

149 **INHIBITORY EFFECT OF RAPAMYCIN ON CCL<sub>4</sub>-INDUCED HEPATIC FIBROSIS IN RATS.** JL Zhu, E Fritzel, SL Liu, J Wu, AA Mirza, and MA Zern. 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 therapeutics, and in an earlier report we showed that FK-506 enhanced the fibrogenic process in *in vivo* and *in vitro* models of liver fibrosis. In the present study, we investigated the effects of a new immunosuppressant agent, rapamycin, in a model of liver fibrosis. Unlike FK506 or cyclosporin A that bind to calcineurin, rapamycin has not been shown to have renal side effects.

**Methods:** Male Sprague-Dawley rats were divided into Control (n=3, no treatment), CCl<sub>4</sub>-treated (n = 5, 50% CCl<sub>4</sub>, 1.0 mL/kg, i.p., twice weekly for 7 weeks), and CCl<sub>4</sub> + Rap. (1.5 mg/kg/day, i.p., n=4) groups. Liver sections were assessed for the degree of fibrosis, and steady-state mRNA levels were determined by Northern blot hybridization analysis. The *in vitro* 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 CCl<sub>4</sub> for seven weeks, liver sections in the CCl<sub>4</sub>-treated group displayed progressive fibrosis. The combination of CCl<sub>4</sub> with rapamycin attenuated the degree of fibrotic infiltration as indicated by histology and a semiquantitative fibrosis score (p < 0.01). The collagen content in the CCl<sub>4</sub>-treated group (438.4±20.0 µg/g) was markedly higher than that in the controls, and the content in the CCl<sub>4</sub> + rapamycin group (260.5±29.4 µg/g, p < 0.01), was significantly lower than that in the CCl<sub>4</sub>-treated group, and similar to the control group (202±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 CCl<sub>4</sub> was abrogated by rapamycin therapy. When freshly isolated hepatic stellate cells were incubated with rapamycin for 24-48 h, there was no significant effect on procollagen or TGF-β1 mRNA levels. However, rapamycin was shown to significantly inhibit <sup>3</sup>H-thymidine incorporation into 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 would be reasonable to select the newer agent for at least some liver transplant patients, especially those who are likely to be subjected to fibrogenic stimulation in the post-transplant period.

150 **IL-4 INVOLVEMENT IN APOPTOSIS INDUCTION DURING LIVER ALLOGRAFT REJECTION.** F Conti, P Grude, P Podevin, C Chereau, D Houssin, Y Calmus. Laboratoire de Recherche Chirurgicale, Hôpital 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. Cytokines, 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 then to 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, IFNγ, IL-2, IL-4) or chenodeoxycholic acid (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 semi-quantitatively graded, and ELISA detection was expressed as enrichment of oligonucleosomes released into the cytoplasm.

Apoptosis was minimal in normal liver and in uncomplicated transplants, present in recurrent hepatitis C and biliary complications, and strongly increased during acute rejection and particularly during chronic rejection. In the *in vitro* study, hepatocyte apoptosis was induced by bile acids and IL-4, the other cytokines having low effects, as summarized in the table:

	IL-1	TNFα	IFNγ	IL-2	IL-4	CDCA
TUNEL	0,6±0,4	0,7±0,3	0,5±0,1	0,5±0,2	2,5±0,5	2±0,4
ELISA	1,3	1,4	1,2	1	2,4	2,2

These results show that hepatocyte apoptosis is an important phenomenon during liver allograft rejection. Apoptosis was strongly induced by IL-4 and CDCA. IL-4 overexpression, which has been previously shown during liver allograft rejections, could thus have an effector role in this process by inducing cell apoptosis.

151 **HEMODYNAMIC CHANGES IN ANHEPATIC VERSUS ISCHEMIC ACUTE LIVER FAILURE EXPERIMENTAL MODEL.** Kostopanagioutou G<sup>1</sup>, Theodoraki K<sup>1</sup>, Pysopoulos N<sup>2</sup>, Rigopoulou E<sup>2</sup>, Dafinos N<sup>1</sup>, Prachalias A<sup>1</sup>, Smirniotis V<sup>1</sup>, Tsantoulas D<sup>2</sup>, Papadimitriou I<sup>1</sup>. Liver Support Unit Aretaieon Hospital Athens University<sup>2</sup> 1<sup>st</sup> Medical Dept. Sismanoglou General Hospital, Athens - Greece.

The removal of the non functioning liver in the case 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 our study was to compare the hemodynamic alterations in the anhepatic and the ischemic experimental model.

**Methods:** Three different groups (n=36) of young landrace pigs, weighting 20-25 kg were studied. Group A (control group n=8) underwent laparotomy and was studied for 8 hours. Group B (anhepatic model n=14) underwent total hepatectomy and bridging of the inferior vena cava with the portal vein via heparin coated tubes and was studied for 8 hours. Group C (ischemic model n=14) underwent ligation of the hepatic artery and portal-caval anastomosis. The duration of the study was 8 hours. A Swan-Ganz catheter was inserted in the internal jugular vein and blood samples were taken at 0, 3, and 8h after the introduction of anesthesia. Heart rate, cardiac index, mean arterial pressure, blood pressure, MPAP, SVO<sub>2</sub>, PCWP, SVR, PVR, PO<sub>2</sub>, PCO<sub>2</sub>, DO<sub>2</sub>, SaO<sub>2</sub>, VO<sub>2</sub>, etCO<sub>2</sub>, pH, Hb, ALT, AST, Blood NH<sub>3</sub>, Albumin, were measured. No inotropic agents were administered during the procedure. Statistical evaluation was performed using one way ANOVA analysis.

Results	CI (l / min / m <sup>2</sup> )			SvO <sub>2</sub> (%)		
	A	B	C	A	B	C
0	5.6±1.1	5.8±1	5.5±1.1	80±7.5	81.2±6.5	80.2±7.5
3	5.3±0.9	3.4±1.4*	4.2±1.5...	78.1±1.1	64.2±4.5*	78.1±1.1
8	4.9±1	3.2±1.2*	4±1.1	79.2±1.0	52.5±6.4*	75.5±1.1

p<0.05

Results	PCWP (mmHg)			SVR (dyn·sec·cm <sup>5</sup> )		
	A	B	C	A	B	C
0	7±2	8±1	8±2	1022±101	1106±113	1210±99
3	10±3	6±1	6±2	1078±94	2712±110*	1702±101*
8	11±2	4±2*	8±1	1111±84	3552±151*	2350±81*

**Conclusions:** 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 maintenance even of a destroyed liver may be preferable in cases of hepatic failure in terms of hemodynamic stability.

152 **HEPATOCTYTE TRANSPLANTATION PREVENTS DEVELOPMENT OF BRAIN EDEMA IN PIGS WITH ISCHEMIC LIVER FAILURE.** N Arkadopoulos, TM Khalili, O Detry, H Lijla, Y Middleton, 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.

Intracranial hypertension leading to brainstem coning is a major cause of death in fulminant hepatic failure (FHF). Mannitol, barbiturates and hyperventilation have been used in this setting, but most patients are either refractory to medical management or cannot be treated because of related complications. In this study, we demonstrate that in pigs with ischemic liver failure, hepatocyte transplantation prolongs survival and prevents development of intracranial hypertension.

**Methods:** Pig liver cells were isolated using two-stage EDTA/collagenase digestion. Viability of cells was always greater than 90%. Adult pigs (30-40 kg) were used.

Group I pigs (n=6) underwent intrasplenic transplantation of 2.5 x 10<sup>9</sup> allogeneic hepatocytes under Cyclosporine A (10mg/kg/day; IM) immuno-suppression. After three days, to allow cell engraftment, pigs underwent portocaval shunt and transection of all liver attachments. Group II pigs (n=7) received no cells prior to induction of liver necrosis. In all animals, a subdural bolt was inserted for intracranial pressure (ICP) monitoring and femoral vessels were cannulated for blood pressure monitoring and glucose supplementation. Body core temperature was kept at 37°C through external heating. ICP, systolic and mean arterial pressure, heart rate and neurologic status were recorded at frequent time intervals.

**Results:** Data are shown as mean±SD. Group I pigs survived longer than Group II controls (28 ± 7 hrs vs. 22 ± 2 hrs; 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 hrs post-op. 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, cerebral perfusion pressure in Group I was significantly higher than in Group II (44 ± 13 mmHg vs. 17±3 mmHg, p<0.05). Transplanted pigs had lower blood levels of NH<sub>3</sub> (727 ± 281 vs. 1248 ± 667 µg/dl), creatinine (0.96 ± 0.25 vs. 1.4 ± 0.2 mg/dl) and alkaline phosphatase (722 ± 170 vs. 1026 ± 158 U/L). At autopsy, all pigs had liver necrosis. In Group I pigs, the spleen sections showed clusters of viable hepatocytes (PAS staining). **Conclusions:** Intrasplenic transplantation of allogeneic hepatocytes prolonged survival in pigs with liver necrosis and prevented development of intracranial hypertension. These findings have important implications for the clinical management of FHF.

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