Abstract# C2058

Oxidative Stress and Apoptosis in a Pig Model of Brain Death (BD) and Living Donation (LD). M. Sereinigg, 1 A. Puntschart, 2 T. Seifert-Heaton, Y. Ma, W. Jassem. 1Department for Transplantation Surgery, Medical University Graz, Graz, Austria; 2Department of General Surgery, Medical University Graz, Graz, Austria; 3Department of Neurology, Medical University Graz, Graz, Austria; 4Division of Biomedical Research and Section for Surgical Research, Medical University Graz, Graz, Austria; 5Division of Oncology, Medical University Graz, Graz, Austria; 6Department of Gastroenterology and Hepatology, Medical University Graz, Graz, Austria.

Background: As organ shortage is increasing, the acceptance of marginal donors including brain death (BD) donors, living donation (LD) becomes more important. The aim of this study was to compare these two groups regarding organ damage caused during brain death (BD), cold ischemic time (CIT) or after reperfusion due to oxidative stress or the induction of apoptosis. The aim of this study was to study a panel of genes involved in oxidative stress and apoptosis and compare these findings with immunohistochemistry from a BD and LD donation (LD) pig model and after cold ischemia time (CIT).

Methods: BD was induced in pigs; after 12 h organ retrieval was performed; heart, liver and kidney tissue specimens were collected in the BD (n=6) and in a LD model (n=6). PCR analysis for NFKB1, GSS, SOD2, PPAR-alpha, OXSR1, BAX, BCL2-L1, and HSP 70.2 were performed and immunostaining was used to show apoptosis and nitrosative stress induced cell damage.

Results: In heart tissue of BD BAX, BCL2-L1 and HSP 70.2 increased significantly after CIT. Only SOD2 was over-expressed after CIT in BD liver tissue. In kidney tissue, BCL2-L1, NFkB, OXSR1, SOD2 and HSP 70.2 expression was significantly elevated in LD. Immunohistochemistry showed a significant increase in activated CCaPase 3 and nitrotyrosine positive cells after CIT in BD liver in kidney tissue but not in heart tissue.

Conclusion: The up-regulation of protective and apoptotic genes seems to be divergent in the different organs in the BD and LD setting; however, immunohistochemistry revealed more apoptotic and nitrotyrosine positive cells in the BD setting in liver and kidney tissue whereas in heart tissue both BD and LD showed an increase.

Abstract# C2059

The Mode of Death of the Liver Donor Imprints Distinct Immune Adaptive Responses On the Hepatic Resident Lymphocytes. E. Xystrakis, X. Huang, M. Cortes, A. Prachalias, M. Rela, S. Fuggle, N. Heaton, Y. Ma, W. Jassem. Institute of Liver Studies, London, United Kingdom.

Background: Experimental liver transplantation studies have shown that brain death (BD) induces a systemic inflammatory response, which increases the immunogenicity of the graft. However, the impact of BD on graft intra-hepatic immune cells is currently unclear. In this study we assessed the phenotype and function of the adaptive immune cells of liver allografts, obtained from brain-dead (BD), deceased after cardiac death (DCD) and living donors (LD), prior to transplantation.

Methods: Liver-resident lymphocytes were isolated from liver perfusates obtained before implantation, to assess their phenotype and function after ex vivo polyclonal stimulation by anti-CD3 and anti-CD28 antibodies. We compared lymphocytes obtained from BD (n=22), DCD (n=10) and from LD (n=10) as control.

Results: We found that liver-resident lymphocytes from DCD donors preferentially produce IL-17 (IL-17 positive cells being 4.5% in DCD, 0.9% in BD and 0.8 in LD p <0.01). This IL-17 secretion is attributed to CD4 T (mean at 3.3% in DCD, 1.6% in BD and 1.2% in LD p<0.05) and CD8 T cells (mean at 1.07% in BD, 2.7% in DCD and 1.2% in LD p<0.05).

In contrast to DCD, lymphocytes from BD donors were enriched in CD8 T cells which exhibit an activated phenotype (mean of CD8+CD69+ cells at 60% in BD, 38% in DCD and 37% in LD, p<0.05).

Assessment of cytokine production shows a significant increase in IFN-gamma production by CD8 T cells of BD grafts (mean at 32% in BD, 15% in DCD, p=0.01 and 11% in LD) which correlates with cold ischemia time and peak level of AST measured in the recipients in the first week post transplantation.

Conclusion: These findings suggest that the events surrounding the mode of death of the donor lead to distinct adaptive immune responses in the graft. This can have an impact on transplant outcome and may represent a possible target for therapeutic interventions.

Abstract# C2060

New Insights in Fatty Liver Preservation: A Role for Carbonic Anhydrase II. M. Bejaoui,1 M. Amine Zaouali,1,2 E. Pantazi,1 E. Folch-Puy,1 H. Ben Abdennabi,1 G. Hotter,1 J. Rosello Catafau. Experimental Pathology, Instituto de Biomedical Research of Barcelona, Barcelona, Spain; 2Molecular Biology and Anthropology Applied to Development and Health (UR12ES11), Faculty of Farmacy, University of Monastir, Monastic, Tunisia.

Purpose: During liver graft preservation, the switch to anaerobic metabolism provokes accumulation of lactic acid and leads to lower pH. Studies on the relevance of pHe and pHi are controversial. It can either activate proteases and induce cell death or protect cell against apoptosis. Carbonic anhydrases (CA) are ubiquitous metalloenzymes involved in many physiological and pathological processes, including pH and CO2[sub]2[sub]/[sub]homeostasis. However, the role of CA in liver graft preservation has not been investigated.

The aim of this work was to evaluate the effects of CAII by its addition to the IGL-1 preservation solution or when it was inhibited by acetazolamide (AZ).

Results: Fatty liver preserved in IGL-1+CAII showed lower injury and better function when compared to IGL-1 alone. IGL-1+CAII solution induced a significant phosphorylation of Akt and significant decreases in MAPKs (ERK, p38 and JNK) during reperfusion. This was consistent with a major decrease of liver apoptosis parameters (caspase 3, caspase 9, and GSK3β). Surprisingly, the AZ pretreatment protected efficiently steatotic liver grafts, as revealed by lower transaminases, higher bile production and decreases in MAPK levels. AZ showed differential protective mechanism of liver as it fails to activate Akt. Also, it reduced vascular resistance which was not observed with CAII addition.

Conclusion: Data reported here, demonstrated that CAII is a potential pharmacological target for preserving liver grafts against cold ischemia reperfusion injury.

Abstract# C2061

Effect of Continuous Hypothermic Oxygenated Machine Perfusion On DCD Liver Organs Following Liver Transplantation. H. Zhou, H. Lu, F. Zhang, X. Wang, L. Lu. Liver Transplantation Center, The First Affiliated Hospital of Nanjing Medical University(NJMU); Key Laboratory of Living Donor Liver Transplantation of Ministry of Public Health, Nanjing, China.

Background: Organ pool was expanded after introduction of liver graft from donors after cardiac death. But owing to the inevitable warm ischemia before liver procurement, these liver showed a higher incidence of several complications compared with primary liver nonfunction. Widespread utilization of DCD liver graft requires novel preservation strategy.

Methods: Liver transplantation (LTx) using Sprague-Dawley rats (250g, male) was employed to assess potential critical preservation methods. All rat donors were randomized into following groups according to liver procurement and preservation: control group, conventional LTx with no warm ischemia and liver grafts were preserved in cold (4 °C) UW solution for 6 hours; DCD group, cardiac arrest was induced, warm ischemia (37 °C) time was 60 minutes and then liver grafts procured were preserved as control group; Continuous hypothermic oxygenated machine perfusion with UW solution group (CHOP-UW), liver graft procurement was similar with DCD as control group; Continuous hypothermic oxygenated machine perfusion with UW solution group (CHOP-UW), liver graft procurement was similar with DCD as control group; Continuous hypothermic oxygenated machine perfusion with UW solution group (CHOP-UW), liver graft procurement was similar with DCD as control group.

Results: Survival rates at 72 hours post transplantation were 78% in control group and 0% in DCD group while most animals died within 3 days after operation. Liver damages were alleviated dramatically in CHOP-UW group and CHOP-NPS group. 72 hours after operation, AST and LDH levels in control group, CHOP-UW group and CHOP-NPS group all fell to normal range and showed no significant difference among three groups but still remained elevating in DCD group. Survival curve showed that survival rate within 2 weeks was 90% in control group and 0% in DCD group while most animals died within 3 days after operation. 70% of animals in either CHOP-UW group or CHOP-NPS group survived.

Conclusion: CHOP can alleviate DCD organ damages and improve animal survival after subsequent liver transplantation. It also provided a promising technique for DCD organ preservation. Further studies are needed to analysis and improve our CHOP-NPS system.