DIFFERENT APROTININ APPLICATIONS INFLUENCING HEMOSTATIC CHANGES IN ORTHOTOPIC LIVER TRANSPLANTATION

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The effect of different aprotinin applications on hemostatic changes and blood product requirements in orthotopic liver transplantation was investigated in a prospective, open, and randomized study.

From November 1989 to June 1990, 13 patients received aprotinin as a bolus of 0.5 Mill. kallikrein inactivator units (KIU) on three occasions in the course of an OLT, whereas 10 other patients were treated with continuous aprotinin infusion of 0.1–0.4 Mill. KIU/hr. Before and after reperfusion of the graft liver, signs of hyperfibrinolysis, measured by thrombelastography, were significantly lower in the infusion group. Tissue-type plasminogen activator (t-PA) activity increased during the anhepatic phase but to a significantly lesser extent in the infusion group. Blood product requirements during OLT were tendentiously higher in the bolus group but not significantly so. However, the use of packed red blood cells was significantly lower in the postoperative period, whereas there was no significant difference in fresh frozen plasma requirements between the two groups.

All 23 patients have survived, and only one woman of each group required retransplantation due to severe host-versus-graft reactions.

Furthermore, we investigated the perfusate of the graft liver in both groups and detected signs of a decreased t-PA release in the infusion group.

Our results demonstrate an advantage of aprotinin given as continuous infusion over bolus application in OLT.

In OLT, hyperfibrinolysis has been recognized as an important cause of intra- and postoperative bleeding (*1–3*), influencing both the short- and long-term outcome (*4, 5*). Previous investigations have indicated that hyperfibrinolysis is due to an increase of tissue-type plasminogen activator (t-PA)* activity (*1, 3*) and a concomitant increase of urokinase-type plasminogen activator (u-PA) activity (*1*).

Aprotinin (Trasylol, Bayer, Leverkusen, Germany), a parenterally applied proteinase inhibitor from bovine lung (*6–8*), inhibits plasmin, kallikrein, trypsin, and to some extent human urokinase (*7*). The toxicity of aprotinin is extremely low (*7*), and even high doses are well tolerated (*6, 9*). As randomized studies in cardiac surgery have demonstrated significantly reduced transfusion requirements when aprotinin was administered during surgery (*10, 11*), we tested its application in patients undergoing OLT. Bolus application of 0.5 Mill. kallikrein inactivator units (KIU) aprotinin given three times during OLT led to reductions in blood product requirement and in the increase of t-PA activity (*1*) as compared with the reports of other investigators (*2, 3, 12*). Nevertheless, thrombelastography (TEG) showed signs of hyperfibrinolysis in most of the OLTs, in spite of aprotinin bolus application.

In the present study, we have investigated the effect of two different aprotinin regimens on the hemostatic changes and blood product requirements in 23 OLTs.

MATERIALS AND METHODS

In an open, prospective, and randomized study 23 patients with terminal liver disease (Table 1) underwent their first OLT at the University Hospital Rudolf Virchow, Berlin, Germany, between November 1989 and June 1990. The patients were randomized by sealed envelopes with serial numbers containing the way of aprotinin administration. Thirteen patients were thereby randomized to receive i.v. boli of 0.5 Mill. KIU aprotinin three times during OLT, given at the beginning of the operation, at the onset of the anhepatic phase, and at the beginning of reperfusion, whereas 10 patients were continuously treated with aprotinin infusion (Table 2). OLT was carried out by established surgical techniques using a venovenous bypass (*13, 14*). Packed red blood cells (RBC) and fresh frozen plasma (FFP) were substituted to compensate for intra- and postoperative blood loss. Postoperatively, RBC concentrates were generally given when the hemoglobin concentration was lower than 9 g/dl, while FFP concentrates were administered when the protein concentration was lower than 6.0 g/dl. For cold storage of the graft liver, Belzer UW-CSS solution (Dupont, Paris, France) was used.

Blood samples were taken from the arterial line; (1) after induction of anesthesia; (2) 5 min before; and (3) 10 min after the beginning of the anhepatic stage. Further samples were collected; (4) 5 min before reperfusion; (5) as well as 5 min; (6) 15 min; (7) 60 min; and (8) 12 hr afterwards. In addition, a sample of the perfusate released from the liver graft vein during the flushing procedure with arterial blood was taken prior to opening of the hepatocaval anastomosis (P). Blood samples were collected in plastic syringes prefilled with 1/10 volume of trisodium citrate. For the determination of t-PA activity anticoagulated blood was immediately acidified with acetate acid. Another blood

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* Abbreviations: α_2-AP, α_2-antiplasmin; AT III, antithrombin III; B, bolus group of aprotinin application; C1-inh, C1-inhibitor; FFP, fresh frozen plasma; I, infusion group of aprotinin application; KIU, kallikrein inactivator units; PAI, plasminogen activator inhibitor; PC, protein C; RBC, packed red blood cells; TAT, thrombin-antithrombin III; TEG, thrombelastography; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; WBLT, whole-blood-clot lysis time.

TABLE 1. Characteristics of 23 patients who underwent their first orthotopic liver transplantation when aprotinin boli (B) or continuous aprotinin infusion (I) were given

Diagnosis	Number		Female		Male		Age (range)	Mean (range)
	B	I	B	I	B	I	B	I
Postnecrotic cirrhosis	5	8	1	4	4	4	50 (43–65)	49 (24–65)
Alcohol-toxic cirrhosis	4	0	3	0	1	0	49 (40–56)	—
Primary biliary cirrhosis	1	1	1	1	0	0	24	55
Secondary biliary cirrhosis (erythrohepatic protoporphyrie)	1	0	1	0	0	0	52	—
Budd Chiari syndrome	1	0	1	0	0	0	47	—
Liver cell carcinoma	1	0	1	0	0	0	60	—
Metastatic liver	0	1	0	1	0	0	—	55
Total	13	10	8	6	5	4	47 (40–65)	53 (24–65)

TABLE 2. Aprotinin application used in 23 patients who underwent their first orthotopic liver transplantation

Bolus group		Infusion group
0.5 Mill. KIU	Induction of anesthesia	0.2 Mill. KIU/hr
0.5 Mill. KIU	Anhepatic phase	0.4 Mill. KIU/hr
0.5 Mill. KIU	Reperfusion	
	Skin closure (heparin 250 IU/hr) (heparin 500 IU/hr starting 12 hr after operation till the 3rd postoperative day)	0.1 Mill. KIU/hr

sample was anticoagulated with a mixture of trisodium citrate, theophyllin, adenosine, and dipyridamol for the determination of plasminogen activator inhibitor (PAI) activity. The specimens were gently mixed, centrifuged at 3000 U/min for 20 min, and the supernatant frozen at −70°C until measurement.

The following parameters were measured using commercially available kits: protein C (PC) activity (Boehringer, Mannheim, Germany), antithrombin III (AT III) activity, plasminogen activity, α_2-antiplasmin (α_2-AP) activity, C1-inhibitor (C1-inh) activity (all: Behring Werke AG, Marburg, Germany), fibrinogen according to Clauss (Hoffmann-LaRoche, Basel Switzerland). t-PA and PAI activities were determined by chromogenic substrate methods, whereas t-PA antigen was measured using a solid-phase enzyme immunoassay (all: Kabi, Stockholm, Sweden). Thrombin-antithrombin III (TAT) complexes were determined by enzyme immunoassays (Behringwerke AG, Marburg, Germany). Aprotinin was estimated by ELISA as previously described (*15*). TEG of recalcified whole blood was performed using a thrombelastograph (Hellige, Freiburg, Germany). When whole-blood-clot lysis time (WBLT), the time between the maximal amplitude and complete lysis in the TEG, was shorter than 90 min, it was defined as severe hyperfibrinolysis, and between 90 and 120 min, as mild hyperfibrinolysis (*3*).

Statistical analysis. Using the Martinez and Iglewicz test (*16*), the distribution of the parameters was found to be abnormal. Therefore the nonparametric Mann-Whitney test was used to test the significance of differences between groups. Values for $P<0.05$ were considered to be significant.

RESULTS

Median values of blood product requirements during OLT were 8 (range: 4–26) units for RBC and 10.5 (7–35) units for FFP in the bolus group and 7 (2–20) units for RBC and 8 (2–28) units for FFP in the infusion group. The difference was not significant. However, the use of RBC was significantly lower ($P=0.045$) in the infusion group (median values (range): 3.5 (0–9) units [bolus group]); 1.5 (0–6) units [infusion group]) in the first three postoperative days after the operation, whereas there was no significant difference in FFP requirements between the two groups (6.5 [0–17] units [infusion group]; 9.5 [4–22] units [bolus group]) at the same stage. The median time of intensive care in the bolus group was 25 days (range: 14–58 days) as compared with 23 days (11–36 days) in the infusion group (NS).

In TEG, hyperfibrinolysis was stated in 7 out of 13 OLTs in the bolus group but only in 1 out of 10 OLTs in the infusion group (Fig. 1). Levels of t-PA activity increased in both groups during the anhepatic phase, reaching their peaks immediately before reperfusion. These maxima, however, were significantly higher in the bolus group (Fig. 2). The t-PA antigen levels in plasma did not differ between the two groups. In both groups PAI levels increased after reperfusion to comparable levels (Fig. 3). The course of C1-inh did not differ for either group (Fig. 3). The levels of α_2-AP were higher in the infusion group, reaching significance only 10 min after the beginning of the anhepatic phase and 12 hr after reperfusion (Fig. 4). Plasminogen concentration showed no difference in either group (Fig. 4). TAT complexes showed significantly higher levels in the infusion group prior to the anhepatic phase and at its onset and after reperfusion (Fig. 5). Similarly, fibrinogen levels were higher in the bolus group shortly before the anhepatic phase and after reperfusion (Fig. 5). The course of PC and AT III activities did not differ in the two groups (Fig. 6). Aprotinin levels were significantly higher in the infusion group (Fig. 7).

In the perfusate the comparison of different hemostatic parameters between the groups revealed a significantly lower level of t-PA antigen and a tendentiously lower level in t-PA activity in the infusion group while activity of the inhibitors PAI and α_2-AP was significantly increased in this group. All other parameters in the perfusate were comparable in both groups (Table 3).

DISCUSSION

Our previous experience (*1*) prompted us to postulate a beneficial effect of aprotinin bolus application on the course of OLT, but signs of hyperfibrinolysis as measured by TEG were still present in most patients undergoing OLT. Here, we have investigated the effect of intermittent bolus versus continuous infusion application of aprotinin during the course of 23 OLTs in an open, prospective, and randomized study.

We confirmed previous investigations suggesting a beneficial effect of aprotinin bolus application in reducing signs of hyperfibrinolysis and bleeding complications in patients undergoing OLT (*1*).

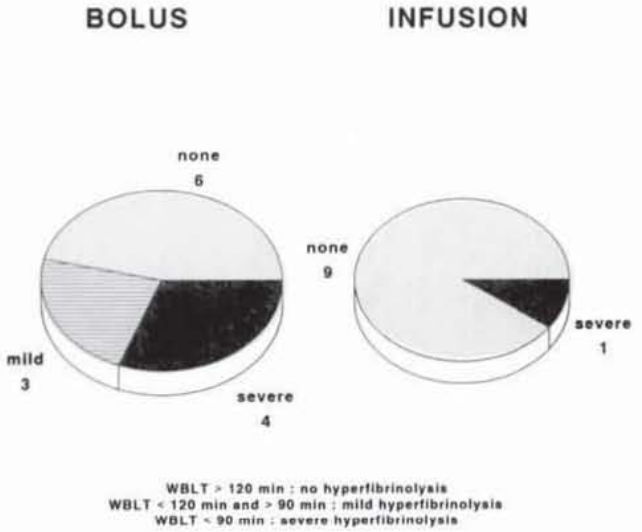

FIGURE 1. Signs of hyperfibrinolysis during orthotopic liver transplantation in thromboelastogram in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion.

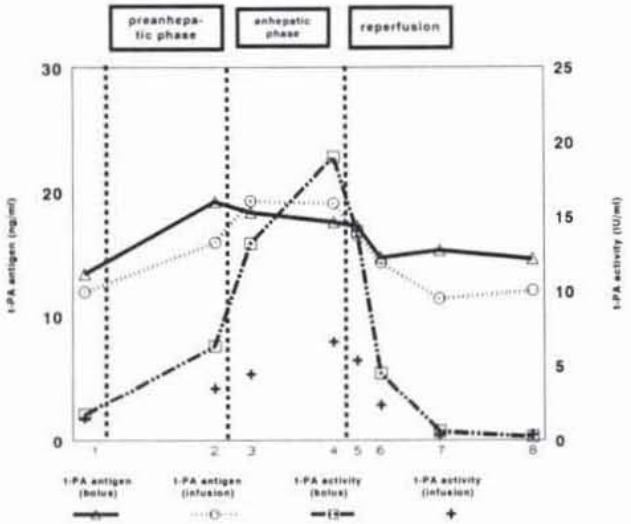

FIGURE 2. t-PA activity and antigen during orthotopic liver transplantation in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion.

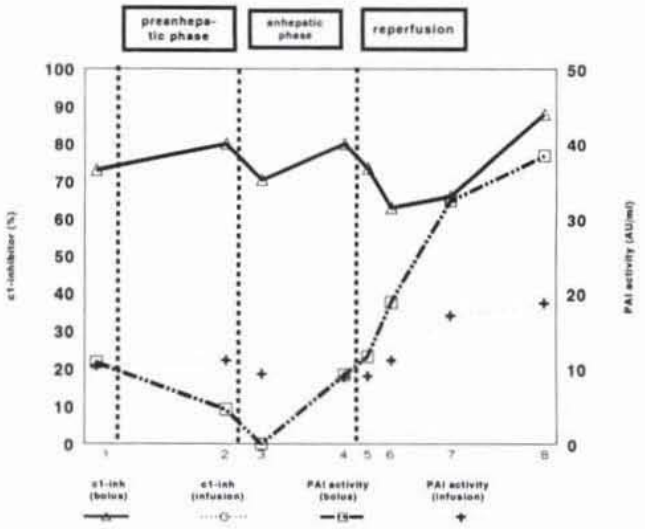

FIGURE 3. PAI and C1-inhibitor activities during orthotopic liver transplantation in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion.

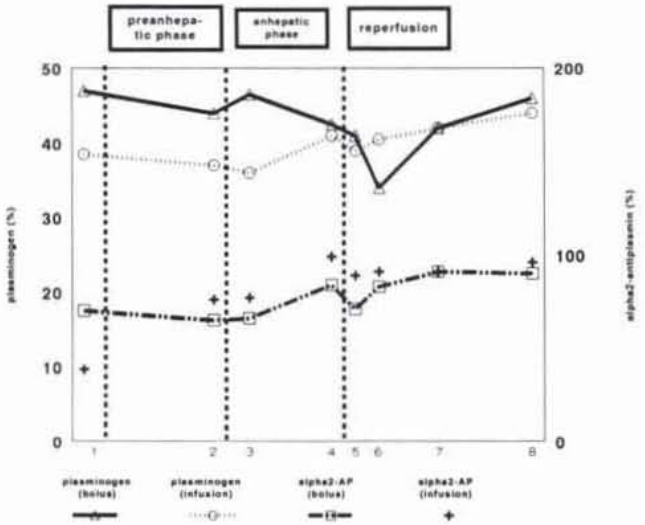

FIGURE 4. Plasminogen concentration and α_2-antiplasmin activity during orthotopic liver transplantation in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion.

Aprotinin levels were higher in the infusion group during the whole length of the OLTs (Fig. 7). Signs of hyperfibrinolysis, according to TEG and increased t-PA activity (Figs. 1 and 2) occurred more often in the bolus group especially before and after reperfusion, whereas PAI and α_2-AP levels (Figs. 3 and 4) were lower during the anhepatic phase and after reperfusion in the infusion group, respectively. These results suggest that higher aprotinin levels maintained by continuous infusion reduce plasminogen activator activity in plasma and diminish signs of hyperfibrinolysis in the infusion group.

After reperfusion a more pronounced increase of TAT complexes and a concomitant decrease of fibrinogen levels in the infusion group indicate an increased prothrombin activation. Higher aprotinin levels may shift the hemostatic balance toward coagulation. As on the other hand activities of PC, AT III, and C1-inh were not seriously decreased in either group no accelerated consumption of coagulation factor inhibitors could be suspected.

Our results showed higher blood product requirements during OLT in the bolus group but not to a significant level. All 23 patients are still alive, and only one woman of the bolus group and one woman of the infusion group needed retransplantation because of severe host-versus-graft reactions. Neither had hepatic artery thrombosis. Our previous investigations of the perfusate showed significantly higher TAT complexes and significantly lower activities of the protease inhibitors PC, AT III, and C1-inh when compared with the systemic circulation 5 min before reperfusion. This indicates increased prothrombin activation and a consumption of inhibitors in the graft liver (*1*). Furthermore, parameters of activated phagocytes such as elastase and cathepsin B were found to be significantly elevated in the perfusate (*17*). In addition, it has recently been demonstrated that aprotinin exerts a protective effect on ischemic hepatocellular damage occurring in the rat liver-transplant model (*18*). We wondered whether considering these results higher systemic levels of aprotinin in the reperfusion phase will

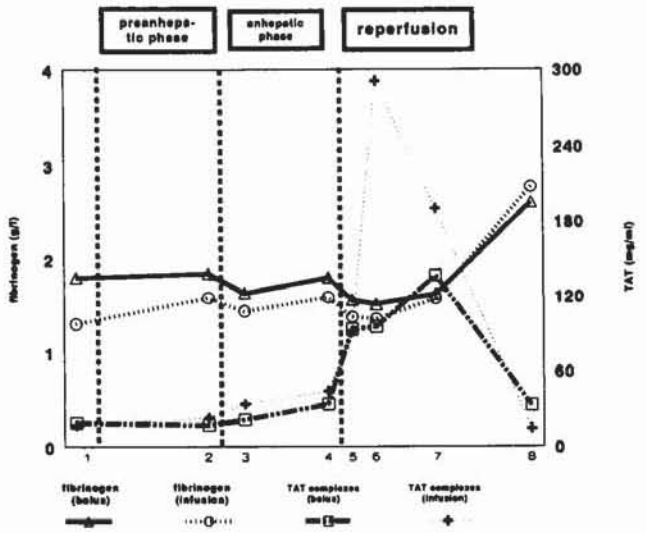

FIGURE 5. Thrombin antithrombin III complexes and fibrinogen concentration during orthotopic liver transplantation in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion.

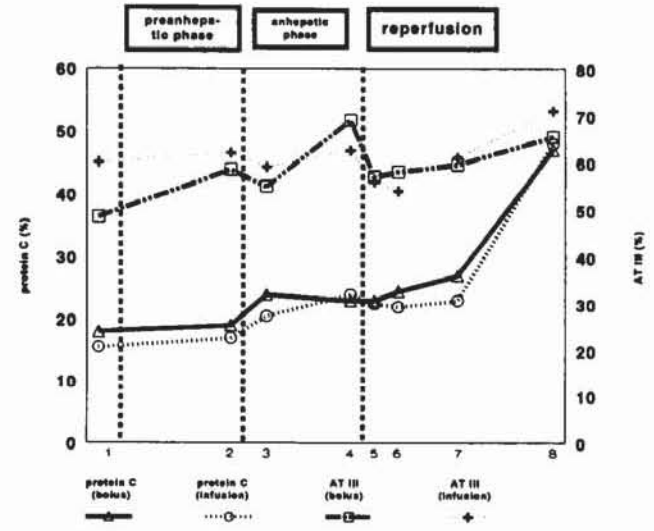

FIGURE 6. Protein C and antithrombin III activities during orthotopic liver transplantation in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion.

change hemostatic parameters of the graft liver perfusate. Indeed, t-PA antigen and activity were lower and PAI and α_2-AP activities were higher in the perfusate of the infusion group. In vitro studies showed no effect of different amounts of aprotinin in t-PA and PAI activity assays (not published). Presuming that high systemic aprotinin levels are reducing endothelial damage in the flushed graft liver, the lower t-PA activity and antigen levels in the perfusate can be explained. In addition, it has to be discussed whether t-PA liberation is reduced by inhibition of kallikrein, a potent stimulator of t-PA release (*19*). However, the diminished t-PA release in the graft liver did not result in a lower t-PA level in the systemic circulation after reperfusion.

Parameters of thrombin formation and protease inhibitors did not differ in the perfusate and the systemic circulation.

In summary, aprotinin application by infusion results in significantly diminished signs of hyperfibrinolysis and a lesser increase in t-PA activity intraoperatively. On the other hand,

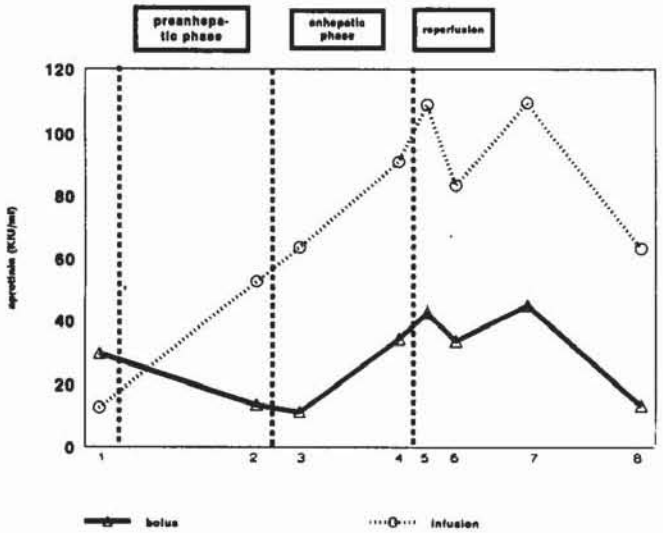

FIGURE 7. Aprotinin concentrations during orthotopic liver transplantation in 13 patients of aprotinin bolus application and 10 patients of aprotinin infusion and *P* (bolus/infusion) are demonstrated.

TABLE 3. Comparison of different hemostatic parameters in the perfusate of 13 patients in the bolus group as compared with 10 patients of the infusion group who underwent their first orthotopic liver transplantation

	Median (B) median (I)	Range (B) range (I)	*P* (B/I)
t-PA activity (IU)	12	0.7–23.6	0.101
	5	0.7–17.4	
t-PA antigen (ng/ml)	12.3	7.2–26.6	0.050
	10.1	1.2–19.3	
PAI activity (AU)	6.4	0–22	0.043
	17.8	8.8–30.4	
α_2-AP (%)	63	5–95	0.010
	93	68–102	
Plasminogen (%)	43	3–77	0.288
	50	35–87	
Fibrinogen (g/L)	1.5	0–2.2	0.476
	1.5	0–2.6	
TAT (mg/ml)	93.2	40.5–324	0.288
	73.5	32.5–1500	
PC (%)	9.5	0–24	0.063
	15	3–50	
AT III (%)	31	3–56	0.476
	37	9–55	
C1-inh (%)	65	6–92	0.254
	73	34–109	

aprotinin given in higher concentrations may influence the hemostatic balance toward hypercoagulation. A beneficial effect of aprotinin for the prevention of endothelial damage in the graft liver is suggested.

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