493 TRANSPLANTATION OF ISOLATED HEPATOCYTES PROLONGS SURVIVAL AND IMPROVES BLOOD CHEMISTRY IN ANHEPATIC RATS. N Arkadopoulos, H Lilja, KS Suh, O Detry, C Mullon, AA Demetriou, J Rozga. Liver Support Research Laboratory, Burns & Allen Research Institute, Dept. of Surgery, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, Circe Biomedical Inc., Lexington, MA.

In acute liver failure, the level of liver-specific support provided by the transplanted hepatocytes could not be distinguished from that of the native liver. We have therefore decided to study the effects of allogeneic hepatocyte transplantation in rats rendered anhepatic. For this purpose, a novel single-stage technique of total hepatectomy in the rat was developed. Methods: Sprague Dawley rats (150-400 g) were used. Rat hepatocytes were isolated using two-stage EDTA/collagenase digestion and highly viable (>90%), hepatocyte-enriched fractions were obtained using Percoll density gradient. Group I rats (n=12) received intrasplenic injection of $2.5x10^7$ allogeneic hepatocytes and Group II rats (n=12) received intrasplenic injection of saline. Both groups were kept under Cyclosporine A immunosuppression (10 mg/kg/day IM). After three days, to allow cell engraftment, all rats were rendered anhepatic. Total hepatectomy was performed as follows: after midline laparotomy, an end-to-side portocaval shunt (PCS) was created and a piece of a 14G Angiocath was inserted into the lumen of the vena cava and positioned above the right renal vein. The stent was secured above the PCS and below the diaphragm and the liver was resected. Postoperatively, all rats were maintained on intravenous glucose (20 mg/100g/hr), which was administered via a jugular vein cannula. Eight rats from each group were used to determine survival time. The remaining rats were euthanized at 12 hours post-hepatectomy for measurement of blood ammonia (NH_3) level, prothrombin time (PT), international normalized ratio (INR), plasma hepatocyte growth factor (HGF) and transforming growth factor ß1 (TGFß1) levels. Results: Anhepatic state was associated with elevated blood HGF and TGFβ1 levels. Transplanted rats survived longer than controls (34 ± 8 *vs.* 16 ± 5 hrs, $p<0.05$). In addition, at 12 hrs postoperatively, Group I rats had significantly lower PT and INR and decreased levels of NH_3 and TGFß1. (Table; data are shown as means ± SD; *$p<0.05$). Sections of transplant-bearing spleens contained numerous clusters of hepatocytes. In 50% of these rats, 8-12% of the transplanted cells stained + for proliferation cell nuclear antigen.

Group	NH_3 (µg/dl)	PT (sec)	INR	HGF(ng/ml)	TGFβ1 (ng/ml)
I	1535±344*	17±1*	1.9±0.1*	9±2	26±16*
II	2137±427	24±4	3.8±1.3	8±4	60±14

Conclusions: In anhepatic rats, intrasplenic transplantation of a relatively small number of allogeneic hepatocytes prolonged survival, lowered blood ammonia, improved blood coagulation, and lowered blood levels of a potent hepatic regeneration inhibitor. The latter effect was associated with transplanted cell proliferation.

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494 PERCUTANEOUS HEPATOCYTE TRANSPLANTATION (PHT) IN LIVER FAILURE. BM. Bilir, D Guenette, A Ostrowska, J Durham, D Kumpe, J Krysl, R Shrestha, T Trouillot, I Teitelbaum, GT Everson. University of Colorado School of Medicine, Denver, CO.

Studies in animal models of liver failure suggests that PHT can improve survival. This prompted the investigation of the safety and feasibility of PHT in patients with acute and chronic liver failure. First, a human liver cell bank was established. The functional and structural integrity of the cryopreserved human hepatocytes was demonstrated by assaying cell morphology (H&E and EM), cell culture characteristics, induction of P4501A1,2, transcription, and survival in vivo by xenotransplantation in a rat model. Because of the differences in the pathophysiology, patients with acute liver failure (ALF) and cirrhosis were investigated separately. Among the six patients enrolled in the **ALF group**, one was withdrawn from the study due to an anaphylactoid reaction. All of the remaining five patients with ALF had grade IV encephalopathy, were ventilator and dialysis-dependent, had factor V level <0.5 and were not OLTx candidates. Three of the five who survived (48-72 hours) beyond PHT had substantial improvement in their encephalopathy score, serum ammonia levels and prothrombin time. Clinical improvement was paralleled by an increase in aminopyrine and caffeine clearance. All three patients lived substantially longer than expected (12, 28, and 52d) after PHT but eventually expired. Postmortem examination revealed the presence of transplanted hepatocytes in livers and spleens by H&E and fluorescent in situ hybridization (FISH). In the **cirrhosis group**, PHT was indicated by refractory encephalopathy and refractory ascites despite TIPS in one, hepatorenal syndrome with anuria in another, and refractory ascites in the third patient. All three patients had a Child's-Pugh score of 15 and tolerated the procedure without immediate complications. The first patient's encephalopathy resolved 24-48 hours after the procedure. Seven months later his Child's-Pugh score was down to 5. The second patient's anuria resolved 48 hours post-PHT. The third patient's encephalopathy resolved and was able to undergo TIPS procedure. All three are currently alive. **Conclusions.** 1) Cryopreserved human hepatocytes are viable and functional upon thawing; 2) PHT in ALF and cirrhosis is feasible and may serve as to bridge patients to OLT; 3) There is an apparent time delay of 24-72 hours between PHT and the first clinical or biochemical sign of improvement. This delay may be due to recovery time from the cryopreservation process or engraftment of the cells. 4) The number of cells needed to assure recovery in patients with severe ALF seems to be higher than 10^{10}. However, in cirrhosis where augmentation rather than replacement of the functional liver cell mass is the goal, 10^9 to 10^{10} hepatocytes may be enough to assure survival. 5) Although the exact pathophysiology of hepatorenal syndrome with anuria is not known, augmentation of liver cell mass by PHT may help to overcome this functional impairment.

495 TREATMENT OF PATIENTS WITH FULMINANT HEPATITIS WITH A BIOARTIFICIAL LIVER BEFORE LIVER TRANSPLANTATION. D Samuel, Ph Ichai, C Féray, F Saliba, M Gigou, JL Arulnaden, F Saurini, P Debat, A Bismuth, D Castaing, H Bismuth. Centre Hépato-Biliaire, Hôpital Paul Brousse, Villejuif, Université Paris-Sud, France.

Fulminant Hepatitis (FH) is a disease leading to a fatal outcome in 80% of cases in the absence of emergency liver transplantation (LT). Patient survival after LT is 70%, however these results are impaired by the death of 15% of patients (pts) before LT mainly by brain oedema, by the death of pts after LT due to the severity of their condition, and by the acceptance of high risk grafts due to the emergency. There is a need for a treatment of liver failure permitting to maintain pts in sufficient good condition until LT.

Patients: 8 pts, 3 M, 5 F, mean age 28 years, suffering from FH who met our criteria for emergency LT were included in a pilot trial of using a bioartificial liver (BAL) during the waiting period for LT. The cause of FH was: Wilson (1), viral A (1), drug-induced (1), unknown (5). **Methods**: These 8 pts were put on an extracorporeal circuit comprising first a plasmapheresis sytem (Cobe), then the plasma of the pts was filtered though a charcoal filter (Gambro), and a capillary filter in which the extracapillary space was filled of porcine hepatocytes on collagen microcarriers in a solution of dextran (Circe Biomedical).These hepatocytes were kept cryopreserved until inclusion of a patient in the trial. Pts were planned to receive one course of 6 hours of bioartificial liver (BAL) every day until LT. **Results**: At time of inclusion, all the pts were either confused (2) or comatose (6), the mean factor V was 13% (Extr. 4-22), 5 were on mechanical ventilation. Three, 3, and 2 pts received 1, 2 and 3 courses of BAL respectively before LT. The main effect observed during BAL was a neurological improvement based on the Glasgow score (mean 7 to 10 after the first course) which seems to disappear at the third course and a diminution of level of ammonemia (125 to 100 µmol/L after one course) and lactates levels. There was no improvement in the coagulation parameters. The main complication were a bleeding in 2 pts secondary to invasive procedures, and transient reversible haemodynamical instability in 2. All pts were transplanted after a mean delay of 2.2 d (1-5 d), 1 died of cerebral bleeding due to an intracranial pressure sensor at day 1, and one of posthepatitis aplastic anemia at 7 months. All other pts are alive and well, with a mean follow-up of 4.5 months (Extr 0.5-10)

Conclusion: This report detailed our experience of treating 8 pts with FH with a Bioartificial liver. The procedure was well supported, all pts could be transplanted and the main effect was a neurological improvement during the BAL treatment.

496 **TREATMENT OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY AND FULMINANT HEPATIC FAILURE BY THE BIOLOGIC-DT® SYSTEM.**

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When acetaminophen overdose patients arrive in the emergency room more than 16 hours after overdose, the acetylcysteine antidote is less effective and overall mortality, including the widely ranging severity of hepatic toxicity, is around 10-15%. (Makin, Wendon, Williams; Gastroenterology, 1995). We identified twelve patients who had arrived in the emergency room 31 (19 hours S.D.) after acetaminophen overdose, with levels predicting severe hepatic toxicity. By 12 to 168 hours, eight patients had established hepatotoxicity [mean peak ALT=4052] and four had developed fulminant hepatic failure with Stage IV coma, acidosis, and renal failure [mean peak AST=9033, mean peak INR=5.2]. All patients were then treated for 4-6 hours with the BioLogic-DT System, a single-access hemodiabsorption system indicated for treatment of acute hepatic failure with coma and serious drug overdose. Treatment was repeated if detectable acetaminophen was measured in plasma. All eight patients with hepatitis recovered intrinsic liver function and general health, with liver enzymes beginning to normalize 24 hours after treatment. Of the four fulminant hepatic failure patients; two recovered liver function, one recovered liver function but died with sepsis, and one died of multi-organ failure. Treatment by the BioLogic-DT System of acetaminophen overdose patients with established hepatic toxicity is safe and in a proportion of patients, may speed the rate of recovery; relatively few patients in this group require emergency liver transplant.