Transplantation of human stem cell-derived hepatocytes in an animal model of acute liver failure

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Introduction. *Hepatocyte cell transplantation can be life-saving in patients with acute liver failure (ALF); however, primary human hepatocyte transplantation is limited by the scarcity of donor hepatocytes. We investigated the effect of stem cell-derived, hepatocyte-like cells in an animal xenotransplant model of ALF.*

Methods. *Intraperitoneal D-galactosamine was used to develop a lethal model of ALF in the rat. Human induced pluripotent stem cells (iPSC), human mesenchymal stem cells, and human iPSC combined with human endothelial cells (iPSC + EC) were differentiated into hepatocyte-like cells and transplanted into the spleens of athymic nude rats with ALF.*

Results. *A reproducible lethal model of ALF was achieved with nearly 90% death within 3 days. Compared with negative controls, rats transplanted with stem cell-derived, hepatocyte-like cells were associated with increased survival. Human albumin was detected in the rat serum 3 days after transplantation in more than one-half the animals transplanted with hepatocyte-like cells. Only animals transplanted with iPSC + EC-derived hepatocytes had serum human albumin at 14 days posttransplant. Transplanted hepatocyte-like cells homed to the injured rat liver, whereas the ECs were only detected in the spleen.*

Conclusion. *Transplantation of stem cell-derived, hepatocyte-like cells improved survival with evidence of in vivo human albumin production. Combining ECs may prolong cell function after transplantation. (Surgery 2015;158:349-59.)*

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CELL TRANSPLANTATION holds great promise as an alternative to whole-organ liver transplantation for patients suffering from some forms of acute, life-threatening liver disease. Despite its promise and preliminary success in human trials, widespread application of cell transplantation for liver failure has been hindered by the scarcity of human donor livers for primary cell isolation.[1,2]

Stem cell-derived hepatocytes are an alternate source for cell transplantation. Induced pluripotent stem cells (iPSC) and mesenchymal stem cells (MSC) are adult-derived cell sources that can be expanded to generate large numbers of cells. These cells display the potential for multilineage differentiation, are devoid of ethical controversy, may be less immunogenic, and are a potential autologous cell source enabling immunosuppression minimization or cessation.[3-6] The iPSC and MSC have been differentiated successfully into hepatocyte-like cells. We recently developed a novel, suspension-based, embryoid body approach for differentiating multipotent stem cells from different sources into hepatocyte-like cells.[7]

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Because the transplantation of stem cell-derived hepatocytes results in transiency in function, we hypothesized that incorporating endothelial cells (ECs) into clusters of stem cell-derived hepatocytes may improve engraftment and prolong function.[8]

In this study, we utilized an animal xenotransplant model of lethal acute liver failure (ALF) to investigate the efficacy of human stem cell-derived hepatocyte-like cells in reversing ALF. Using an identical differentiation protocol, we evaluated the in vivo function of hepatocyte-like cells derived from human iPSC, human MSC, and human iPSC + EC.

METHODS

Rat model of ALF. The Institutional Animal Care and Use Committee approved the use of rats for experimentation in this study. The goal for the model of ALF was reproducible mortality within 3 days of onset of liver failure. Sterile D-galactosamine (Sigma-Aldrich, St. Louis, MO) dissolved in Hank's balanced salt solution (HBSS) was used to induce ALF. Anesthesia was induced by placement of the rats in an isofluorane induction chamber. Intraperitoneal injection of 800–1,000 mg/kg of D-galactosamine was evaluated during the developmental stage of this model of ALF to determine the dose responsiveness. The model was initially developed in male Lewis rats (LEW/SsNHsd; Harlan Laboratories, Indianapolis, IN) and repeated and translated in male nude athymic rats (Crl:NIH-*Foxn1*^{rnu} [Charles River Laboratories, Wilmington, MA] and Hsd:RH-*Foxn1*^{rnu} [Harlan Laboratories]).

The Lewis rats were housed in our institutional vivarium with 12-hour light–dark cycles and received standard chow and water ad libitum; the athymic nude rats were housed in a specialized barrier facility and received irradiated chow and water. All rats were weighed daily and signs of hepatic encephalopathy scored using published neurologic criteria.[9] Liver injury was quantified by measuring rat serum alanine transferase (ALT) by tail venipuncture (VetScan 2.0, Abaxis, Union City, CA). Animals were monitored for 14 days after transplantation, and when humanely killed, liver and spleen samples were retrieved. Animals were killed at earlier time points if they met the following humane criteria: moribund state, weight loss of ≥30% from before induction of ALF, or increase in ALT to >15 times baseline.

The data were analyzed to determine the optimal dosage required to achieve a reproducible increase in ALT and mortality within 72–96 hours after induction of ALF.

Xenotransplant model. To develop the model, primary hepatocytes isolated from mouse livers were transplanted into the spleens of athymic nude rats 16–18 hours after induction of ALF. ALF was induced in 270–350 g male, athymic nude rats with 950–975 mg/kg of sterile D-galactosamine dissolved in HBSS at a concentration of 97.5 mg/mL.

Isolation of hepatocytes. Primary mouse hepatocytes were isolated from 6- to 8-week-old male mice (C57BL/6NHsd, Harlan Laboratories) weighing 25–30 g. We used 22 mice to establish and optimize the technique. An additional 16 mice were used to obtain donor hepatocytes for culture and transplantation. Under ketamine anesthesia, the inferior vena cava was cannulated with a 24-gauge angiocath (Becton-Dickson, Franklin Lakes, NJ) and the liver flushed and digested retrograde. The flush and digestion solutions were oxygenated for 10 minutes before perfusion and warmed to 37°C. The flush solution consisted of 6.67 g/L NaCl, 0.16 g/L KCl, 2.1 g/L $NaHCO_3$, 0.34 g/L KH_2PO_4, 0.11 g/L $MgCl_2$, and 0.14 g/L $MgSO_4$. The digestion solution consisted of the flush solution with 1.2 mmol/L calcium chloride and 1 mg/mL Type 2 Collagenase (Worthington Biochemical Corp, Lakewood, ND). Both solutions were administered for 7 minutes each at a flow rate of 6 mL/min. After digestion, the peritoneal and diaphragmatic attachments of the liver were transected and the liver was removed in a 50-mL, conical tube with sterile Krebs-Henseleit buffer (25 mmol/L $NaHCO_3$, 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L NaH_2PO_4, 10 mmol/L glucose and 1.3 mmol/L $CaCl_2$) at 4°C. Using a sterile, cotton-tipped applicator, the liver capsule was ruptured and cells released. The solution was strained through a sterile nylon mesh, and the resultant solution was washed twice at 50*g* for 2 minutes with cold Krebs-Henseleit buffer. The final pellet was re-suspended in 5 mL of cold, hepatocyte maintenance medium (HMM) consisting of enriched Iscove's Modified Dulbecco's Medium (Invitrogen, Grand Island, NY) with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), 1 × penicillin/streptomycin (100 U/mL penicillin + 0.1 mg/mL streptomycin), 2 g/L glucose, 1 g/L galactose, 0.5 g/L nicotinamide, 0.110 g/L sodium pyruvate, 10 mg linoleic acid, 0.5 mL of insulin–transferrin–selenium, 50 μg/L dexamethasone, and 10 μg/L epidermal growth factor (all from Sigma-Aldrich). The resulting sample was analyzed for yield and viability using Trypan blue exclusion. Using our isolation protocol, the mean yields were 1–5 × 10^7 hepatocytes per