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

Bill Chiu[a], Hector Melin-Aldana[b], Riccardo A. Superina[a,*]

[a]*Department of Surgery, Children's Memorial Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL 60614, USA*
[b]*Department of Pathology, Children's Memorial Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL 60614, USA*

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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% ± 0.75% vs 0.11% ± 0.21%). The percentage of apoptotic cells was similar between the 2 livers (0.09% ± 0.13% vs 0.09% ± 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.

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.

* Corresponding author. Tel.: +1 773 883 6187; fax: +1 773 975 8534.
E-mail address: RSuperin@childrensmemorial.org (R.A. Superina).

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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′-AAAACTTGCAGCAGAATTTGC-3′; reverse primer, 5′-ACTCTGTGGTATAGCAGGACTTCA-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′-ACTCGTACTACTCTTCAGTCA-3′; reverse primer, 5′-GAACATGTGGGGAGCTACTCA-3′; annealing temperature, 54°C; 35 cycles; and
protein C: forward primer, 5′-TAACAAGCACACCGGCCT-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