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The intra-operative use of trasylol (aprotinin) in liver transplantation

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Abstract. Aprotinin has been reported to reduce blood loss in difficult cases requiring cardiopulmonary bypass surgery and more recently in liver transplantation. Over a 9-month period we compared the effects of an intra-operative infusion of aprotinin on transfusion requirements and coagulation profiles in 12 patients undergoing liver transplantation for end-stage cirrhosis with an equal number of consecutive transplants in patients with similar pathology who did not receive aprotinin. Transfusion of blood and blood products was reduced to one-third in the aprotinin-treated group. Operative time was also significantly reduced, as was ICU stay post-operatively. Aprotinin profoundly inhibits fibrinolysis and this is likely to be the major effect by which blood loss is reduced. Thromboelastography revealed severe fibrinolytic changes in the anhepatic stage in 4 of 6 controlled patients; this accelerated in 3 following reperfusion of the new graft. By contrast, only 1 patient of 12 in the aprotinin-treated group showed fibrinolytic activity in the anhepatic period, and none showed evidence of fibrinolysis following reperfusion of the new graft.

Key words: Liver transplantation, trasylol – Trasylol, liver transplantation – Aprotinin, liver transplantation

Clinical liver transplantation has evolved by the identification and resolution of a series of major obstacles. Patient selection, technical aspects, operative and post-operative management and the use of immunosuppression have all improved in recent years. Nevertheless, for the end-stage cirrhotic, who is likely to have severe portal hypertension and who may have previously undergone major upper abdominal surgery, the liver transplant procedure may be accompanied by severe and life-threatening blood loss, both during and after the operation.

In addition, patients with end-stage liver disease have an increased likelihood of developing fibrinolysis. In 11%–15% of cirrhotic patients, an ongoing fibrinolytic state can be demonstrated prior to surgery [1]. Superimposed on the haemostatic defects seen pre-operatively in liver transplant candidates are the intra-operative changes in coagulation that may occur. These are particularly marked during the anhepatic stage and for a variable time after reperfusion of the new graft and may coincide with periods of major blood loss. Recent work has indicated that in some patients intra-operative derangement of coagulation is, in part, due to an increase in fibrinolytic activity and thus measures to counter fibrinolysis should be taken [3],

Fibrinolysis is likely to result from an imbalance of inhibitors and activators of the fibrinolytic system. End-stage cirrhotics undergoing orthotopic liver transplantation frequently show a decreased hepatic clearance of circulating tissue plasminogen activator (tPA) and also decreased synthesis of plasminogen activator inhibitor (PAI) [5].

Trasylol (aprotinin; Bayer, Newbury, UK) is a serine protease inhibitor derived from bovine lung and apocrine tissue. In 1964 Tice et al. [7] demonstrated the efficacy of aprotinin in the prevention and inhibition of fibrinolysis in patients undergoing cardiopulmonary bypass. Royston et al. [6] reported the effect of aprotinin in reducing transfusion requirements during open-heart surgery.

In liver transplantation, Neuhaus et al. [4] have reported a reduction in intra-operative bleeding and fibrinolysis with the use of aprotinin.

We have conducted a pilot study of the effect of trasylol (aprotinin) on blood transfusion requirements and coagulation during liver transplantation. Specifically studied were the effects of aprotinin on:

(1) Blood and blood product requirements intra-operatively and post-operatively.

(2) The incidence and severity of fibrinolysis during the operative procedure, as measured by thromboelastography and specific factor assays.

(3) The length of the operative procedure.

(4) The length of the stay in the intensive care unit (ICU).

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Patients and methods

Twenty-four consecutive patients undergoing liver transplantation for end-stage cirrhosis were studied over a 9-month period. The first 12 patients served as controls and did not receive intra-operative aprotinin. The second cohort of 12 patients (the study group) received intra-operative infusions of aprotinin as detailed below. All transplant procedures were performed by a single experienced surgeon. A standard anaesthetic technique was used. No form of extracorporeal bypass was used in any of the patients.

Three patients in the study group and three patients in the control group were permanently hospitalized before transplantation; the remaining patients were called in from home. Ethics committee approval and relevant patient informed consent were obtained.

Patients in the study group were given a loading dose of 2 million kallikrein inhibitory units (KIU) of preservative-free aprotinin via a central line. A infusion of 500000 KIU per hour was then continued until the patient was transferred to the ICU. In addition, 70000 KIU was added to each unit of blood transfused intra-operatively.

Fresh frozen plasma (FFP) and blood in a ratio of 1:1 were transfused to maintain clotting factors and a haematocrit of 30%. Platelets and cryoprecipitate were given as indicated by laboratory and thromboelastographic results.

Operative procedure

For comparative purposes, the operative procedure was divided into three stages. Stage 1 was pre-anhepatic (first incision to recipient hepatectomy), stage 2 was anhepatic and stage 3 was post-anhepatic (reperfusion of new graft to skin closure). Blood samples were taken in each case at the following times: (1) Anaesthesia + 30 min, (2) Stage 1 + 30 min, (3) Stage 2 + 10 min, (4) Stage 3 – 10 min, (5) Stage 3 + 5 min, (6) Stage 3 + 30 min, (7) Stage 3 + 60 min, (8) Stage 3 + 120 min.

Each blood sample was analysed for: (1) haemoglobin, (2) prothrombin time/international normalised ratio (INR), (3) partial thromboplastin time (PTT), (4) platelets, (5) potassium, (6) sodium, (7) calcium, (8) glucose, and (9) thromboelastography.

In a smaller patient group, specific factor assays, tPA, antiplasmin and PAI were measured.

Thromboelastography

Thromboelastography (TEG) is a technique that measures the viscoelastic properties of clot, giving information on the whole coagulation process from a single blood sample. Within 20–30 min it is possible to identify clotting factor activity, platelet function and any significant fibrinolytic process (Fig. 1). It has obvious applications in settings where there are potentially a multitude of haemostatic defects requiring rapid on-site diagnosis and intervention. It is now routinely used in many centres for the monitoring of coagulation during liver transplantation [3] and in cardiac surgery.

Post-operative procedure

Patients were electively ventilated in the ICU for a minimum period of 24 h. Following endotracheal extubation, patients who were haemodynamically stable with good renal and respiratory function were discharged from the ICU to a surgical ward.

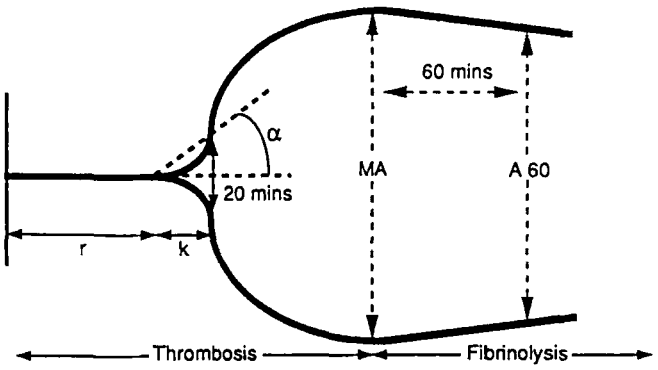

Fig. 1. Thromboelastogram trace. Normal thromboelastographic parameters: *r* Reaction time (represents intrinsic clotting and is broadly equivalent to the PTT); *rk* time to presence of a small but fixed level of viscoelasticity attained by the forming clot; *α* speed of clot strengthening function of fibrinogen; *ma* greatest transverse amplitude of the graph and is a direct function of the maximum dynamic properties of fibrin and platelets; *CLI* clot lysis index (the ratio of the amplitude at 60 min, A 60, to the *ma;* reflects loss of clot integrity due to lysis). *r* = 10–15 min; *rk* = 12–18 min; *α* = 45°; *MA* = 50–60 mm; *A 60* = MA-5 mm; paper speed = 2 mm/min

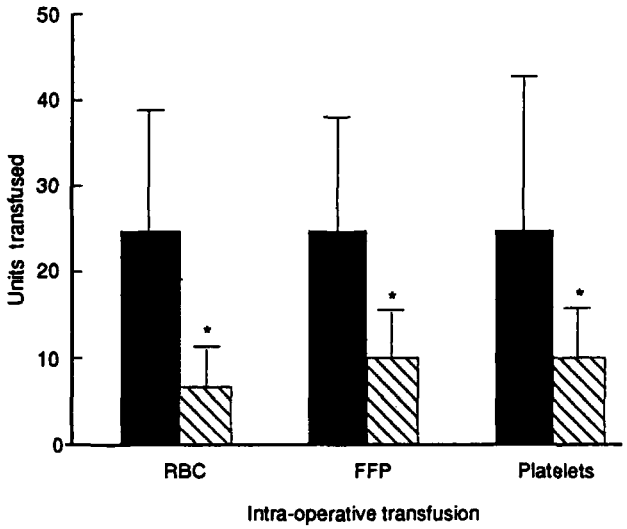

Fig. 2. Blood and blood product usage. Requirements of blood, fresh frozen plasma and platelets during orthotopic liver transplantation in patients receiving aprotinin infusions compared to patients not receiving it (controls). ■ Control; ▨ aprotinin. * $P < 0.002$ (Mann-Whitney U-test)

Statistics

The Mann-Whitney U-test was used, on advice of our Department of Clinical Epidemiology and Statistics, as the most appropriate and powerful non-parametric test for data from two populations of this size.

Results

Haematological data (Fig. 2)

Red blood cell requirements. A mean of 23.6 units with a median of 21.5 units was transfused in the untreated (control) group (range 6–55). In the aprotinin group a mean of

Table 1. Thromboelastographic parameters (mean values and standard deviations) for control and study groups during various stages of liver transplantation. Normal values: r = 6–8 min, rk = 8–10 min, ma = 50–70 min

Stage	Pre-operative (Stage 1)		Anhepatic (Stage 2)		Reperfusion (Stage 3)		Stage 3 + 60 min	
Group	Control	Aprotinin	Control	Aprotinin	Control	Aprotinin	Control	Aprotinin
r	11 + 2	12 + 4	13 + 6	11 + 2	19 + 11	22 + 7	11 + 2	13 + 2
rk	14 + 3	15 + 4	23 + 19	13 + 3	28 + 20	31 + 10	14 + 3	15 + 3
ma	40 + 13	40 + 14	26 + 12	45 + 7	26 + 11	31 + 11	51 + 5	57 + 6

7.5 units with a median of 6.5 units was required (range 1–17). The difference between the two groups was significant ($P < 0.002$, Mann-Whitney U-test).

Fresh frozen plasma requirements. A mean of 23.6 units with a median of 22 units was required for the control group (range 6–51). For the aprotinin group the mean was 9.3 units with a median of 8 units (range 2–17). Again, the difference between the two groups was significant ($P < 0.002$, Mann-Whithney U-test).

Platelet requirements. For the control group, a mean of 24 units with a median of 24 units was transfused (range 0–70). The aprotinin group required a mean of 9.3 units with a median of 10 units (range 0–20). Once more there was a significant difference between the two groups ($P < 0.002$, Mann-Whitney U-test).

Post-operative red blood cell requirements. A mean of 11 units with a median of 3 units was required for the control group. The aprotinin group required a mean of 2.5 units with a median of 2 units.

Stage 3 time. The mean length of stage 3 in the control group was 118 min with a median of 110 min. This contrasted with a mean of 67 min and a median of 66 min in the aprotinin group. The difference was significant at $P < 0.002$ (Mann-Whitney U-test).

ICU stay. The mean length of time spent post-operatively in the ICU was 6 days for the control group (range 1–17) and 3 days for the aprotinin group (range 1–6). This difference was also significant ($P < 0.002$, Mann-Whitney U-test).

Thromboelastographic data

Table 1 shows thromboelastographic parameters (mean values and standard deviations) for control and study groups during the various stages of this liver transplant procedure.

All patients had pre-operative TEG tracings showing some prolongation of "r" and "rk" times with reduced "ma" in most cases. This was consistent with baseline prothrombin times, partial thromboplastin times and platelet counts.

In the control group there was a significant deterioration in all TEG parameters during the anhepatic period despite FFP and platelet transfusions. In the aprotinin group there was no change from pre-oeprative values.

On reperfusion there was marked deterioration of the TEG in all patients, consistent with the observations of other workers [3].

At 60 min, presumably reflecting the effect of the new graft function, all patients had normal thromboelastograms.

Fibrinolysis

Fibrinolysis was assessed from thromboelastographic data and euglobulin lysis time (ELT). Table 2 demonstrates a semi-quantitative assay of fibrinolysis (the clot lysis index), based on the thromboelastogram.

Two patients in the control group and three patients in the aprotinin group showed moderate fibrinolysis on baseline (pre-operative) samples.

Four patients in the control group developed severe fibrinolysis during the anhepatic period, and these worsened markedly on reperfusion of the new graft in three of the four patients. The degree of fibrinolysis had a direct effect on blood loss and subsequent replacement. In these three patients mean transfusion requirements were 28 units (SD ± 12 units) of whole blood compared to 7.6 units (SD ± 5 units) in the other patients in the control group.

Figure 3 shows a typical sequence of thromboelastograms from one of the three patients developing severe intra-operative fibrinolysis, resulting in massive blood loss and replacement. In contrast, in the aprotinin group, one patient showed evidence of moderate fibrinolysis in the anhepatic period, but there was no evidence of lysis in any patient following reperfusion.

Euglobulin lysis time

ELT was within the normal range (60–200 min) in the aprotinin-treated group. In one patient, ELT was shortened to the normal lower limit in the anhepatic phase and immediately after reperfusion, and this correlated with evidence of some fibrinolytic activity on the TEG tracing.

Table 2. A semi-quantitative assay of fibrinolysis based on the thromboelastogram. Clot lysis index: A60/ma: (Amplitude at 60 min/maximum amplitude). Moderate lysis: CLI: 65%–45%; Severe lysis: CLI: 45%–0%

Clot lysis index	Controls (6/12)		Aprotinin (12/12)	
	Moderate	Severe	Moderate	Severe
	(Number of patients)		(Number of patients)	
Baseline	2	0	3	0
Anhepatic	1	4	1	0
Reperfusion	0	3	0	0
+ 60 min	0	0	0	0

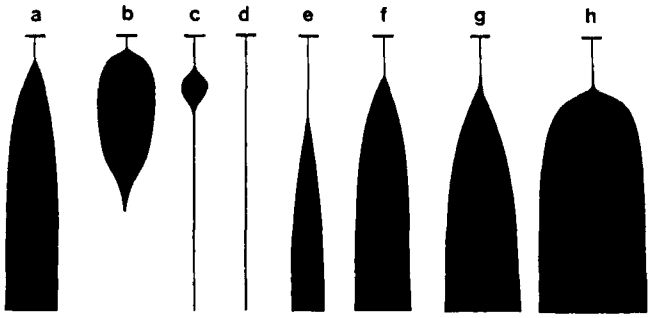

Fig. 3 a–h. Sequence of thromboelastographic tracings in a patient undergoing orthotopic liver transplantation. **a** Baseline TEG (low in clotting factors and platelets); **b** anhepatic + 10 min (fibrinolysis developing); **c** anhepatic + 45 min (severe fibrinolysis); **d** reperfusion + 5 min (straight line TEG – no clot formation); **e** reperfusion + 15 min (post-tranexamic acid); **f** reperfusion + 30 min (some spontaneous correction in coagulation); **g** reperfusion + 90 min (further FFP and platelets given); **h** reperfusion + 120 min (normal TEG apart from prolonged *r* time; INR = 2.3)

Tissue plasminogen activator levels

In three study patients, tPA levels were surprisingly constant throughout the procedure (mean tPA baseline 12 + / – 5 ng/ml, anhepatic 16 + / – 9 ng/ml, reperfusion 14 + / – 3 ng/ml, closure 11 + / – 4 ng/ml).

Discussion

Aprotinin is a serine protease inhibitor with inhibitory effects on human plasmin, trypsin and both tissue and plasma kallikrein. The anti-fibrinolytic activity of aprotinin was first recognised and described by Tice et al. [7]. In 1987, Royston et al. [6] reported the remarkable effect of aprotinin on blood transfusion requirements following repeat open-heart surgery.

In the field of liver transplantation, the beneficial effects of aprotinin on intra-operative bleeding and fibrinolysis were recently reported by Neuhaus et al. [4]. These workers saw a significant reduction in blood loss and consequent transfusion requirements after administration of a 2 million-IU prophylactic dose of aprotinin.

Our study differs from this in that a continuous, intraoperative infusion of aprotinin was also given, as in the protocol of Royston et al. [6]. We have also demonstrated marked differences between patients treated with aprotinin and controls with respect to coagulation profiles performed intra-operatively using thromboelastography, confirming the powerful anti-fibrinolytic effect of aprotinin.

Our study entirely supports the observations of other workers. Significant reductions in intra-operative transfusion requirements of red blood cells, fresh frozen plasma and platelets in the aprotinin-treated group have been shown. Duration of surgery from the time of reperfusion of the new graft to closure (stage 3) was also significantly reduced.

Furthermore, in our study, there is a striking difference between our two patient groups in transfusion requirements in stage 3, the mean transfusion requirements in the aprotinin group being only 0.6 + / – 0.8 units. This contrasts with the usual pattern of transfusions during liver transplantation, which tends to show a fairly even distribution during the three defined stages, except where there is a significant coagulopathy post-reperfusion, when very large volumes may need to be transfused both in stage 3 and post-operatively.

The mechanism by which blood loss is reduced by administration of aprotinin during liver transplantation is not entirely clear. Certainly the profound inhibition of fibrinolysis (as measured by TEGs and ELT) must be a major component. The tPA levels in patients treated with aprotinin were lower than would be expected, particularly during the anhepatic phase and immediately post-reperfusion. This may be due to inhibition of kallikrein and, thus, bradykinin, which is a potent stimulator of tPA release [2].

Despite TEGs with relatively poor initial coagulation profiles post-reperfusion, in the aprotinin-treated group, the surgical field remained remarkably dry and haemostasis, when required, was easily and rapidly achieved, leading to early wound closure and decreased total operating time.

The consistent reductions in blood and blood product requirements, duration of surgery and length of ICU stay in patients treated with aprotinin implies a substantial reduction in the costs of liver transplantation.

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