Effect of ketorolac, bupivacaine and low-dose heparin on thrombelastographic variables in vitro

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Summary
We have studied the effects of ketorolac, bupivacaine and low-dose heparin on three thrombelastographic (TEG) variables (r, α, MA) in vitro. Blood samples were obtained from 12 healthy volunteers and 88 ASA I–II elective surgical patients. Clinically relevant concentrations of ketorolac (4.1 μg ml⁻¹) and bupivacaine (2.7 μg ml⁻¹) had little or no effect on the TEG variables. However, low concentrations of heparin (0.07–0.28 u. ml⁻¹) had marked effects on all three TEG variables. Thus the interpretation of TEG abnormalities during anaesthesia and surgery may be confounded by the presence of heparin, but not by clinical concentrations of ketorolac or bupivacaine. (Br. J. Anaesth. 1995; 75: 27–30)

Key words

Thrombelastography (TEG) has recently become popular for assessment of whole blood coagulation in the perioperative period [1]. Thrombelastography provides a viscoelastic measure of clot formation from initial fibrin formation, through clot strengthening, to eventual clot lysis [1]. The reaction time of the TEG (r, time to 2 mm divergence) measures the time to initial fibrin formation, the angle (α) measures the rate of clot formation and the maximum amplitude (MA, maximum divergence) is an index of the maximum strength of the clot [1]. An advantage of thrombelastography is that it assesses platelet function in addition to fibrin generation [1]. It has proved useful for the diagnosis of clotting disturbances in a variety of situations, including cardiac surgery [2], liver transplantation [3] and pre-eclampsia [4].

Patients presenting for anaesthesia may be exposed to many drugs that are known to affect platelet function or coagulation, including non-steroidal anti-inflammatory drugs (NSAID) [5], local anaesthetics [6] and low-dose heparin [7]. At present, the direct effects of these drugs on TEG variables are unknown. Therefore, it is difficult to interpret TEG changes in patients receiving these medications.

Ketorolac is a water soluble NSAID with anti-platelet effects similar to those of aspirin [8]. Bupivacaine is a commonly used local anaesthetic agent that has been shown previously to inhibit platelet aggregation during extradural analgesia [9]. The aim of the current study was to examine the direct effects of clinically relevant concentrations of ketorolac, bupivacaine and heparin on TEG variables in vitro.

Patients and methods
The study was approved by the local hospital Ethics Committee and informed consent was obtained from all participants. Venous blood samples were obtained from 12 healthy adult volunteers and 88 adult ASA I–II patients undergoing general anaesthesia for elective surgery. In the surgical patients, blood samples were obtained from a peripheral venous cannula at the time of insertion (i.e. before induction of anaesthesia). None of the volunteers or patients had received or was receiving medications known to affect coagulation or platelet function. In particular, none had received NSAID, local anaesthetic agents or heparin within the previous 10 days. Patients with a history of haematological, renal or hepatic disease were excluded.

GROUPS 1–6
Blood samples were obtained using a double syringe technique. Blood (2 ml) was collected in the first syringe which was discarded. A second syringe was used to collect another 2.7 ml of blood which was transferred to a siliconized collection tube containing one part sodium citrate 0.3 mol litre⁻¹ per nine parts blood (Becton Dickinson, Rutherford, NJ, USA). The samples were stored at room temperature for 15–30 min before thrombelastography was performed.

A computerized thrombelastograph D (Haemoscope Corporation, Skokie, IL, USA) with disposable cups and pins was prewarmed for 30 min before each run. Both channels of the TEG were calibrated and aligned daily using calibration and
alignment pins. During each run, one of the channels was used as a “control” and the other as a “test”. A micropipette was used to place 300 μl of blood in each cup. A separate micropipette was used to place an additional 10 μl of normal saline in the control cup and 10 μl of test solution in the test cup. In group 1 (n = 12), both channels were treated as controls (to assess the variability between the two channels). The test solutions and final concentrations (in TEG cup) for groups 2–6 were as follows: group 2 (n = 12), heparin 0.07 u. ml⁻¹; group 3 (n = 12), heparin 0.14 u. ml⁻¹; group 4 (n = 12), heparin 0.28 u. ml⁻¹; group 5 (n = 13), ketorolac 4.1 μg ml⁻¹; and group 6 (n = 13), bupivacaine 2.7 μg ml⁻¹. Heparin (Hepsal) was supplied by CP Pharmaceuticals, Wrexham, UK, ketorolac tromethamine (Toradol) by Syntex, Palo Alto, CA, USA and bupivacaine HCl by Baxter Healthcare, Thetford, UK.

All samples were recalculated by adding 50 μl of CaCl₂ solution 0.105 mol litre⁻¹ to each cup. The contents of the cups were mixed gently by lowering and raising the TEG pins several times. The TEG runs were then initiated. The r time, α angle and MA were recorded for each channel. The k time was not analysed because similar information is provided by the α angle. Moreover, the k time cannot be calculated if MA is < 20 mm. The runs were continued for a minimum of 60 min. If there was no divergence in the TEG trace at 60 min, the runs were continued until r was reached (2 mm divergence), up to a maximum of 90 min.

GROUPS 7 AND 8

In groups 7 and 8, the test solutions were incubated with the sample for 30 min before thromboelastography was performed in order to provide additional time for the test drugs to interact with platelets. Two 2.7-ml samples were collected into identical collection tubes. Normal saline 100 μl was added to the first tube (control) and 100 μl of test solution was added to the second tube (test). After 30 min incubation, 300 μl from the control sample was placed in the control cup. At the same time 300 μl from the test sample was placed in the test cup. The test solutions and final concentration (in TEG cup) for groups 7 and 8 were: group 7 (n = 13), ketorolac 4.1 μg ml⁻¹; and group 8 (n = 13), bupivacaine 2.7 μg ml⁻¹. The samples were then recalculated and the TEG runs were initiated as in groups 1–6.

STATISTICAL ANALYSIS

In each group, the TEG variables from control and test channels were compared using one-sample Wilcoxon signed rank tests. A Bonferroni correction was performed (for multiple simultaneous comparisons within each group), and only P values < 0.016 were considered significant. Systematic and random errors between the two channels were assessed by calculating the mean difference (bias) and the sd of the differences (precision) between the two control channels in group 1.

Results

The data are displayed in table 1. There were no significant differences between the two control channels (group 1) for r, α or MA (P > 0.05). Mean differences between the two channels in group 1 were 0.7 (sd 1.8) min for r, 2.2 (5.4)° for α and 0.4 (3.1) mm for MA. Similarly, there were no significant differences between control and test data in groups 5–8 (P > 0.05). However, there were significant differences between test and control data for all three TEG variables in groups 2–4 (P < 0.016). In group 4, seven of the 12 r values were > 90 min. An example of a marked effect of heparin 0.07 u. ml⁻¹ in one patient is given in figure 1.

Table 1 Effects of ketorolac, bupivacaine and heparin on TEG variables (*P < 0.05 vs control). Data shown as mean (sd).

<table>
<thead>
<tr>
<th>Group</th>
<th>r Time (min)</th>
<th>α Angle (°)</th>
<th>MA (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>1 (n = 12)</td>
<td>Normal saline</td>
<td>19.7 (8.0)</td>
<td>20.4 (8.2)</td>
</tr>
<tr>
<td>2 (n = 12)</td>
<td>Heparin 0.07 u. ml⁻¹</td>
<td>19.3 (8.9)</td>
<td>46.6 (14.6)*</td>
</tr>
<tr>
<td>3 (n = 12)</td>
<td>Heparin 0.14 u. ml⁻¹</td>
<td>14.0 (4.6)</td>
<td>60.7 (23.2)*</td>
</tr>
<tr>
<td>4 (n = 12)</td>
<td>Heparin 0.28 u. ml⁻¹</td>
<td>19.3 (8.6)</td>
<td>76.0 (17.9)*</td>
</tr>
<tr>
<td>5 (n = 13)</td>
<td>Ketonolac 4.1 μg ml⁻¹</td>
<td>14.4 (4.0)</td>
<td>15.4 (5.2)</td>
</tr>
<tr>
<td>6 (n = 13)</td>
<td>Bupivacaine 2.7 μg ml⁻¹</td>
<td>19.8 (6.3)</td>
<td>20.0 (6.2)</td>
</tr>
<tr>
<td>7 (n = 13)</td>
<td>Ketorolac 4.1 μg ml⁻¹</td>
<td>14.1 (4.6)</td>
<td>12.6 (4.6)</td>
</tr>
<tr>
<td>8 (n = 13)</td>
<td>Bupivacaine 2.7 μg ml⁻¹</td>
<td>12.6 (2.9)</td>
<td>11.6 (4.4)</td>
</tr>
</tbody>
</table>
Discussion

In this in vitro study, we have demonstrated significant effects on the TEG of low-dose heparin but not ketorolac or bupivacaine. Ketorolac is a potent inhibitor of cyclo-oxygenase with antiplatelet effects similar to those of aspirin and other NSAID [8]. The peak plasma concentration of ketorolac following 30 mg i.m. in adults is about 3.0 μg ml⁻¹ [8]. Therefore, the concentration used in this study was of similar order or slightly higher than that likely to be encountered clinically. An antiplatelet effect of ketorolac should be manifest by a reduction in MA [1]. However, there was no effect on MA or other TEG variables, even after 30 min incubation (groups 5 and 7). This suggests that clinical concentrations of ketorolac do not affect the TEG. A minor effect cannot be excluded because of the possibility of a type II error. Nevertheless, there was no trend to abnormal values in the ketorolac group. The results are in keeping with a previous clinical study that found that aspirin had little effect on TEG variables ex vivo [10].

The peak plasma concentration of bupivacaine after extradural or brachial plexus anaesthesia is 2.0–2.5 μg ml⁻¹ [11]. The concentration used in this study was at the upper limit of the clinical range. Again, despite incubation for up to 30 min, we observed no effect on TEG variables (groups 6 and 8). However, as with ketorolac, a minor effect cannot be excluded because of the possibility of a type II error.

There have been no previous studies on the direct effects of local anaesthetics on the TEG. However, it has been shown that extradural anaesthesia is associated with slight reductions in MA in the postoperative period [12]. The current results suggest that a reduction in MA during extradural anaesthesia is more likely an indirect effect of extradural block than a direct effect of systemically absorbed bupivacaine.

Although the results indicated that ketorolac and bupivacaine have no obvious effects on TEG variables in vitro, it does not necessarily follow that these drugs have no clinically significant effects on overall coagulation in vivo. Haemostasis and thrombosis are complex processes that involve dynamic interactions between platelets, clotting factors and vascular endothelium. The TEG does not assess the contribution of vascular endothelium. At present, the only routine coagulation test that assesses the contribution of vascular endothelium is the bleeding time.

In contrast with the effects of ketorolac and bupivacaine, low concentrations of heparin had major effects on the TEG. The concentrations used in this study were “subtherapeutic” and were in the range commonly found during prophylaxis against deep venous thrombosis (DVT) [13]. At 0.28 u. ml⁻¹ there was either no evidence of fibrin generation (i.e. r > 90 min) or the r values were markedly increased (group 4). At 0.14 u. ml⁻¹, all samples showed some evidence of fibrin generation, but all r values were increased, and all α and MA values were decreased (group 3). At 0.07 u. ml⁻¹ the changes were less marked, but there were still significant differences for all variables (group 2). As such, in the subtherapeutic range, heparin appeared to produce dose-related increases in r values and dose-related decreases in α and MA values. These changes are consistent with the known effects of heparin on fibrin generation.

Studies on haemostasis are often difficult to analyse because of wide inter-patient variability. In the current study, inter-patient variability was excluded by collecting control and test samples from the same patient. Similarly, variations in platelet activation were minimized by collecting the control and test samples at the same time through the same venepuncture. The low variability was reflected in the similarity between the two channels in group 1 (i.e. control vs control). As expected, there were minor differences in TEG variables between groups. However, this did not affect the results, because all comparisons were made within groups.

Many patients presenting for anaesthesia receive heparin prophylaxis against DVT (e.g. 10 000–15 000 u. day⁻¹ s.c.) [7]. Many patients also receive heparin during cardiac or vascular surgery. After cardiopulmonary bypass, heparin is usually reversed with protamine. Nevertheless, small amounts of residual heparin may be present in the postoperative period. There are many situations where patients may have low concentrations of heparin. Our data indicate that the TEG may be altered markedly by these low concentrations, and unless the effect of heparin is accounted for, the interpretation of the TEG may be erroneous. The effect of heparin on the TEG can be reversed by the addition of heparinase to the TEG sample in vitro [14]; this manoeuvre should be considered if the presence of heparin is suspected.

The results suggest that the TEG may be a useful method of detecting or monitoring low concentrations of heparin. There appeared to be a dose–response relationship in the subtherapeutic range, and a separate channel is available for a heparinase control. However, the presence of several r values > 90 min with heparin 0.28 u. ml⁻¹ suggests that the TEG is too sensitive to monitor heparin activity in the therapeutic range. Moreover, the results of this in vitro study need to be confirmed.
by follow-up studies *in vivo*. Further studies are also required to determine if low molecular weight heparins have similar effects to unfractionated heparin.

**Acknowledgement**

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**References**