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CASE REPORT

Portal vein thrombosis after intraportal hepatocytes transplantation in a liver transplant recipient

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Summary

Hepatocytes transplantation is viewed as a possible alternative or as a bridge therapy to liver transplantation for patients affected by acute or chronic liver disorders. Very few data regarding complications of hepatocytes transplantation is available from the literature. Herein we report for the first time a case of portal vein thrombosis after intraportal hepatocytes transplantation in a liver transplant recipient. A patient affected by acute graft dysfunction, not eligible for retransplantation, underwent intraportal infusion of 2 billion viable cryopreserved ABO identical human allogenic hepatocytes over a period of 5 h. Hepatocytes were transplanted at a concentration of 14 million/ml for a total infused volume of 280 ml. Doppler portal vein ultrasound and intraportal pressure were monitored during cell infusion. The procedure was complicated, 8 h after termination, by the development of portal vein thrombosis with liver failure and death of the patient. Autopsy showed occlusive thrombosis of the intrahepatic portal vein branches; cells or large aggregates of epithelial elements (polyclonal CEA positive), suggestive for transplanted hepatocytes, were co-localized inside the thrombus.

Introduction

Novel strategies, such as hepatocytes transplantation, aimed at reducing the growing disparity between the number of livers and the disproportionately large number of patients awaiting hepatic transplantation have been successfully studied and tested in preclinical animal models [1–2] and recently applied in humans [3–6]. Hepatocytes transplantation has several practical and theoretical advantages over whole organ or partial liver transplantation, such as cryopreservation of the cells for later use in emergencies or scheduled; a single donor could potentially provide hepatocytes for more than one recipients and cell transplantation should not interfere with subsequent liver transplantation or liver directed gene therapy. Moreover the minimally invasive characteristic and the presumed low morbidity, mortality and reversibility of hepatocytes transplantation has been largely advocated as main advantages of over whole organ or partial liver transplant [7]. However, clinical human hepatocytes transplantation is still in its early infancy, as only isolated small series and case reports, and no controlled clinical trials have been reported in the literature to date [3–6]. Due to the very limited clinical experience in this field, reports focusing on morbidity and mortality of hepatocytes transplantation are lacking and several works have

studied this problem only in animal models [8]. In this paper we report a case of portal vein thrombosis after hepatocytes transplantation in a liver transplant recipient.

Case report

A 56-year-old white male affected by alcoholic cirrhosis who had undergone primary transplantation and retransplantation for primary non function, developed severe graft dysfunction characterized by coagulopathy (INR = 3.23), hyperbilirubinaemia (37.7 mg/dl) and hyperammonaemia 20 days after retransplantation. A transjugular liver biopsy showed 70% necrosis of the graft. AST and ALT, which had been markedly elevated after retransplantation, were within normal range. Patient presented anuric in acute renal insufficiency requiring haemodialysis. Infection of the upper airway due to *Pseudomonas aeruginosa* was diagnosed. The patient was not judged eligible for a third liver transplant given the high risk of perioperative death, acute renal failure and *P. aeruginosa* infection. In order to try to stimulate the regeneration of the residual normal parenchyma and improve the function of the graft we decided to perform human hepatocytes transplantation. Informed consent was obtained from the relatives of the patient. The route of human hepatocytes infusion was the main trunk of the portal vein through a percutaneously placed catheter (7 French of diameter) [Fig. 1]. A second catheter was placed inside the lumen of the 7 French catheter to monitor [Fig. 1] portal vein pressure during infusion of the cells. Portography revealed a stenosis of the recipient portal vein, with a pressure gradient of 10 mmHg, that was treated by pneumatic balloon dilatation resulting in improvement of the pressure gradient to 5 mmHg [Fig. 1]. Viable and functioning cryopreserved human hepatocytes were obtained from the National Bank of Human Hepatocytes [9]. Viability of the hepatocytes, measured by trypan blue dye exclusion test, after thawing was 50%. Dead hepatocytes were removed by centrifugation at 50 ***g*** for 5 min; finally, after centrifugation, 2×10^9 viable cryopreserved ABO identical human hepatocytes were infused inside the portal vein over a period of 5 h. Immunosuppression regimen consisted of steroids (500 mg i.v. before hepatocytes infusion) plus tacrolimus (target level 10–15 ng/ml already achieved in a previously transplanted patient). Hepatocytes were transplanted at a concentration of 14 million/ml for a total infusion volume of 280 ml, separated in four times at 30 min interval. The cells were infused at a flow rate of 60 ml/h by a syringe pump. Portal pressure was continuously monitored during infusion; mean basal value was 27 mmHg. A basal Doppler ultrasound showed a mean portal vein flow of 20 cm/s. A Swan-Ganz catheter was placed for pulmonary pressure monitoring during hepatocytes infusion: mean basal value was 30 mmHg. Portal pressure increased to 40 mmHg 30 min after initiation of hepatocytes infusion and simultaneously portal vein flow, measured by Doppler ultrasound, decreased to 10 cm/s. Thereafter, portal pressure and portal flow showed only minimal variations during the entire period of infusion; the maximal value of portal pressure measured was 46 mmHg and the minimal value of portal flow was 9 cm/s. Pulmonary pressure steadily increased to a maximal value of 45 mmHg at the end of the procedure. PaO_2 saturation was maintained greater that 95% throughout the period of infusion except for the last 30 min when the value fell to 90%. Eight hours after termination of hepatocytes infusion pulmonary pressure and PaO_2 saturation returned to basal value: radiographic signs of pulmonary embolism were not present. Portal pressure decreased to 30 mmHg 8 h after discontinuation of cell infusion. However, hepatic colour-Doppler ultrasound performed the morning after the procedure revealed portal vein thrombosis, the hepatic artery and veins were patent with normal resistive index and triphasic flow respectively. A portography performed through the percutaneous catheter confirmed thrombosis of the right and left portal vein branch while the common portal vein was patent [Fig. 2]. Local thrombolysis with urokinase (100 000 UI bolus + 50 000 UI/h for a total dosage of 300 000 UI) infused through the percutaneous portal catheter was performed. Continuous local heparin infusion was also carried out maintaining a PTT greater

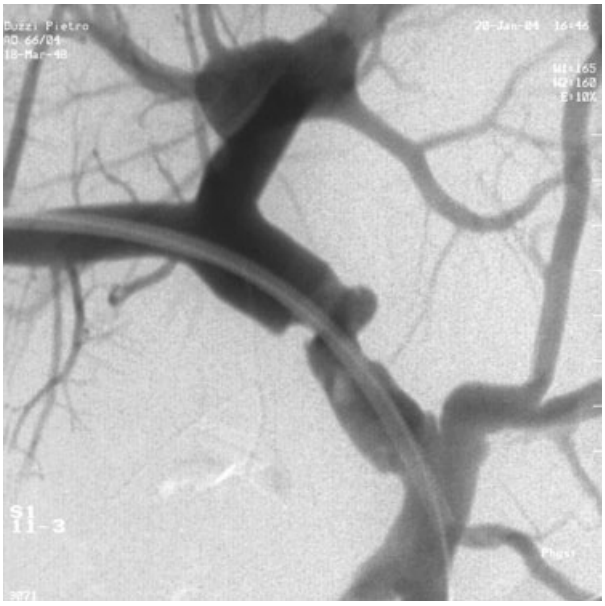

Figure 1 Percutaneous portography showing the 7 French catheter placed inside the common trunk of the portal vein for hepatocytes infusion.

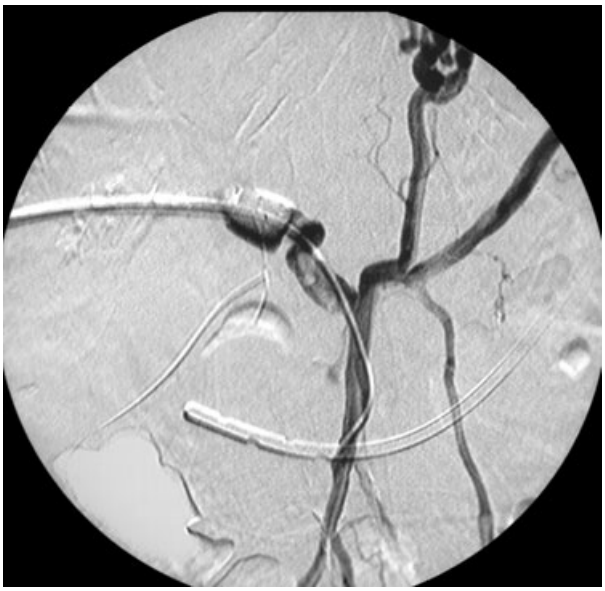

Figure 2 Percutaneous portography showing thrombosis of the right and left portal vein branch; the common portal vein was patent.

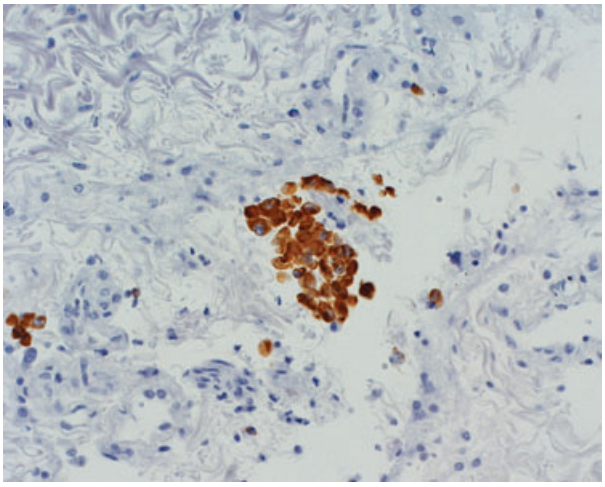

Figure 3 Lung histology (20×) showing small subpleural cells aggregate with evident reactivity for epithelial marker (CK 8).

than 50 s. AST and ALT during intraportal hepatocytes transplantation had a progressive increase (from normal value pre-infusion to 625 U/l and 253 U/l, respectively, at the end of the procedure). Platelets counts remained stable. Thirty-six hours after hepatocytes transplantation and 24 h after thrombolytic therapy a colour-doppler ultrasound showed a partial recanalization of the right trunk of the portal vein, while the left branch was still closed. The patient, however, developed progressive liver failure with AST, ALT and LDH increasing up to 4000 and 3100 UI and 13 000 UI, respectively, and died 48 h after

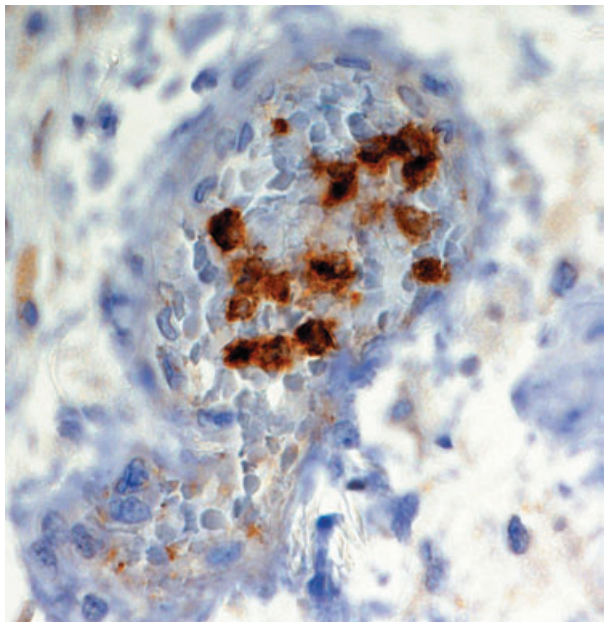

Figure 4 Lung histology (20×) showing epithelial cells, inside a subpleural vascular lumen, reactive for polyclonal CEA exclude their mesothelial nature.

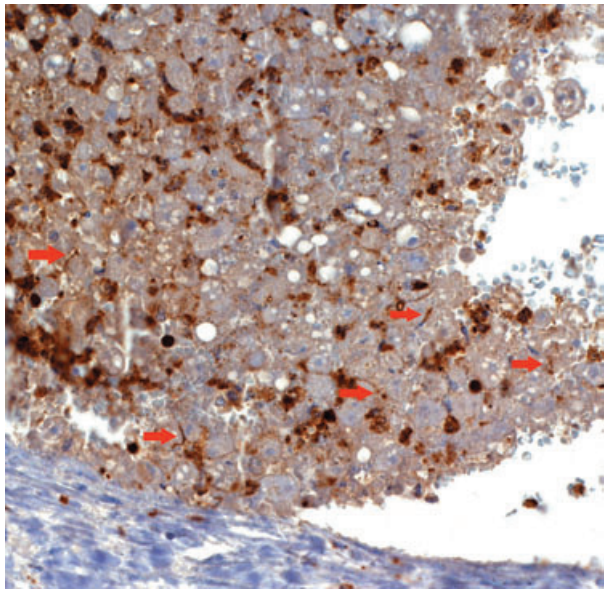

Figure 5 Liver histology (20×) showing large epithelial cells group inside a portal channel; red arrows show canalicular reactivity for polyclonal CEA confirming the hepatocytic nature and viability of the cells.

intraportal transplantation of cryopreserved human hepatocytes. Autopsy confirmed the thrombosis of the right and left portal branches, the main trunk and the portal vein anastomosis were patent. Multiple thrombi were also present inside the intrahepatic small branches

of the portal vein. The hepatic veins and artery were patent. No macroscopic signs of pulmonary embolisms were evident. Histological slides from lung parenchyma showed micro thrombosis in arterioles and septal capillaries, with areas of haemorrhagic alveolitis. A definite group of hepatocyte like cells was present at subpleural level [Fig. 3]. Cell reactivity either for epithelial markers or for polyclonal calcinoemblyonic antigen (CEA), within a vascular lumen, guarantee the nonmesothelial nature of this group of cells [Fig. 4]. Liver histology showed thrombosis of multiple intrahepatic large portal vein channels; within the thrombus cells or large aggregates of epithelial elements, showing canalicular reactivity pattern for polyclonal CEA [Fig. 5], cytoplasmic vacuolization, possibly steatosis, were present; these cells were only partially preserved, but still viable.

Discussion

Based on the studies performed in animal models and on the physiology, the liver and the spleen are the most reliable sites for hepatocytes engraftment and function [10]. Moreover most of the clinical attempts performed so far, and notably those achieving the best results, have utilized the intraportal approach for hepatocytes transplantation. Hepatocytes transplantation has been used to bridge patients with acute liver failure to liver transplantation; patients have been treated with between 10^7 and 10^{10}, or from <1% to 4% of the native hepatocyte mass, allogeneic hepatocytes infused through either the splenic artery or the portal vein, with localization of transplanted cells in the liver and spleen and anecdotal improvements in ammonia and encephalopathy [5,11–13]. Complications reported have been few and include transient hemodynamic instability during intraportal infusion, sepsis and embolization of hepatocytes into the pulmonary capillary bed. Moreover, transient portal hypertension during intraportal infusion have been reported [3,4,5,12]. Liver-based metabolic disease is probably the best indication for hepatocytes transplantation as a low number of functioning hepatocytes might engraft and correct the metabolic defect. Familial hypercholesterolaemia, ornithine transcarbamylase deficiency (OTC) deficiency, Crigler–Najjar syndrome type I and glycogen storage disease type I have been the indication for clinical trials of allogenic hepatocytes transplantation for metabolic liver disease [3,4,5,13,14]. Results have been encouraging with evidence of function of the lacking enzyme and clinical improvement. In all those cases the route of hepatocytes infusion was the portal vein and complications reported include transient portal hypertension, portal flow reduction and transaminases elevation (no cases of portal vein thrombosis have been reported), hypoxaemia, requiring oxygen for normalization, and infiltrates on chest X-ray; lung examination at autopsy confirmed the presence of hepatocytes in the alveoli and blood vessels without causing macroscopic infarction. Clinical intraportal pancreatic islets infusion had been reported in the literature for the treatment of diabetes; the morbidity related to that procedures is low with only two reversible cases of portal branch thrombosis, however the amount of volume infused was only 13±11 ml and none of those attempts had been performed on a portal vein of a liver transplant recipient subjected to vascular anastomosis [15].

Muraca *et al.* [9] showed an inverse linear relationship between portal pressure and portal flow and pulmonary arterial pressure increase by 11–62% during intraportal infusion of up to 2.4% of total hepatocyte mass in a pig model; they also found hepatocytes containing thrombi in segmental and smaller portal branches and hepatocytes in lung capillaries. Schneider *et al.* [16] reported successful monitoring of ^{99m}Tc-MAA/hepatocytes mixture translocation into the lungs during intraportal infusion in an animal model; however they do not report any significant impairments of portal haemodynamic and liver function.

The amount of hepatocytes infused through the portal vein in clinical trials ranged from 1 to 3 billion hepatocytes with viability ranging from 58 to 90%. The time of infusion have been reported only in the paper by Fox *et al.* [3] and Muraca *et al.* [4] being, respectively, 15 and 4 h for infusion of 7.5×10^9 and 2×10^9 allogeneic freshly isolated human hepatocytes. Hepatocyte transplantation for metabolic liver defect has been always performed with freshly isolated human hepatocytes, while attempts for acute liver failure have been performed with both fresh and cryopreserved cells.

In the case reported herein we attempted intraportal hepatocytes transplantation to temporary support liver function and stimulate hepatic regeneration in a liver transplant recipient (second transplant) affected by primary graft dysfunction judged not eligible for a third transplant. This procedure was highly experimental as it was performed after two prior liver transplantation; no previous attempts in liver transplant recipients have been done so far. We infused 2 billion viable cryopreserved human hepatocytes over a period of 5 h, thereby not differing from previous reports of intraportal hepatocytes transplantation, also for the technique utilized. We did not use heparin for prophylaxis as the patient already suffered from severe coagulopathy (INR = 3.23) because of liver insufficiency. During the infusion we noted an increase in portal pressure from 30 mmHg to 40–45 mmHg, returning to basal value 8 h after the procedure. A parallel reduction of portal blood flow from 20 cm/s to 10 cm/s was noted during infusion. This procedure was complicated by the development of complete

thrombosis of the right and left portal vein branches. Probably a major factor for the development of this severe life-threatening complication was the presence of a stenosis of the recipient portal vein, with a pressure gradient of 10 mmHg, that, despite pneumatic balloon dilatation resulting in improvement of the pressure gradient to 5 mmHg, gave a contribution to the development of complete thrombosis of the left and right portal branches after hepatocytes infusion. Moreover, previous surgery with multiple portal vein vascular anastomosis (the patient have had two liver transplantation) might have favoured the insurgence of this complication. We conclude that intraportal hepatocytes transplantation, although very promising, might have an important morbidity and mortality especially when performed in very sick patients, already subjected to liver surgery and vascular anastomosis. Accurate portal vein pressure and flow monitoring should be always carried out during infusion, and alternative routes of hepatocyte transplantation should be pursued in order to reduce the risk of an intraportal approach.

References

1. Regimbeau JM, Mallet VO, Bralet MP, Gilgenkrantz H, Houssin D, Soubrane O. Transplantation of isolated hepatocytes. Principles, mechanisms, animal models, clinical results. *Gastroenterol Clin Biol* 2002; **26**: 591.
2. Allen KJ, Soriano HE. Liver cell transplantation: the road to clinical application. *J Lab Clin Med* **2001138**: 298.
3. Fox IJ, Chowdhury JR, Kaufman SS, *et al.* Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med* 1998; **338**: 1422.
4. Muraca M, Gerunda G, Neri D, *et al.* Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet* 2002; **359**: 317.
5. Strom SC, Fisher RA, Thompson MT, *et al.* Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation* 1997; **63**: 559.
6. Fox IJ, Chowdhury JR. *Hepatocyte Transplantation. Am J Transplant* 2004; **4**(Suppl 6):7.
7. Strom S, Fisher R. Hepatocyte transplantation: new possibilities for therapy. *Gastroenterology* 2003; **124**: 568.
8. Muraca M, Neri D, Parenti A, Feltracco P, Granato A, Vilei MT, *et al.* Intraportal hepatocyte transplantation in the pig: hemodynamic and histopathological study. *Transplantation* 2002; **73**: 890.
9. Baccarani U, Sanna A, Cariani A, *et al.* Isolation of human hepatocytes from livers rejected for liver transplantation on a national basis: results of a 2-year experience. *Liver Transpl* 2003; **9**: 506.
10. Laconi E, Laconi S. Principles of hepatocyte repopulation. *Semin Cell Dev Biol* 2002; **13**: 433.
11. Sass DA, Shakil AO. Fulminant hepatic failure. *Gastroenterol Clin North Am* 2003; **32**: 1195.
12. Di Campli C, Nestola M, Piscaglia AC, *et al.* Cell-based therapy for liver diseases. *Eur Rev Med Pharmacol Sci* 2003; **7**: 41.
13. Bilir BM, Guinette D, Karrer F, *et al.* Hepatocyte transplantation in acute liver failure. *Liver Transpl* 2000; **6**: 32.
14. Sokal EM, Smets F, Bourgois A, *et al.* Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation* 2003; **76**: 735.
15. Bucher P, Mathe Z, Bosco D, *et al.* Morbidity associated with intraportal islet transplantation. *Transplant Proc* 2004; **36**: 1119.
16. Schneider A, Attaran M, Gratz KF, *et al.* Intraportal infusion of 99mtechnetium-macro-aggregrated albumin particles and hepatocytes in rabbits: assessment of shunting and portal hemodynamic changes. *Transplantation* 2003; **75**: 296.