nonimmunogenic feto-maternal interface. The absence of MHC class II expression on the JAR–UC fusions suggests the presence of dominant trans-acting suppressor factor(s) in trophoblasts. Isolation and identification of these factor(s) may provide a method to suppress MHC class II expression.

REFERENCES

HEPATOZYME TRANSPLANTATION IN ANHEPATIC RATS: EFFECT ON SURVIVAL, BLOOD CHEMISTRY, AND GROWTH FACTOR PROFILE

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PATIENTS WITH FULMINANT HEPATIC FAILURE (FHF) and liver necrosis often die before a liver becomes available for transplantation. Staged total hepatectomy has been carried out to remove the necrotic liver and stabilize patients until transplantation. We hypothesized that in FHF patients that are rendered anhepatic, hepatocyte transplantation can provide liver-specific support. We have developed a novel single-stage technique for total hepatectomy in rats and used it to study the effects of intrasplenic hepatocellular transplantation in anhepatic rats.

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MATERIALS AND METHODS

Adult male Sprague-Dawley rats (150–350 g) were used. Donor hepatocytes were isolated by in situ 2-step ethylenediaminetetraacetic acid/collagenase digestion and enrichment through a Percoll gradient. Hepatocyte viability was always greater than 90%, as determined by trypan blue exclusion. Group I rats \((n = 16)\) underwent intrasplenic injection of \(2.5 \times 10^7\) hepatocytes. Group II rats \((n = 12)\) underwent intrasplenic injection of saline. In all animals, 3–6 out of 6–7 splenic venous branches were permanently ligated to prevent immediate migration of transplanted cells to the liver. Both groups were kept under daily cyclosporine immunosuppression \((10 \text{ mg/kg, intramuscularly})\). After 3 days, to allow cell engraftment, all rats were rendered anhepatic. Briefly, an end-to-side portocaval shunt (PCS) was created and the hepatic artery and bile duct were transected. Next, a 3–4-cm-long piece of a 14-gauge Angiocath was introduced (via direct puncture) into the lumen of the inferior vena cava (IVC) at a level between the left renal and the right iliacombrar vein. The stent was advanced into the IVC and secured above the PCS and the liver dome. The liver was then removed, including tissue surrounding the intrahepatic portion of the IVC. Postoperatively, all rats were maintained on continuous intravenous (jugular vein) glucose supplementation \((20 \text{ mg/100 g/hour})\). Eight rats from each group were monitored until death to determine survival time. The remaining rats were euthanized at 12 hours posthepatectomy for measurement of blood ammonia \((\text{NH}_3)\) levels, prothrombin time (PT), international normalized ratio (INR), plasma hepatocyte growth factor (HGF), and transforming growth factor \(\beta1\) (TGF\(\beta1\)) levels (enzyme-linked immunosorbent assay). Spleen sections were immunostained for expression of proliferation nuclear cell antigen (PCNA). Data are expressed as means \(\pm\) standard deviation. Student’s \(t\)-test was used for statistical analysis.

RESULTS

Group I transplanted rats survived significantly longer than group II sham-transplanted controls \((34.1 \pm 8.5 \text{ vs. } 15.5 \pm 4.8 \text{ hours}, P < 0.05)\). In addition, group I rats progressed to stage IV encephalopathy (no righting reflex to pain stimuli) later than group II rats \((29.5 \pm 7.7 \text{ vs. } 10.6 \pm 3.9 \text{ hours}, P < 0.05)\). At 12 hours posthepatectomy, transplanted rats had lower \(\text{NH}_3\) levels \((1.35 \pm 3.44 \text{ vs. } 2.137 \pm 427 \text{ mg/dL}, P < 0.05)\) and lower PT \((17 \pm 1 \text{ vs. } 24 \pm 4 \text{ seconds}, P < 0.05)\) and INR \((1.9 \pm 0.1 \text{ vs. } 3.8 \pm 1.3, P < 0.05)\) than controls. Blood levels of HGF were similar in both groups \((9.0 \pm 2.2 \text{ vs. } 8.3 \pm 4.0 \text{ ng/mL})\), whereas blood TGF\(\beta1\) levels were significantly lower in transplanted rats compared to controls \((25.5 \pm 16.0 \text{ vs. } 60.1 \pm 2)\).
13.8 ng/mL, \( P < 0.05 \)). Sections of transplant-bearing spleens contained numerous clusters of hepatocytes. In 50% of these animals, intrasplenic hepatocytes showed signs of proliferation (PCNA labeling index = 8–12%).

DISCUSSION

We have demonstrated that in anhepatic rats, intrasplenic transplantation of a relatively small number of allogeneic hepatocytes delayed the onset of encephalopathy and prolonged survival. Additionally, intrasplenically transplanted hepatocytes exhibited detoxifying and synthetic activity, as demonstrated by lower NH₃ levels and improved blood coagulation.

We have previously shown that in rats the anhepatic state was associated with the progressive rise in blood HGF and TGFβ1 levels. In this study, hepatocyte transplantation had no immediate effect on blood HGF levels. It did, however, slow the rise in blood TGFβ1 levels. This growth factor profile may have been responsible for the observed transplanted cell proliferation. In conclusion, hepatocyte transplantation may prove to be a useful bridge to transplantation in patients with fulminant hepatic failure.

REFERENCES


IN UTERO HEPATOCYTE TRANSPLANTATION IN A RAT MODEL

George B. Mychaliska, MD, Marcus O. Muench, PhD, Matthew H. Swartz, Jacquelyn J. Maher, MD, Joseph P. Burns, BA, Craig T. Albanese, MD, and Michael R. Harrison, MD, FACS

PEDIATRIC LIVER TRANSPLANTATION is successful in treating a variety of diseases but has a number of limitations including donor

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