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New potential cell source for hepatocyte transplantation: Discarded livers from metabolic disease liver transplants☆,☆☆

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Abstract Domino liver transplantation is a method used to increase the number of liver grafts available for orthotopic liver transplantation (OLT). Reports indicate that livers from patients with metabolic liver disease can be safely transplanted into select recipients if the donor's defect and the recipient's metabolic needs are carefully considered. The liver of patients with many types of metabolic liver disease is morphologically and biochemically normal, except for the mutation that characterizes

Abbreviations: HTx, hepatocyte transplant; OLT, orthotopic liver transplantation; CYP, cytochrome P450; DLT, domino liver transplantation; OD, organ donor; MD, metabolic and diseased livers; MMA, methylmalonic acidemia; PHO, primary hyperoxaluria; MSUD, Maple Syrup Urine Disease; CN, Crigler–Najjar syndrome; OTC, ornithine transcarbamylase deficiency; CPS-1, carbamoyl-phosphate synthetase 1 deficiency; Cit, citrullinemia; CHF, congenital hepatic fibrosis; PNALD, parenteral nutrition associated liver disease; A1AT, alpha-1-antitrypsin deficiency; FC, familial cholestasis; BA, biliary atresia; ALF, acute liver failure; HMM, hepatocyte maintenance medium; PE, plating efficiency; EROD, ethoxyresorufin-O-deethylase; LCU, Luminescent Counting Unit; BNF, beta-naphthoflavone; PB, phenobarbital; Rif, rifampicin; UGT, uridine 5′-diphospho-glucuronosyltransferase; FRG, $Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-}$; MMF, mycophenolate mofetil.

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that disease. Other biochemical functions normally performed by the liver are present and presumably "normal" in these hepatocytes. Hepatocytes were isolated from the liver of 35 organ donors and 35 liver tissues taken at OLT from patients with liver disease were analyzed for 9 different measures of viability and function. The data indicate that cells isolated from some diseased livers performed as well or better than those isolated from organ donors with respect to viability, cell yield, plating efficiency and in assays of liver function, including drug metabolism, conjugation reactions and ammonia metabolism. Cells from metabolic diseased livers rapidly and efficiently repopulated a mouse liver upon transplantation. Conclusions: As with domino liver transplantation, domino cell transplantation deserves consideration as method to extend the pool of available organs and cells for transplantation.

Introduction

Transplantation of hepatocytes (HTx) has been shown to be useful for patients with chronic or acute liver failure or genetic defects in liver function (Strom et al., 1997; Fisher et al., 2000; Fisher and Strom, 2006; Strom et al., 2006; Fox et al., 1998; Horslen et al., 2003; Muraca et al., 2002; Dhawan et al., 2006; Stephenne et al., 2006; Lee et al., 2007; Meyburg et al., 2009a; Mitry et al., 2004). The most common indications of HTx are liver-based inborn metabolic disorders. Because of large redundancies of function, the entire hepatic capacity is not normally required to maintain homeostasis. The main source of cells for HTx is livers rejected for transplantation (Strom et al., 2006; Meyburg et al., 2009b; Hughes et al., 2006). Steatosis is the most common cause for rejection of a tissue for transplantation. Low cell viability, yield and drug-metabolizing enzyme activity has been reported in hepatocytes obtained from fatty livers (Donato et al., 2006).

Domino liver transplantation (DLT) of organs from patients with metabolic diseases has been used to increase the number of organs available for transplant (Ericzon et al., 2008; Mazariegos et al., 2012; Popescu and Dima, 2012). The world registry lists 790 such transplants through 2009 (Roels and Rahmel, 2011). The metabolic disease in the donor liver either should not induce the disease in the recipient, or would be expected to produce symptoms of the disease only after many years. Unlike DLT, where the entirety of the liver is rapidly replaced with one with a metabolic disease, HTx generally results in replacement of <10% of the recipient liver with donor cells. Since most metabolic disease hepatocytes would not induce the symptoms of the disease in the recipient, hepatocytes from donors with metabolic diseases might be useful for transplantation if they could be isolated in useful numbers and retained sufficient viability and function. Here we examined the viability, cell yield and metabolic activity of hepatocytes isolated from organ donors and tissues obtained from patients receiving OLT for metabolic and other types of liver diseases. The results indicate that hepatocytes with high viability and function can be isolated from organs removed at the time of OLT from patients with diseased livers. If the diseases of the donor and recipient are carefully considered, these organs could provide a useful source of cells for clinical transplantation.

Materials and methods

Human liver tissues were collected under IRB protocol 0411142 and hepatocytes were isolated from 35 organ donors (OD) and from 35 liver tissues obtained from patients receiving OLT for metabolic and other liver diseases (MD). Liver tissue from organ donors was flushed with either Belzer's UW solution or HTK depending on the solution preferred by the procurement agency. Metabolic disease cases were recovered in the OR and flushed with either Belzer's UW solution if stored for more than 90 min or ice-cold Eagle's MEM if the tissues were taken to the lab immediately for isolation. Hepatocyte isolation and culture were performed as previously described (Gramignoli et al., 2012; Kostrubsky et al., 1999). Methods to assess cell viability, plating efficiency, or ammonia, testosterone and 7-ethoxyresorufin metabolism, resorufin conjugation, media and culture conditions were as previously described (Gramignoli et al., 2012). Cell-based assays for specific CYP (1A2; 2C9; 3A7; 3A4, Promega Corporation, Madison, WI, U.S.A.) were used according to the manufacturer's instructions. Results were expressed as Luminescent Counting Unit (LCU)/min and normalized to a million of viable cells (suspension cultures) or to the double strand DNA content (adherent cultures) measured, after the Glo™-assays are complete, by Quant-iT™ PicoGreen® dsDNA kit according to the manufacturer's instructions (Molecular Probes, Invitrogen, Camarillo, CA).

Animal transplant

Animal studies were reviewed and approved by the Institutional Animal Care and Use Committee. Human hepatocytes (10^6/mL in Eagle's Minimal Essential Medium; Lonza) were transplanted in FRG mice as previously described (Azuma et al., 2007).

Statistical analysis

Statistical differences were determined by comparing means using analysis of variance with repeated measurements and Dunnett's post hoc test, $p < 0.05$ was chosen as the minimum level of significance. Data were analyzed by GraphPad Prism (version 5.03, GraphPad Software Inc.).

Results

Hepatocytes were successfully isolated from 70 hepatic tissues; 35 were obtained from organ donors (OD) rejected for OLT, and 35 were tissues were obtained from the explanted liver of patients who received OLT for metabolic and other types of liver disease (MD) including, biliary atresia,