Transfusion requirements in orthotopic liver transplantation
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2004

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Citation for published version (APA):

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Chapter 4

Reduced transfusion requirements by recombinant factor VIIa in orthotopic liver transplantation

A Pilot Study

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*Transplantation 2001;71:402-405*
Abstract

Background
Large transfusion requirements, i.e. excessive blood loss, during orthotopic liver transplantation (OLT), are correlated with increased morbidity and mortality. Recombinant factor VIIa (rFVIIa) has been shown to improve hemostasis in a variety of conditions, but has never been studied in liver transplantation.

Methods
We performed a single centre, open label, pilot study in adult patients undergoing OLT for cirrhosis Child-Pugh B or C, to assess efficacy and safety of rFVIIa. rFVIIa (80μg/kg) was administered at the start of the operation, to be repeated according to predefined criteria. Packed red blood cell concentrates (RBC), fresh frozen plasma, and platelet concentrates were administered according to predefined criteria. Perioperative transfusion requirements in study patients were compared with matched controls.

Results
Six patients were enrolled in the study. All received a single dose of rFVIIa. Transfusion requirements (given as median, with range in parentheses) were lower in the study group than in matched controls: 1.5 (0-5) versus 7 (2-18) units of allogeneic RBC ($P = 0.006$), 0 (0-2) versus 3.5 (0-23) units of autologous RBC ($P = 0.043$), total amount of RBC 3 (0-5) versus 9 (4-40) units ($P = 0.002$). Transfused fresh frozen plasma was 1 (0-7) versus 8 (2-35) units ($P = 0.011$). Blood loss was 3.5 L (1.4-5.3) versus 9.8 L (3.7-35.0) ($P = 0.004$). One study patient developed a hepatic artery thrombosis at day 1 postoperatively.

Conclusions
A single dose of 80 μg/kg rFVIIa significantly reduced transfusion requirements during OLT. Further study is needed to establish the optimally effective and safe dose of rFVIIa in orthotopic liver transplantation.
Introduction
Orthotopic liver transplantation (OLT) is often associated with excessive blood loss and hence, the need for transfusion of large amounts of blood products (1). Increased blood loss is correlated with a lower survival rate (2,3). Patients with higher transfusion requirements stay longer in the intensive care, suffer more infections and have a higher rate of graft loss (2). Primary causes of massive blood loss are surgical bleeding and impaired hemostasis. Impaired hemostasis in end-stage liver disease results from lowered plasma levels of coagulation factors, hyperfibrinolysis, thrombocytopenia and thrombocytopenia (3). Blood loss has been reduced by improved surgical and anesthesiological techniques, by better graft preservation and by appropriate supplementation of coagulation factors and platelets (4). The antifibrinolytic drug aprotinin is often given to improve hemostasis. Its efficacy has been debated, but recent data are favorable (5,6). Nevertheless, further reduction of blood loss and transfusion requirements is needed. Thrombosis of the hepatic vessels is a serious complication in liver transplantation. Hepatic artery thrombosis is the most common vascular complication. Recent studies, including one from our center, show an incidence of about 5%, with most cases occurring early after transplantation (7,9). Hepatic artery thrombosis may result in graft loss. Therefore, any measure to improve hemostasis should not increase the risk of thrombosis. Recombinant factor VIIa (rFVIIa, NovoSeven®, Novo Nordisk, Copenhagen, Denmark) has been shown to be effective in a variety of hemostatic disorders. Its efficacy has been demonstrated in hemophilia patients with inhibitors to factor VIII or IX, whereas its benefit has been suggested in an increasing number of other bleeding disorders (10,11). In patients with liver cirrhosis, correction of a prolonged prothrombin time was observed after administration of rFVIIa (12). Recently, its effective use was reported in two cases of successful liver transplantation in children with fulminant hepatic failure (13).
We performed a single-center, open-label, dose-exploring pilot study to assess the efficacy and safety of rFVIIa in patients who undergo OLT.
Patients and methods

Patients
Eligible patients were adults (≥18 years of age) who were scheduled for elective OLT because of end-stage chronic liver disease, classified as Child-Turcotte (Pugh’s modification) score B or C (14). Exclusion criteria included overt major bleeding or a known hereditary bleeding disorder; a history of venous or arterial thrombosis or a known hereditary thrombophilic state; clinically overt atherosclerosis; renal insufficiency (serum creatinine level > 1.7 mg/dl); fulminant hepatic failure i.e. hepatic encephalopathy within eight weeks of the onset of symptoms in the absence of preceding liver disease; administration of plasma products, platelets and/or hemostatic drugs less than 7 days before transplantation; and/or non steroidal anti inflammatory drugs less than two weeks prior to transplantation. The study protocol was approved by the hospital Ethical Board. All patients gave written informed consent.

Controls
Data on transfusion requirements and blood loss in patients in the present study were compared with matched controls. We previously analysed blood loss and transfusion requirements in all adult patients with cholestatic or non-cholestatic liver disease who underwent a primary OLT in our hospital (15). Multivariate analysis identified year of transplantation, Child-Pugh classification, and urea nitrogen level as independent predictors of transfusion requirements. Cases and controls in the present study were matched for these variables and for cholestatic versus non-cholestatic liver disease.

Transplantation and transfusion procedures
Transplantation was performed either with preservation of the retrohepatic caval vein (the piggyback technique) or with the conventional technique with excision of the retrohepatic caval vein and use of venovenous bypass. Packed allogeneic and autologous red blood cell concentrates (RBC) were transfused to maintain a hematocrit between .25 and .30. Units of RBC had a volume of 250 ml and a hematocrit of 0.70. Autologous blood was returned to the patient when 1.5 liters were collected in the cell saver reservoir (Fresenius CATS®, Fresenius AG, Bad Homburg, Germany). Fresh frozen plasma (FFP) was administered when hemostasis
was clinically insufficient and prothrombin time (PT) and/or activated partial thromboplastin time (APTT) were ≥ 1.5 times the upper limit of normal or fibrinogen levels were below 100 mg/dl. Fibrinogen concentrate (FC) was given when fibrinogen levels fell below 70 mg/dl despite administration of FFP. Platelet concentrates (PC) were administered when hemostasis was insufficient and platelet count was less than 50.10^9/L. Each PC contained five donor units (U) of platelets. Blood samples were drawn for repeated measurement of blood cell counts, chemistry, and coagulation during and after surgery. Aprotinin or other hemostatic drugs were not given. Thromboprophylaxis with subcutaneous nadroparine (2850 U) once daily was started after transplantation. Follow-up was completed at day 5 after surgery.

Study drug administration
An intravenous injection of 80 μg of rFVIIa/kg of bodyweight was administered within 10 min before the start of surgery, to be repeated if blood loss after the previous dose exceeded the patient’s estimated circulating volume. An interval of at least two hours was required between doses. rFVIIa was not to be given after the completion of bile duct anastomosis. This dose regimen was to be adapted if, in two patients, after rFVIIa administration clinically significant bleeding still occurred or thrombosis was observed. Plasma samples for measurement of FVIIa:C levels were collected at 0.5, 1, 2, 4, 6 and 8 hours after the first dose, and before any additional dose.

Laboratory measurements
Routine assays were used for blood cell counts, chemistry, PT (normal range 11-16 s), APTT (26-36 s), fibrinogen (170-350 mg/dl) and antithrombin (74-113%). Levels of FVIIa:C were measured as previously described (16).

Endpoints
Efficacy was primarily assessed by evaluation of perioperative (from start until 24 hours after the end of OLT) transfusion requirement of RBC, FFP, PC and FC. In addition, perioperative blood loss was estimated from blood collected in suction containers, autologous transfusion system and sponges during surgery and in drains after surgery. Measurement of blood loss was
considered less accurate than requirement of RBC transfusion to indicate true blood loss, because of dilution of fluid in suction containers by ascites and intraperitoneal hypersecretion in addition to practical difficulties. Safety was assessed by Doppler ultrasonographic examination of the hepatic vessels on days 1, 3 and 5 after surgery, followed by angiography if flow was not recorded by Doppler; by electrocardiography at 12 hours, 24 hours and 5 days after surgery; and by monitoring laboratory parameters, vital signs and adverse events.

Statistics
Each patient was matched with two controls. Continuous variables are presented by median (range); cases were compared with controls by the two-sample Wilcoxon test. Categorical variables are presented by frequencies and tested by the Fisher’s exact test. P values < 0.05 are considered statistically significant.

Results
Between December 1998 and September 1999, six eligible patients were enrolled in the study. Matched controls were transplanted between November 1997 and September 1999. The piggyback technique was applied in all study patients and in 5 of 12 controls; in the remaining seven control patients, a venovenous bypass was used. Baseline characteristics of both groups are summarized in Table 1. The proportion of patients who had previously undergone upper abdominal surgery, and values of hemoglobin, PT, APTT, fibrinogen, antithrombin and platelet count in study patients and controls were similar at baseline.

Efficacy
None of the patients received more than one dose of rFVIIa, as blood loss was less than the estimated circulating volume in all. Table 2 shows the transfusion requirements in patients and matched controls. Allogeneic RBC transfused were 1.5 (range, 0-5) and 7 (range, 2-18) U in study patients and controls, respectively (P = 0.006). Autologous RBC transfused were 0 (range, 0-2) versus 3.5 (range, 0-23) U (P = 0.043). The total amount of transfused RBC was 3 (range, 0-5) versus 9 (range, 4-40) U (P = 0.002). Autologous blood was returned in 2/6 study patients and 9/12 controls. Median transfusion requirements of FFP were 1 (range, 0-7) and 8
(range, 2-35) U in study patients and controls, respectively. Although study patients received less PC than controls, this difference was not statistically significant. Median usage of fibrinogen concentrate was 0 (range, 0-2) in both study patients and controls. Two of the six study patients received no blood products at all, whereas all controls required transfusions.

**Table 1 Baseline characteristics of study patients and controls**

<table>
<thead>
<tr>
<th></th>
<th>Study patients n=6</th>
<th>Controls n=12</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>43 [37-61]</td>
<td>48 [34-63]</td>
<td>0.74</td>
</tr>
<tr>
<td>Female sex, no. (%)</td>
<td>2 (33)</td>
<td>4 (33)</td>
<td>1</td>
</tr>
<tr>
<td>Child-Pugh, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3 (50)</td>
<td>6 (50)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3 (50)</td>
<td>6 (50)</td>
<td></td>
</tr>
<tr>
<td>Cholestatic liver disease, no. (%)</td>
<td>2 (33)</td>
<td>4 (33)</td>
<td>1</td>
</tr>
<tr>
<td>Previous upper abdominal surgery, no. (%)</td>
<td>1 (17)</td>
<td>2 (20)</td>
<td>1</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>10.1 [6.7-26.6]</td>
<td>12.9 [10.1-27.7]</td>
<td>0.15</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.8 [8.4-13.2]</td>
<td>11.6 [9.0-13.4]</td>
<td>0.81</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>21.8 [15.2-23.6]</td>
<td>19.0 [14.2-30.0]</td>
<td>0.51</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>38.5 [32.8-51.9]</td>
<td>38.0 [31.0-46.5]</td>
<td>0.61</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>170 [140-390]</td>
<td>230 [100-720]</td>
<td>0.64</td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>39 [27-69]</td>
<td>42 [20-109]</td>
<td>0.71</td>
</tr>
<tr>
<td>Platelet count (x10^3/mm^3)</td>
<td>78 [38-134]</td>
<td>60 [21-245]</td>
<td>0.62</td>
</tr>
<tr>
<td>Cold ischemia time (min)</td>
<td>481 [379-794]</td>
<td>669 [372-862]</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*Unless otherwise denoted, values are in median [range]. PT, prothrombin time; APTT, activated partial thromboplastin time*

The difference in estimated blood loss between study patients and controls (3.5 [range, 1.4-5.3] versus 9.8 [range, 3.7-35.0] L) was statistically significant (P = 0.004). The median postoperative hemoglobin level in the study group was 9.2 g/dl versus 9.1 g/dl in the control group. The median difference between individual pre- and postoperative hemoglobin levels was 2.2 g/dl in the study group and 2.7 g/dl in the controls. FVIIa clotting activity levels (FVIIa:C) are presented in Figure 1. At 8 hours after injection (mean 1 hour before the end of surgery), FVIIa:C levels in all patients were approaching baseline values. The course of fibrinogen levels, antithrombin levels, and platelet counts during OLT were similar in both
groups (data not shown). Operating time (median, 554 versus 598 minutes) was shorter in study patients but this difference was not statistically significant ($P = 0.26$).

**Table 2**  
Transfusion requirements in study patients and matched controls*

<table>
<thead>
<tr>
<th></th>
<th>Study patients (n = 6)</th>
<th>Controls (n = 12)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>total RBC (U)</td>
<td>3 [0-5]</td>
<td>9 [4-40]</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Allogeneic RBC (U)</em></td>
<td>1.5 [0-5]</td>
<td>7 [2-18]</td>
<td>0.006</td>
</tr>
<tr>
<td><em>Autologous RBC (U)</em></td>
<td>0 [0-2]</td>
<td>3.5 [0-23]</td>
<td>0.043</td>
</tr>
<tr>
<td>FFP (U)</td>
<td>1 [0-7]</td>
<td>8 [2-35]</td>
<td>0.011</td>
</tr>
<tr>
<td>PC (U)</td>
<td>0 [0-5]</td>
<td>5 [0-15]</td>
<td>0.24</td>
</tr>
<tr>
<td>FC (g)</td>
<td>0 [0-2]</td>
<td>0 [0-2]</td>
<td>0.63</td>
</tr>
</tbody>
</table>

*Values in median [range]. RBC, red blood cell concentrates; FFP, fresh frozen plasma; PC, platelet concentrate; FC, fibrinogen concentrate.

**Figure 1**  
Levels of factor VIIa activity after administration of 80µg/kg rFVIIa, in individual patients.
Safety
In the fourth patient, Doppler ultrasonography at day 1 after surgery did not detect flow in the hepatic artery. Thrombosis of the proper hepatic artery was confirmed by angiography. Thrombectomy was performed, followed by local infusion of urokinase and intravenous infusion of heparin. A repeat angiography two days later showed patency of proper and right hepatic artery, but occlusion of left hepatic artery. Graft function remained intact during the study period without further intervention. No other thrombotic events or other study drug-related adverse events occurred.

Discussion
Our data demonstrate a remarkable reduction in transfusion requirements of OLT patients who received a single dose of 80 µg/kg rFVIIa just before surgery. Compared to a group of matched controls, their need for total RBC transfusions decreased from median 9 to 3 U (67% reduction).

This difference was not caused by a restrictive transfusion policy in this non-comparative open label study, as the difference between pre- and postoperative hemoglobin was not larger in the study patients than in the controls. Improved hemostasis in the study patients by more infusion of coagulation factors and platelets cannot explain the observed difference in transfusion requirements either. On the contrary, overall transfusion requirements including plasma (components) were significantly lower in study patients, whereas baseline values of coagulation parameters were comparable in both groups.

Patients were matched to controls for variables that were shown to be independent predictors of transfusion requirements in a recent retrospective analysis in our center (15). Patients and controls had the same Child-Pugh classification, and were matched for urea nitrogen within 8 mg/dl. Controls were transplanted no more than one year before the study patients. Study patients were not matched to controls for the use of autologous blood, although this might be a predictor of increased transfusion requirements (15): most study patients simply did not lose enough blood to wash and return to them. The minimal blood loss is therefore the cause rather than the result of not using autologous blood. The piggyback technique was used in all 6 study patients, compared to five of 12 controls. There has been one report of lowered transfusion
requirements with this technique, but other studies have not shown this to be the case (17-19). We did not match for surgical technique, as this variable was not tested in our previous study (15). In our series of 12 controls, RBC requirements were similar with both techniques (allogeneic 7 versus 7 U, autologous 4 versus 2 U).

FVIIa binds to tissue factor that is exposed by subendothelial tissue. This complex induces thrombin generation (20). Debate still continues whether tissue factor exposed on activated platelets plays an additional role (21,22). The necessity of subendothelial tissue factor ensures a localized effect of rFVIIa, without systemic activation of coagulation.

Considering the localized effect, diffuse intravascular coagulation or deep vein thrombosis as a side effect of rFVIIa is neither expected nor has been observed (10). However, in OLT, extensive endothelial injury with expression of tissue factor is present within the hepatic sinusoids as a result of ischemia/reperfusion injury (23), and at the sites of vascular anastomosis. Therefore, even in the absence of rFVIIa or any other prohemostatic drug, thrombosis of the hepatic vessels is a recognized complication, occurring in about 5% of adult OLT patients (7-9). To minimize the risk of thrombosis at sites of vascular anastomosis, we decided to administer rFVIIa with intervals of at least two hours (the half life in hemophilia patients who are treated for a bleeding is 2.30 hr) (24), and not after the construction of the anastomosis of the bile duct. After the single dose that all patients received, plasma levels of FVIIa:C returned to almost baseline before the end of surgery, which renders an increased risk of postoperative thrombosis less likely. A prolonged effect of rFVIIa cannot be precluded from these data, however, as non-detectable levels of FVIIa can still be pharmacologically active. Furthermore, fibrinolysis may have been inhibited through a thrombin-activable fibrinolysis inhibitor that is activated by the relatively large amounts of thrombin generated in the presence of rFVIIa (25). We did not observe any systemic thrombotic complication, whereas frequent Doppler ultrasonography after OLT showed patent hepatic vessels in five of six patients. One patient, however, did experience hepatic artery thrombosis. Although this may be explained by chance, we would not recommend a higher dose or dose frequency in subsequent studies either, as an increased incidence of thrombosis would clearly be unacceptable. Indeed, the observed efficacy of a single 80 μg/kg dose provides no reason for increasing the dose. The combination of rFVIIa with aprotinin should be discouraged in
further studies, until the safety of rFVIIa has been established. In conclusion, a single dose of 80 µg/kg rFVIIa significantly reduced transfusion requirements during orthotopic liver transplantation. The presented data call for a prospective, placebo-controlled study to assess the optimally effective and safe dose of rFVIIa in orthotopic liver transplantation.
References