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CASE REPORT

Treatment of antibody-mediated rejection with high-dose immunoglobulins in ABO-incompatible liver transplant recipient

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Summary

ABO-incompatible liver transplantation (LT) entails high risk of antibody-mediated rejection (AMR) and poor graft survival. Different treatment modalities have been reported, but none with use of a 2-week course of high-dose polyclonal i.v. immunoglobulins (IVIg) associated with plasmapheresis without the use of steroid pulses or monoclonal antibody. A 60-year-old male patient with blood-group O, Caucasian, underwent urgent LT for acute liver failure after hepatectomy for HCV-related hepatocellular carcinoma. He was grafted with a 66-year-old, blood-group A, HCV-positive liver graft. Pretransplant conditioning consisted of plasmapheresis and immunosuppression was triple with tacrolimus (TAC), steroids, and mycophenolate mofetil with anti-IL2-R monoclonal antibodies, plasmapheresis if hemagglutinin level >1:8, and extracorporeal photopheresis. After reduction of liver function tests to baseline, the patient presented a tenfold increase in alanine aminotransferases (ALT) levels 7 days post-transplantation. AMR was confirmed on histology. Treatment consisted of IVIg (1.5 g/Kg/daily for the first 7 days, and 1 g/Kg/daily from day 8 to 14) with a 14-day course of plasmapheresis. No side effect was observed and daily blood IgG levels ranged between 24.4 and 36.4 g/l. At the end of the scheduled course ALT returned to baseline. A control liver biopsy 55 days after LT showed no rejection and replacement of necrosis with fibrous strands. This case

Introduction

Liver transplantation (LT) across the ABO barrier is associated with a high risk of antibody-mediated rejection (AMR), poor patient and graft survival, and a high risk of vascular thrombosis and ischemic bile duct complications [1]. Recently, significant improvements in patient and graft survival rates have been reported through the use of novel immunosuppressive modalities, such as total plasma exchange, splenectomy, hepatic perfusion, and the use of anti-CD20 monoclonal antibodies [2–3]. We report on a case of AMR in ABO-incompatible liver transplant recipient treated with high-dose i.v. immunoglobulins (IVIg).

Case report

A blood-group-O-positive, 60-year-old, Caucasian male with diagnosis of hepatocellular carcinoma (HCC) in the setting of hepatitis C virus (HCV)-related cirrhosis (viral genotype 2a/2c) developed acute liver failure (ALF) after right hepatectomy. ALF presented with relentless jaundice,

ascites, bacterial translocation and encephalopathy. Histology of the surgical specimen showed a grade I–II, 9-cm HCC with satellite nodules, vascular microinvasion and clear resection margins. The patient was transplanted 16 days after surgery with a 66-year-old, blood-group-A, HCV-positive, male donor liver graft after a single course of plasmapheresis. Post-transplant immunosuppression consisted of a 14-day course of plasmapheresis when hemagglutinins >1:8; extracorporeal photopheresis (ECP); anti-IL2-receptor monoclonal antibodies (basiliximab, Simulect™; Novartis, Basel, Switzerland) 20 mg/day on day 1 and 4; steroids; tacrolimus, and mycophenolate mofetil (MMF). Graft function recovered immediately after transplantation. Fig. 1 shows the hemagglutinin level during the early post-transplant course and its correlation with serum ALT. Hemagglutinin ranged from as low as 1:256 on post-transplant day 1 to as high as 1:1056 on day 6, while ALT returned to baseline within post-transplant day 6 (64 UI/ml). On day 7 the patient presented a nearly tenfold increase in ALT. Biopsy of the liver graft was consistent with humoral rejection (Fig. 2) and showed the presence of fibrin deposits and neutrophils in portal venules and sinusoids, vascular congestion and diffuse coagulative necrosis of hepatocytes. Immunofluorescence and immunohistochemistry showed IgG deposits on sinusoids (Fig. 3a) and C3 deposits on vascular endothelium (Fig. 3b). To reduce side-effects of high-dose immunosuppression, the patient was started on class G polyclonal IVIg (Sandoglobulin™; ZLB Behring AG, Bern, Switzerland), with no further drug adjustment, but with prosecution of both plasmapheresis and ECP. We decided not to use steroid boluses or anti-CD20 monoclonal antibodies due to the patient's poor conditions and administered IVIg at a dosage of 1.5 g/kg

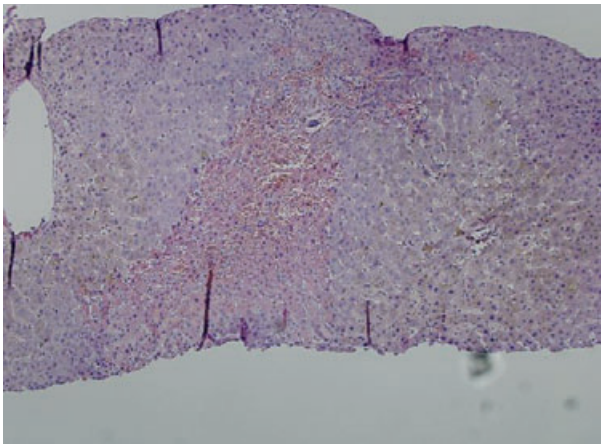

Figure 2 Liver biopsy performed on post-transplant day 7. The optic microscopy shows fibrin deposits and neutrophils in portal venules and sinusoids, vascular congestion and diffuse coagulative necrosis of hepatocytes (hematoxylin and eosin stain, 100×).

for the first 7 days and 1 g/kg during the following week. IVIg were administered at the end of each plasmapheresis. Median IVIg serum level during the whole scheduled course was 33.4 g/l (range 24.4–36.4) with no side effects. Two weeks after IVIg withdrawal the serum IgG level was 23 g/l and dropped to 13.1 g/l 21 days 3 weeks after completion of IVIg administration. Upon IVIg withdrawal ALT were within normal range despite high hemagglutin levels (between 1:1024 and 1:4096). As for HCV kinetics, in the early post-transplant course blood viremia rose to 3.5×10^6 U/ml on post-transplant day 7, whereas it fell to 7×10^5 U/ml on completion of the IVIg scheduled course. On post-transplant day 41, due to increase in total bilirubin (28 mg/dl), the patient

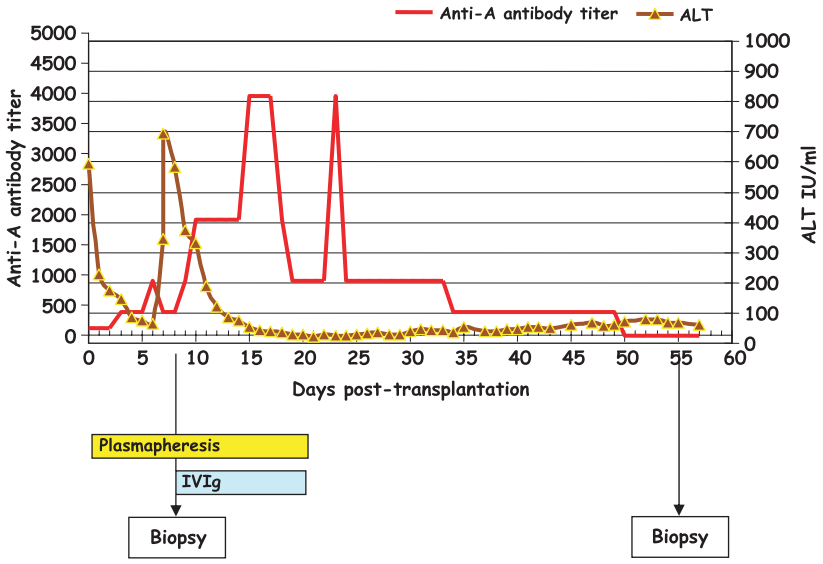

Figure 1 Hemagglutinin levels and ALT in the post-transplant course. Hemagglutinins rose despite daily plasmapheresis from 1:256 on post-transplant day 1 to 1:1024 on day 6. Liver function test returned to baseline by day 6 (ALT 64 IU/ml). On day 7 a steep increase in ALT was observed (693 IU/ml). Liver biopsy is consistent with antibody-mediated rejection and i.v. immunoglobulins are administered for 14 days. A trans-tube cholangiography performed on day 41 showed normal biliary tree. A control biopsy on day 55 showed replacement of necrosis with fibrous strands.

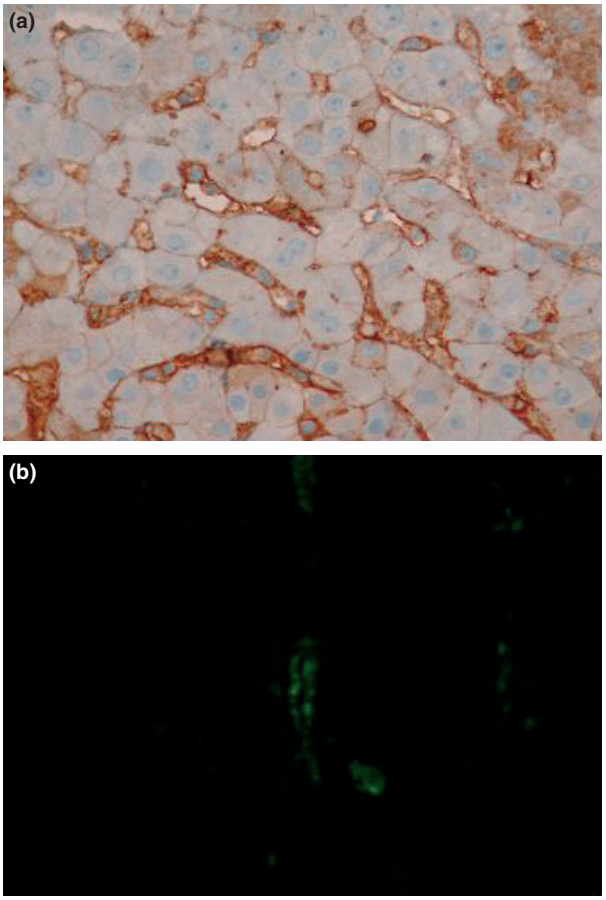

Figure 3 (a) Liver biopsy performed on post-transplant day 7. Immunohistochemistry on paraffin section demonstrating IgG deposits in sinusoids by anti-human IgG rabbit polyclonal antibodies (Nexes Ventana Medical System, Tucson, AZ, USA) (400×). (b) Liver biopsy performed on post-transplant day 7. The frozen section incubated with rabbit anti-human C3 complement/FITC (Dako A/S, Glostrup, Denmark) shows C3 deposits on vascular endothelium (400×).

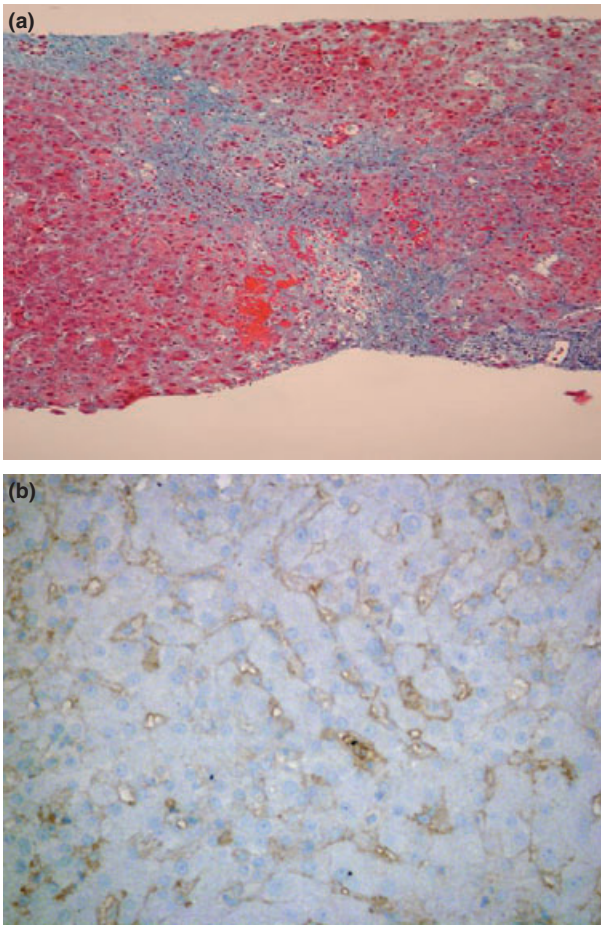

Figure 4 (a) Liver biopsy performed on post-transplant day 55. The optic microscopy shows resolution of humoral rejection with replacement of necrotic areas by fibrous strands (Masson trichrome, 100×). (b) Liver biopsy performed on post-transplant day 55. The immunohistochemistry on paraffin sections demonstrates dramatic reduction of IgG deposits in sinusoids by anti-human IgG rabbit polyclonal antibodies (Nexes Ventana Medical System, Tucson, AZ, USA; 400×).

underwent trans-T-tube cholangiography which showed normal intra- and extra-hepatic biliary tree. Despite wide-spectrum antibiotic prophylaxis, bile culture tested positive for multi-resistant *Pseudomonas* and *Serratia* spp. and both MMF and tacrolimus were tapered. Due to persistence of elevated bilirubin, a repeat liver graft biopsy was performed 55 days after transplantation (embedded in paraffin) and showed resolution of humoral rejection with replacement of necrotic areas by fibrous strands (Fig. 4a), while immunohistochemistry showed dramatic reduction of IgG deposits (Fig. 4b). A repeat trans-T-tube cholangiography was performed on day 58 and showed findings consistent with ischemic-type biliary lesion (ITBL). No arterial thrombosis was detected on Doppler-US. The patient underwent retrograde cholangio-pancreatography for treatment of ITBL, but died on day 67 post-transplantation of gram-negative sepsis and consequent multiorgan failure.

Discussion

Despite the unfavorable outcome and the occurrence of rejection-related ITBL, the present case may pave the way to a novel approach for ABO-incompatible LT based on the following:

1 Plasmapheresis alone was unable to reduce hemagglutin level; furthermore, administration of fresh plasma during the procedure might worsen humoral rejection by providing factors of the complement cascade pathway.
2 Rejection was treated with IVIg in association with plasmapheresis. IVIg have shown immunomodulatory

properties and have been employed for treatment of autoimmune diseases [4]. They were also used successfully in two cases of hyperacute rejection in ABO-incompatible LT at a dosage of 0.5 g/kg for 5 days in association with anti-CD20 monoclonal antibodies and steroid boluses [5]. The protocol of IVIg dose was based on the evidence of a dose–effect relationship in the treatment of hyperacute rejection in xenotransplantation [6] and on the observation that 14 days are required on average to achieve host-to-graft accommodation in the presence of elevated anti-graft antibody titers [2]. To the best of our knowledge this is the first report of use of high-dose IVIg for such a long period in solid organ transplant recipients and without additional immunosuppressive therapy (steroid pulses or monoclonal antibodies). However, blood IgG levels were not higher than the levels achieved in the setting of long-standing chronic diseases.

3 Accommodation was achieved despite increase in hemagglutinin level up to 1:4096, as testified by normal ALT and liver reserve function (PT) and the fact that the liver graft biopsy performed on post-transplant day 55 excluded rejection. The eventual decrease in hemagglutinin levels observed starting on day 25 was likely to be accounted for by IVIg-induced clonal exhaustion of antibody-producing lymph cells. Accordingly, consequent to the clonal exhaustion was likely to be the dramatic reduction of IgG deposits in hepatic sinusoids.

4 However, in the present case IVIg were unable to prevent ITBL which may be accounted for by immunological mechanisms and was the result of humoral rejection. Immunohistochemistry of the biopsy samples did not prove evidence of direct damage to the biliary epithelium, while antibody-complement deposits were only observed in the vascular endothelium. The hypothesis of direct antibody-induced damage to the biliary epithelium cannot be excluded, as reported by previous authors [7]. However, the most credited mechanism responsible for biliary damage is dependent upon alterations of microcirculation. Whether IVIg might turn useful in preventing biliary lesions occurring in the setting of immunological, infectious and ischemic lesions is an area that deserves further investigation.

5 Collaterally, it is worth noting that IVIg may play a role in modifying HCV post-transplant viral kinetics. We may speculate that IVIg played a role in HCV clearance in the post-transplant course.

The value of the present case relies in the histological confirmation of the role of IVIg in blocking the cascade pathway leading to the vascular damage consequent to AMR. However, IVIg seem to be ineffective in treating biliary complications once AMR has established. Therefore, in the past we used a combination of pretransplant plasmapheresis (irrespective of hemagglutin level) associated with a 14-day course after transplantation if hemagglutinins were >1:8. Currently, we use routine pretransplant plasmapheresis and IVIg (1 g/Kg) after graft reperfusion and boost IVIg levels at the end of each plasmapheresis, whenever plasmapheresis is required in the post-transplant course. Prevention of acute cellular rejection is achieved with calcineurin-based triple immunosuppression and immunomodulation with ECP [8]. Use of ECP was meant to prevent acute cellular rejection, based on our previous experience in a patient affected with fulminant hepatic failure and treated with ABO-incompatible LT [8]. Currently, there is no proven evidence on the role of ECP in prevention/treatment of AMR.

Based on the present experience, a regimen with IVIg is under ongoing assessment at our center and it may provide favorable graft survival rates, with no need for splenectomy, hepatic drug infusion or monoclonal antibodies.

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