired even when compared with the similarly diluted control. This impairment, therefore, represents an additional effect of the levobupivacaine/enoxaparin combination.

This is the first study to assess whether an amide local anaesthetic can enhance the anti-clotting effects of enoxaparin. We have demonstrated that such an effect does occur at a concentration of levobupivacaine of 2.5 mg ml⁻¹. This observation may have important clinical implications. Spinal haematoma is a rare but potentially devastating consequence of neuraxial block in patients receiving LMWH. One of the factors, which appear to increase the risk, is the use of epidural catheters. This effect may at least in part be explained by the influence of the local anaesthetic solution infused or injected into the epidural space.

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