149 INHIBITORY EFFECT OF RAPAMYCIN ON CCL4-INDUCED HEPATIC FIBROSIS IN MICE. T. Zhao, H. Hirai, S. I. Wu, AA Muna, and MA Zarn. Jefferson Medical College, Philadelphia, PA.

Background: The accelerated course of hepatic fibrosis that occurs in some patients post liver-transplantation is a major clinical problem. There is speculation that this response may be secondary to the anti-rejection therapies, and in an earlier report we showed that FK-506 enhanced the fibrogenic process in vivo and in vitro models of liver fibrosis. In the present study, we investigated the effects of a new immunosuppressive agent, rapamycin, in a model of liver fibrosis. Unlike FK506 or cyclosporine A that bind to calcineurins, rapamycin has not been shown to have renal side effects.

Methods: Male Sprague-Dawley rats were divided into Control (n=3, no treatment), CCl4-treated (n=5, 50% CCl4, 1.0 ml/kg, i.p., twice weekly for 7 weeks), and CCl4 + Rap (1.5 mg/kg/d, i.p., 3 times/week). Liver sections were assessed for the degree of fibrosis, and steady-state mRNA levels were determined by Northern blot hybridization analysis. The in vivo effects of rapamycin on extracellular matrix production by hepatic stellate cells, as well as proliferation of the cells, were also investigated.

Results: After treatment with CCl4 for seven weeks, liver sections in the CCl4-treated group displayed progressive fibrosis. The combination of CCl4 with rapamycin attenuated the degree of fibrosis as assessed by histology and a semiquantitative fibrosis score (p < 0.01). The collagen contents in the CCl4-treated group (458 ± 20.0 μg/g) was markedly higher than that in the controls, and the content in the CCl4 + rapamycin group (252.5 ± 20.4 μg/g, p < 0.01), was significantly lower than that in the CCl4-treated group, and similar to the control group (228 ± 30.1 μg/g, p > 0.05). Northern blot hybridization analysis demonstrated that the increase in mRNA levels of procollagen type I (α2) and TGF-β1 induced by CCl4 was abrogated by rapamycin therapy. When freshly isolated hepatic stellate cells were cultured with rapamycin for 24-48 h, there was no significant effect on collagen synthesis or TGF-β1 mRNA levels. However, rapamycin was shown to significantly inhibit H-2Kd/detoxification induced by cultured hepatic stellate cells. Conclusion: If rapamycin proves to be as effective an anti-rejection agent as FK-506 (preliminary clinical studies are quite encouraging), then our findings suggest that it may be useful for at least some liver transplant recipients, especially those who are likely to be subjected to fibrogenic stimuli in the post-transplant period.

151 HEMODYNAMIC CHANGES IN ANEUPLECTIC UREMIC ACUTE LIVER FAILURE EXPERIMENTAL MODEL. Kostopouloua G, Theodora, K. Pappas, N. Lykouda, D. Dafni, N. Prastaki, A. Smirniota, V. Paspatakis, G. Katsarou, and I. Liver Support Unit, Athens University Medical School, Greece.

The occurrence of the non-function liver is the core of terminal stage of fulminant hepatic failure or primary non-function of the liver graft, and the patient's support by various systems until a proper graft is found is under investigation.

The aim of the study was to observe the hemodynamic alterations in the anephric and the anephric experimental model.

Methods: Three different groups (n=6) of young pigs weighing 20-25 kg were studied. Group A (control) pigs were anesthetized with sodium pentobarbital and was studied for 6 hours. Group B (anephric model) pigs underwent total heparinisation and bridging of the inferior vena cava with the portal vein via hepatic central vessels and was studied for 6 hours. Group C (anephric model) pigs underwent total heparinisation of the hepatic artery and portal caval anastomosis. The duration of the study was 8 hours. A Swan-Ganz catheter was inserted into the femoral artery and blood samples were taken at 0, 5, 15, 30, 45, 60, 90 and 120 minutes. The monitoring of the heart rate, cardiac output, mean arterial pressure, pulmonary artery pressure, MAP, 

Results:

<table>
<thead>
<tr>
<th>Group</th>
<th>CI (ml/min/m²)</th>
<th>SV (ml/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.41 ± 1.1</td>
<td>2.58 ± 1</td>
</tr>
<tr>
<td>B</td>
<td>2.38 ± 0.2</td>
<td>4.84 ± 0.1</td>
</tr>
<tr>
<td>C</td>
<td>4.94 ± 0.8</td>
<td>5.22 ± 0.1</td>
</tr>
</tbody>
</table>

Conclusion: The results of this study demonstrate a greater hemodynamic deterioration after total hepatectomy compared to the ischemic liver model, while biochemical deterioration was observed in both groups. It is possible that the surviving remnants of a destroyed liver may be perfusionable with case of hepatic failure in terms of hemodynamic stability.

150 IL-4 INVOLVEMENT IN APOTOSIS INDUCTION DURING LIVER ALLOGRAFT REJECTION. P. Costa, P. Grise, P. Pedrin, C. Charnes, D. Huynh, V. Delaunay Laboratoire de Recherche Chirurgicale Hopital Cochin, Paris, France.

The mechanisms that govern allograft rejection remain unknown. Recently, it has been suggested that apoptotic hepatocyte death is a component of liver rejection. Cytoxins, which play a central role in rejection lesions, could also be involved in the induction of apoptosis.

The aims of this study was to confirm that apoptosis is involved in liver allograft rejection in human, and to then determine the factors contributing to apoptosis induction in human hepatocytes.

Thirty-five frozen biopsies have been studied. Five were from normal livers and 30 from transplanted patients. Apoptosis has been detected by TUNEL method (Boehringer-mannheim). In a second part of the study, human hepatocytes have been isolated and cultured with or without cytokines (IL-1, TNFα, IFN-γ, IL-2, IL-4) and cholesteroxydase (CDCA). Which has been shown to increase IL-4 production by mononuclear cells. Cell apoptosis has been detected by TUNEL method and confirmed by ELISA detection of histone-associated-DNA-fragments (Boehringer-mannheim). TUNEL-detected apoptosis has been blocked with DNA-competitive blocker, and ELISA detection was expressed as enrichment of deoxynucleosides released into the cytoplasm.

Apoptosis was minimal in normal liver and in unencapsulated transplants, present in recurrent hepatitis C and biliary complications, and strongly increased in IL-4 and CDCA, both a suppressor, which has been previously shown during liver allograft rejections and could have an effect role in this process by inducing cell apoptosis.

152 HEPATOCYTE TRANSPLANTATION PREVENTS DEVELOPMENT OF BRAIN EDEMA IN PIGS WITH UREMIA. J. E. Furlong, T. A. Moore, J. L. Rieger, J. M. Huddleston, C. Muller, A. Bolande. Liver Transplantation Research Laboratory, Burn's Aller Research Institute, Dep. of Surgery, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, Citro Biomedical Inc., Lexington, MA.

Intercellular hypertension leading to brain edema is a major cause of death in fulminant hepatic failure (FHF). Minimal, barbiturate and hyperventilation have been used in this setting, but most patients are either refractory to medical management or cannot be treated because of respiratory failure. Here, we demonstrate that in pigs with ischemic liver failure, hepatocyte transplantation provides survival and prevents development of interstitial hypertension. Method: Pig liver cells were isolated using two-stage EDTA/Acentiloxane digestion. Viability of cells was always greater than 90%. Adult pigs (50-60 kg) were used. Group 1 pigs (n=4) underwent transplantation of 2.5 x 10^6 allogeneic hepatocytes under Cylosporine A (10mg/kg/day). IM) immunosuppression. After three days, to allow cell engraftment, pigs underwent portal shunt and transaction of all liver attachments. Group II pigs (n=7) received no cells prior to induction of liver necrosis. In all animals, a subdural belt was inserted for intracranial pressure (ICP) monitoring and femoral vessels were cannulated for blood pressure monitoring and glancing supputation. Body core temperature was kept at 37° C through external heating. ICP, systemic and mean arterial pressure, heart rate and neurogenic stimuli were recorded at frequent time intervals. Results: Data are shown as mean±SEM. Group II pigs survived longer than Group I controls (P<0.05) and at all time points studied, their ICP remained below 15 mmHg. In contrast, all Group II pigs had ICP>20 mmHg as early as 14-16 h postop. At the time of death, ICP in Group I pigs was 12 ± 3 mmHg and in Group II pigs it was 29 ± 4 mmHg (p<0.05). At the same time, central perfusion pressure in Group I was significantly higher than in Group II (44 ± 12 mmHg vs. 17 ± 3 mmHg, p<0.05). Transplanted pigs had lower blood levels of TGF-α (177 ± 25 vs. 124 ± 67 ng/ml), creatinine (0.4 ± 0.2 vs. 1.4 ± 0.2 mg/dl) and albumin (7.2 ± 10 vs. 10.6 ± 2.8 mg/dl). All animals, all pigs had liver necrosis. In Group I pigs, the spleen sections showed clusters of viable hepatocytes (H4 staining). Conclusion: Hepatocyte transplantation of allogeneic hepatocytes prolonged survival in pigs with liver necrosis and prevented development of interstitial hypertension. These findings have important implications for (the clinical management of FHF).

This research was funded in part by Citro Biomedical Inc., Lexington, MA.