



# Human extrahepatic portal vein obstruction correlates with decreased factor VII and protein C transcription but increased hepatocyte proliferation

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## Abstract

**Purpose:** A 3-year-old girl developed extrahepatic portal vein obstruction (EHPVO) after a liver transplant. She had sequelae of portal hypertension that required another transplantation. The circumstances allowed for comparison of liver-dependent coagulation factor production between the second donor liver and the explanted liver with EHPVO.

**Methods:** Liver samples from the explanted first graft and the second transplant were obtained. Fresh tissue was used to perform reverse transcription–polymerase chain reaction with primers against factors V, VII, as well as VIII, protein C, and paraffin-embedded sections for hepatocyte proliferation using Ki-67 antibody as well as for apoptosis using TUNEL assay.

**Results:** The transcription of factor VII and that of protein C were decreased in the explant as compared with the newly transplanted liver (factor VII, 77% of the donor; protein C, 88% of the donor). The transcription of factor V and that of factor VIII were unchanged. The explant had a greater percentage of proliferating hepatocytes than the new organ ( $0.85\% \pm 0.75\%$  vs  $0.11\% \pm 0.21\%$ ). The percentage of apoptotic cells was similar between the 2 livers ( $0.09\% \pm 0.13\%$  vs  $0.09\% \pm 0.13\%$ ).

**Conclusions:** Idiopathic EHPVO is associated with a reduction in liver-dependent coagulation factor transcription and an increase in hepatocyte proliferation. Portal blood flow deprivation alters hepatic homeostasis and initiates mechanisms that attempt to restore liver-dependent coagulation factors.

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Extrahepatic portal vein obstruction (EHPVO) causes chronic portal hypertension in children [1]. Despite having a near-normal liver histology, patients with EHPVO have a reversible decrease in serum levels of liver-dependent coagulation factors [2]. Deprivation of portal blood flow appears to alter normal liver synthetic function, resulting in

diminished production by the liver of coagulation factors. In this report, we describe the case of a patient who developed EHPVO after a liver transplant and subsequently received a second transplant to correct the portal hypertension. This unique situation lent itself to a comparison with what we had previously only been able to do in a laboratory setting: testing the hypothesis that EHPVO causes a reversible decrease in the synthesis of liver-dependent coagulation factors.

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## 1. Methods

### 1.1. Case history

A 3-year-old girl with biliary atresia underwent her original transplantation before she was 1 year old for liver failure after a failed Kasai procedure. The transplant was performed at an outside institution with a live donor. The portal vein was occluded shortly after the first transplant. The patient began having gastrointestinal bleeding when she was 2 years old and was treated by banding and sclerotherapy. She was referred to our institution for intractable gastric, esophageal, and intestinal bleeding.

At our institution, a mesorex bypass was attempted, but it failed because of the patient's diseased intrahepatic portal vein. The histology of the liver was normal, with no evidence of rejection or fibrosis. The patient then underwent a second transplant with the left lobe of a 10-year-old child. An iliac vein graft from the donor was anastomosed to the patient's superior mesenteric vein to supply the transplanted liver. The right lobe was not suitable for transplantation. However, it served to provide normal liver tissue that could be compared biochemically for evidence of coagulation factor synthesis with the explanted liver that was removed from the patient.

The recipient did well after the transplant, and her portal hypertension symptoms resolved. Before her second liver transplant, her prothrombin time (PT) ranged from 17.6 to 21.9 seconds, and her liver function panel was within the reference range. After the second transplant, her PT normalized to 15.5 seconds. The donor also had a normal PT level 12.1 to 14.8 seconds before the organ harvest.

### 1.2. Reverse transcription–polymerase chain reaction

Liver tissue collected at the end of the operation was used to harvest total RNA using a protocol recommended by the manufacturer of the RNeasy Mini (Qiagen, Valencia, Calif), and the concentration of RNA was determined with a spectrophotometer. An RNA polymerase chain reaction (PCR) core kit (Applied Biosystems, Foster City, Calif) was used to perform reverse transcription–PCR. The conditions and primers used in the various PCRs are as follows:

factor V: forward primer, 5'-AAACTTGCAGCA-GAATTTGC-3'; reverse primer, 5'-ACTCTGTGGTATAG-CAGGACTTCA-3'; annealing temperature, 56°C; 35 cycles;  
factor VII: forward primer, 5'-CCTTCATTGCTGGAGACAGT-3'; reverse primer, 5'-TGTGCATCTGTGTGTGCATA-3'; annealing temperature, 55°C; 27 cycles;  
factor VIII: forward primer, 5'-ACTCGTACTACTCTT-CAGTCA-3'; reverse primer, 5'-GAACATGTGGGGAGC-TACTCA-3'; annealing temperature, 54°C; 35 cycles; and

protein C: forward primer, 5'-TAACAAGCACACCCGGCCT-3'; reverse primer, 5'-TAAGGCATGTGACATACAACAGG-3'; annealing temperature, 56°C; 35 cycles.

The PCR products then underwent gel electrophoresis with the use of 1.5% agarose in 1× Tris-acetate-EDTA buffer. GAPDH was used as a loading control.

### 1.3. Hepatocyte proliferation and immunohistochemistry

Formalin-preserved liver specimens were sectioned and embedded in paraffin blocks. Standard immunohistochemistry techniques were used to stain the liver sections with antibodies against Ki-67 (Dako, Carpinteria, Calif) for proliferation. After the staining, each liver section was divided into 4 quadrants, and 2 random visual fields at 400× were selected from each quadrant. The percentage of positively stained hepatocytes in all 8 visual fields was tabulated, and the mean ± 1 SD was calculated for each liver section.

### 1.4. Hepatocyte apoptosis

Liver specimens in paraffin blocks were used, and the protocol recommended by the TUNEL assay manufacturer (In Situ Cell Death Detection Kit, TMR Red, Roche Applied Science, Indianapolis, Ind) was followed. After the TUNEL assay treatment, the sections were viewed using a fluorescent microscope with a rhodamine filter, and the mean percentage ± 1 SD of positively stained cells for each liver section was evaluated as described.

### 1.5. Institutional review board

This study was approved by the review board of our institution (IRB No. 12729).

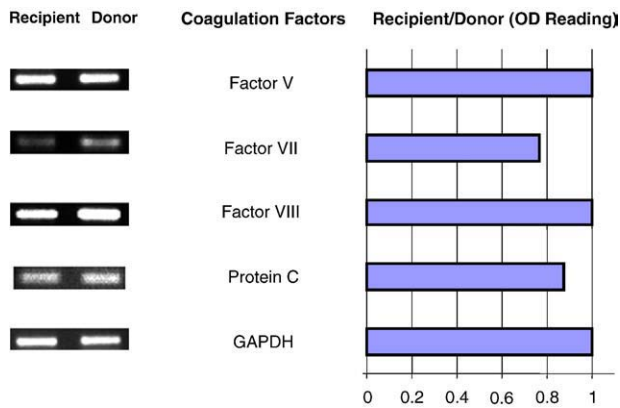
## 2. Results

### 2.1. Reverse transcription–PCR

The DNA transcription of factor VII and that of protein C were decreased in the recipient's explanted liver as compared with the donor's liver (factor VII, 77% of the donor; protein C, 88% of the donor). The DNA transcription of factor V and that of factor VIII were similar between the 2 livers (Fig. 1).

### 2.2. Hepatocyte immunohistochemistry

The mean percentage of proliferating cells in the recipient's explanted liver was markedly increased as



**Fig. 1** Reverse transcription–PCR results for factors V, VII, as well as VIII and protein C. GAPDH was used as a loading control. The optical density (OD) reading of each band was used to calculate the recipient/donor ratio.

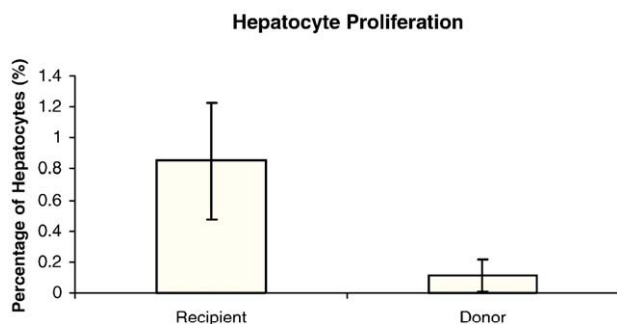
compared with that of the donor's liver ( $0.85\% \pm 0.75\%$  vs  $0.11\% \pm 0.21\%$ ) (Fig. 2).

### 2.3. Hepatocyte apoptosis

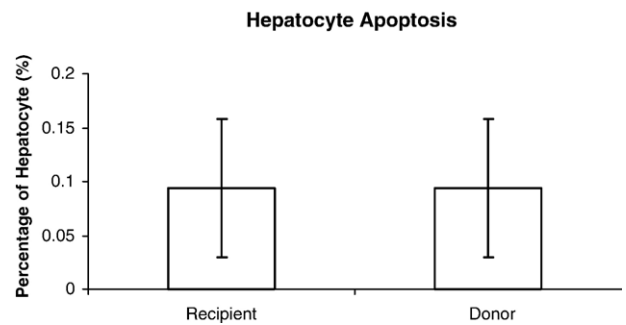
The mean percentage of apoptotic cells in the recipient's explanted liver was similar to that in the donor's liver ( $0.09\% \pm 0.13\%$  vs  $0.09\% \pm 0.13\%$ ) (Fig. 3).

## 3. Discussion

Portal vein obstruction has been clinically associated with a reversible decrease in liver-dependent coagulation factor levels. A rat model of EHPVO has been used in our laboratory to study the mechanism by which EHPVO results in the decrease in levels. Our preliminary results indicate that a decrease in transcription rather than an increase in consumption is responsible for the observed change in factor levels. Similar planned studies of human beings would be impossible to perform. This case provided us with a unique



**Fig. 2** Percentage of proliferating hepatocytes. Eight readings were taken from each patient. The error bar represents 1 SD.



**Fig. 3** Percentage of hepatocytes in apoptosis. Eight readings were taken from each patient. The error bar represents 1 SD.

set of circumstances that allowed us to compare coagulation factor DNA transcription in a normal donor liver with that in the recipient's first transplanted liver that was normal, except for the portal vein obstruction. Because only half of the donor's liver was used, ample tissue was available to carry out further testing. We were able to demonstrate for the first time in a human setting simultaneous factor-level transcription analysis between a liver with EHPVO and a normal control liver.

We found that the liver deficient in portal blood flow had a cell proliferation rate approximately 8 times higher than that in the healthy transplanted liver. This observation has also been demonstrated in our rodent models (personal unpublished data). Kahn et al [3] showed that rats with portal hypertension and diminished portal blood flow had a normal capacity to regenerate hepatic mass after hepatic resection. Uemura et al [4] also reported that after partial portal vein ligation, DNA synthesis in rats was significantly increased in the nonligated as compared with the ligated hepatic lobe.

However, we found no change in the percentage of apoptotic cells between the healthy transplanted liver and the liver deprived of portal blood flow. This clinical observation also corroborated with our findings using a rodent EHPVO model [5]. Rodent liver 3 months after the deprivation of portal blood flow also had rates of apoptosis similar to those in the control liver (personal unpublished data).

The decreased levels of factor VII and protein C transcription also corroborate our clinical findings and may also be the mechanism that leads to lower serum levels of liver-dependent coagulation factors as reported in our clinical experience [2]. These are restored to normal levels 1 year after successful restoration of portal flow in children with idiopathic EHPVO.

The observation in this report and in our laboratory experience with the rat of an increase in hepatocyte proliferation after EHPVO is an interesting one. It suggests that once portal flow is diminished, repair mechanisms may be initiated. However, the mechanisms appear to be incomplete in restoring the hepatocyte mass to normal perhaps because of the lack of sufficient portal flow and a

reduction in hepatotropic factors and nutrients that the portal vein brings to the liver. Despite having increased hepatocyte proliferation, patients with deficient portal blood flow still had decreased production of both factor VII and protein C. This suggested that the liver might initiate changes to compensate for the decreased portal blood flow, but the synthetic function might not fully return to normal. The direct comparison between these 2 livers supports this hypothesis.

Deprivation of portal blood flow could initiate complex changes within the liver. Further studies are needed to understand the regulatory mechanisms that control synthesis of coagulation factors in the liver and how EHPVO results in a disruption of liver homeostasis.

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