Use of heparinase modified thrombelastography in liver transplantation

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Summary
Severe coagulopathies can occur during liver transplantation, particularly after reperfusion of the grafted liver. Heparin release has been proposed as one of the factors contributing to this coagulopathy. We have analysed the thrombelastograph (TEG) traces of 55 patients after reperfusion using native and heparinase-treated samples. In almost all cases an abnormal native TEG was improved in vitro by heparinase, demonstrating the presence of heparin or a heparin-like substance. The heparinase-modified TEG allowed assessment of the underlying coagulation status, providing a rational guide to blood component replacement or treatment of fibrinolysis. (Br. J. Anaesth. 1997; 78: 175–179)

Key words

Liver transplantation is frequently complicated by severe coagulopathies, particularly after reperfusion of the grafted liver. The cause of the marked deterioration in coagulation that accompanies reperfusion is incompletely understood but many potential factors have been implicated, including hyperfibrinolysis, DIC, platelet activation, trapping of platelets in the graft and a heparin-like effect.1

The thrombelastograph (TEG) has been shown to be a useful monitor of coagulation during liver transplantation and provides a rational approach to the use of blood component therapy or pharmacological intervention.2 A normal TEG is shown in figure 1. Characteristic changes in the TEG trace occur after reperfusion of the grafted liver and have been described by several authors.2–4 There is prolongation of the reaction time (r) and coagulation time (r+k), and a decrease in maximum amplitude (MA) and clot formation rate (α). In our experience, the first TEG after reperfusion (r+10 min) frequently shows a straight line (no trace), demonstrating no clot formation during the 60 min that the sample is running. Several factors are thought to be responsible for this coagulopathy, including heparin release from the implanted liver graft. There have been several reports that document the presence of heparin-like activity immediately after reperfusion.

The frequency of this heparin effect is reported to be between 25% and 95% of cases.5,6 Kang and colleagues have suggested that where there is demonstrated correction of the reaction time in blood treated with protamine sulphate in vitro, administration of protamine 50 mg can be used to antagonize the heparin effect in vivo.1 Two cases have been reported where a coagulopathy developing 30 min after graft reperfusion was reversed clinically by protamine sulphate 50 mg, resulting in cessation of oozing from cut surfaces and normalization of the TEG.7 Using protamine titration in vitro, Bakker and colleagues demonstrated a heparin effect in all of 15 recipients where heparin had been administered to the donor before removal of the liver graft and in one of five recipients whose donor had not been heparinized.8 This suggests that heparin may bind to the endothelium during the donor procedure and subsequently be released into the circulation on reperfusion. It is standard practice in the UK to administer heparin 300 u. kg⁻¹ to all donors before organ harvesting. There may also be an additional component of endogenously derived heparin-like substance released by the donor liver from hepatic vascular endothelium damaged during ischaemia or reperfusion.

Heparinase is an enzyme obtained from Flavobacterium heparinum which specifically cleaves the polysaccharide portion of the anticoagulant
proteoglycans heparin and heparan sulphate. Dietrich, Silva and Michelacci demonstrated that heparinase catalyses an eliminase reaction directly within the antithrombin III binding site of heparin, making it a potent reagent for eliminating the anticoagulant effect of heparin. Heparinase has been shown to reverse the TEG effects of heparin and studies on normal volunteers have demonstrated that heparinase does not affect TEG variables of whole blood not containing heparin. Protamine antagonizes the effects of heparin but may adversely affect coagulation if the dose of protamine is not matched to the quantity of heparin in the sample.

We have shown previously that the hypo-coagulable state demonstrated on the TEG is largely reversed by administration of heparinase in vitro. Since 1994 we have been using heparinase-coated sample pots (Heparinase I, 2 u.; Haemoscope Corp) in the TEG run simultaneously with the native sample after reperfusion. Where an abnormal TEG is improved by heparinase, this demonstrates the presence of heparin or heparan sulphate. In this study, we analysed the TEG tracings of 55 patients undergoing liver transplantation.

**Patients and methods**

We examined the TEG tracings of 55 consecutive patients undergoing liver transplantation. Patients undergoing liver transplantation for fulminant hepatic failure were excluded. The diagnosis and Child’s classification of the patients studied are given in table 1. Miscellaneous diagnoses were Wilson’s disease (1), glycogen storage disease (1) and chronic active hepatitis (3). All blood samples for TEG analysis were obtained from a non-heparinized arterial cannula. Paired TEG traces were run simultaneously for all samples obtained after reperfusion. One sample had nothing added to it (native blood) and the other sample was treated with heparinase.

Patients were classified into four groups, according to the appearance of the TEG tracing at reperfusion + 10 min (R+10) (fig. 2). The native TEG sample was placed into one of two groups: a straight line (no trace) at 60 min or an interpretable trace within 60 min. The corresponding heparinase traces were also allocated to one of two groups: normal or abnormal, as defined by TEG variables. For the purposes of this study an abnormal trace was considered to be a coagulation time in excess of 60 mm of trace or a maximum amplitude of less than 35 mm, these being the values which we consider to require treatment with blood components at this stage of operation.

Total transfusion of blood and blood components were recorded for all three stages of the procedure. Red blood cells were transfused to maintain packed cell volume at 27–30%. Blood component therapy was administered on the basis of TEG criteria and the presence or absence of bleeding from surgical sites.

Statistical analysis of blood transfusion requirements after reperfusion (unclamping of portal venous clamp and reperfusion of the liver to end of surgery) was carried out using the Kruskal–Wallis test with adjustment for ties. The Mann-Whitney U test was applied to individual groups.

Protamine 1 mg kg$^{-1}$ was given if a heparin effect was demonstrated on the TEG and there was clinical evidence of non-surgical bleeding caused by coagulopathy. Aprotinin (2 million kiu loading dose at the start of the procedure, followed by infusion of 500 000 kiu h$^{-1}$) was used according to our institutional criteria (patients with hepatic cirrhosis and those with markedly abnormal preoperative coagulation studies).

**Results**

Analysis of the R+10 TEG traces revealed only four of 55 traces which were unchanged by heparinase
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39 of the native samples had a straight line TEG, of which all but one were improved by heparinase. The remaining patient had a straight line TEG which was unchanged by heparinase. This particular patient, who had received aprotinin, developed a severe coagulopathy after reperfusion which was not improved by infusion of FFP, cryo-precipitate and platelets, or administration of protamine. She died several hours after operation with intractable coagulopathy and circulatory collapse.

Sixteen patients had an interpretable trace at reperfusion; 13 of these were improved by heparinase. The three patients whose TEG was unchanged by heparinase at R\textsubscript{10} min developed prolongation of coagulation time in the native sample at R\textsubscript{30} min and demonstrated a heparin effect at that time.

Protamine was administered in 26 cases. In cases where protamine was not administered, the heparin effect lasted 60–200 min. One patient who had received protamine developed recurrence of the heparin effect immediately after operation and received a second dose. In all cases where protamine was administered, correction of the TEG changes occurred (fig. 3).

Aprotinin was administered in 42 cases. In three of the 13 patients who did not receive aprotinin, the TEG after reperfusion was a straight line but the heparinase TEG revealed significant fibrinolysis which was treated with an i.v. bolus of tranexamic acid 500 mg. None of the patients who received aprotinin developed evidence of fibrinolysis on the TEG.

Mean blood transfusion for the four groups of patients is shown in figure 4. Statistical analysis of stage III (after reperfusion) transfusions revealed that patients who exhibited both a straight line native TEG and abnormal coagulation on the heparinase-modified trace (St Ab) had significantly higher transfusion requirements at this stage than any other group (P<0.01). There was no significant difference in stage III red blood cell (RBC) transfusion between patients who had an interpretable trace (irrespective of whether or not the heparinase-modified TEG was normal) and those patients who had a straight line TEG at reperfusion but had normal underlying coagulation (P=0.99).

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<th>Table 2</th>
<th>TEG findings at reperfusion + 10 min</th>
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<td>StAb</td>
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| Figure 3 | Example of heparin effect reversed by protamine. |

| Figure 4 | Mean blood transfusion. StN = Straight line native trace, normal underlying coagulation (n=18). Median transfusion stage III: 0 u. of red blood cells (RBC) (range 0–9 u.); StAb = straight line native trace, abnormal underlying coagulation (n=21). Median transfusion stage III: 3 u. of RBC (range 0–25 u.); TrN = interpretable native trace, normal underlying coagulation (n=11). Median transfusion stage III: 1 u. of RBC (range 0–4 u.); TrAb = interpretable native trace, abnormal underlying coagulation. (n=5). Median transfusion stage III: 1 u. of RBC (range 0–3 u.). In stages I and II (dissection and anhepatic stage) blood loss (and therefore RBC transfusion) is generally related to surgical factors and the presence of portal hypertension, and is therefore presented separately to stage III (after reperfusion) blood loss which is more usually related to coagulopathy. |
Mean core temperature of the patients was 36.4°C and at no time did any patient have a central temperature less than 35°C.

Discussion

The use of heparinase-modified TEG demonstrated the presence of heparin or a heparin-like substance after reperfusion of the grafted liver in all but one of the 55 patients studied. This “heparin effect” was variable both in magnitude and duration. The clinical significance of this heparin effect is uncertain as in many cases it is self-limiting and requires no specific treatment; however, in some patients it undoubtedly contributes to reperfusion bleeding. A “straight line” TEG in patients with good underlying coagulation rarely results in obvious bleeding at the wound site. There was no significant difference in stage III transfusion requirements between patients with good underlying coagulation irrespective of whether or not a marked heparin effect was demonstrated.

Our results indicate that patients who have an underlying coagulopathy are most sensitive to this effect. Transfusion requirements after reperfusion were significantly greater in patients who had both a “straight line” native TEG and abnormal underlying coagulation. In the absence of a hepatic effect, poor coagulation treated according to conventional criteria did not result in significantly greater blood loss after reperfusion, but the numbers of patients in this particular group were insufficient to draw definitive conclusions.

It is possible that some liver transplant recipients may have a greatly increased sensitivity to heparin at reperfusion because of a combination of underlying coagulopathy and reduced heparin clearance. Clearance of heparin and heparan sulphate is known to be significantly prolonged in patients with liver disease13 and it is possible that until the grafted liver achieves adequate function, that this is also the case in transplant recipients.

The origin of this heparin effect remains open to speculation but there is evidence for both exogenous and endogenous sources. All our donors had received heparin 300 u. kg⁻¹ according to standard UK practice. It is likely that this exogenously administered heparin contributes to this phenomenon,14 even though the donor liver is flushed routinely with 4.5% albumin 500 ml before completion of the venous anastomoses. It is unclear if there is a significant component caused by endogenous heparan sulphate released from the damaged vascular endothelium of the ischaemic liver graft. Increased plasma concentrations of thrombomodulin are a marker of endothelial cell damage15 and a sudden marked increase in concentrations have been demonstrated in liver transplant recipients within 5 min of reperfusion.16 It has been shown that even in circumstances where the donor received no heparin, heparin-like activity, as demonstrated by protamine titration, may be present after reperfusion of the grafted liver.8

McKee and colleagues demonstrated increased concentrations of heparan sulphate in cirrhotic patients who bled from varices compared with matched controls who did not bleed but were unable to demonstrate a correlation with any other coagulation profile in the two groups. They concluded that the presence of this anticoagulant may have an important role in determining bleeding risk.13 It is possible that heparan released into the systemic circulation from the ischaemic hepatic vascular endothelium at reperfusion similarly results in increased bleeding in patients who are excessively sensitive to its presence by virtue of poor underlying coagulation.

Where a marked heparin effect is demonstrated and the patient has clinical evidence of abnormal bleeding because of coagulopathy, administration of protamine sulphate 50–100 mg is of value. Correction of clotting factor and platelet deficiency should be undertaken simultaneously according to institutional procedures.

In conclusion, the presence of heparin or a heparin-like substance is demonstrable on the TEG in virtually all liver transplant recipients after reperfusion of the graft. This effect is so consistent, although variable in magnitude, that it most likely represents an inevitable accompanying effect of reperfusion. Provided graft function is adequate, it is generally not a significant cause of bleeding after reperfusion and corrects spontaneously within 1–2 h. However, some patients may be excessively sensitive to these trace amounts of heparin or heparan and where a heparin effect is demonstrated and the patient has clinical evidence of abnormal bleeding caused by coagulopathy, protamine sulphate 1 mg kg⁻¹ should be administered. Neutralase (Heparinase I) has recently been used successfully in human studies to reverse the effects of heparin17 and may prove to be a preferable alternative to protamine as it lacks the potentially serious side effects of anaphylaxis and pulmonary vasoconstriction.

In our series, the native TEG trace after reperfusion was rendered uninterpretable in more than 50% of patients because of the presence of a marked heparin effect. Conventional criteria for administration of blood products based on the assessment of the native TEG at this stage could result in unnecessary or excessive treatment with blood component therapy. Use of the heparinase-modified TEG allows rapid assessment of the contribution of heparin to coagulopathy after reperfusion. In addition, the underlying coagulation profile is revealed, thereby facilitating the diagnosis of fibrinolysis, clotting factor or platelet deficiency and enabling appropriate therapy to be instituted.

The use of heparinase-modified TEG to monitor coagulation during liver transplantation provides useful additional information over native TEG alone and we would recommend its routine practice in this group of patients.

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References


