RO-075 PRESERVATION OF NORMAL MORPHOLOGY OF HUMAN LIVERS AFTER 24 HOURS OF HYPOTHERMIC MACHINE PERFUSION. A FIRST-IN-MAN STUDY

D. Monbaliu[1], Q. Liu[1], L. Libbrecht[2], R. De Vos[1], K. Vekemans[1], O. Detry[3], J. van Pelt[1], T. Roskams[1], J. Pirenne[1]. *[1]Abdominal Transplant Surgery, University Hospitals, Leuven, Belgium; [2]Pathology, University Hospital, Ghent, Belgium; [3]Surgery and Transplantation, University Hospital, Liège, Belgium*

Background: Hypothermic Machine Perfusion (HMP) of kidneys preserves organ integrity better and longer than Simple Cold Storage (SCS). Interest in liver HMP is increasing but there are no data on the capacity of HMP to preserve the morphology of human livers for prolonged periods. We developed an HMP device for human livers with dual arterial/portal perfusion and separate pressure/flow controls.
Methods: After ethical and Belgian Liver Intestine Committee (BLIC) approval, 6 human livers considered potentially transplantable but discarded due to mild changes, recipient contra-indication and eventually failed ET-reallocation were machine-perfused during 24hrs at 4-6°C using preservation solution KPS-1™. Metabolic/biochemical/hemodynamics parameters and standard/electron microscopy were assessed.
Results: During HMP, pO2 decreased whereas pCO2 increased, suggestive of initial aerobic metabolism rapidly replaced by anaerobic metabolism as indicated by rising lactate and decreasing pH. AST in perfusate progressively increased: 538±484, 631±515, 927±657 and 945±573 IU/L at 30min, 1, 6 and 24hrs. Arterial and venous vascular resistances decreased from 1.29±0.67 and 0.25±0.30 (at the start) to 0.52±0.47 and 0.13±0.13 mmHg/min/ml after 24hrs HMP ($p=0.13$ and $p=1$, respectively). On detailed light/electron microscopic examination after 24hrs HMP, morphology/architecture was well-preserved. Some sinusoidal dilatation and enlargement of Disse space were seen. Hepatocytes, Kupffer and sinusoidal cells ultrastucture was well-maintained. Anoxic vacuoles were seen in some hepatocytes. Occasionally, hepatocytes contained slightly swollen mitochondria with less electron-dense matrix (reversible changes) or more rarely, flocculent densities (irreversible changes). Other cytoplasmic organelles appeared normal.
Conclusion: This study shows -for the first time- that HMP is capable to preserve the morphology and cellular integrity of human livers for prolonged periods (>24hrs). Randomized control trials of transplantation of machine-perfused livers are planned to determine the added value of HMP compared to SCS to preserve human livers.

RO-076 LIVER WASHOUT PRECONDITIONING: A USEFUL TOOL TO PROTECT THE GRAFT AGAINST REPERFUSION INJURY

Mohamed Amine Zaouali[1], Sussagna Padrissa-Altés[1], Hassen Ben Abdennebi[2], Agustín Garcia-Gil[3], Olivier Boillot[4], Joan Roselló-Catafau[1]. *[1]Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Barcelona, Spain; [2]Physiologie Humaine, Faculté de Pharmacie, Université de Monastir, Monastir, Tunisia; [3]Servicio de Cirugía, Hospital Clínico, Zaragoza, Spain; [4]Unité de Transplantation Hépatique, CHU-Edouard Herriot, Lyon, France*

Antecedents: Hepatic ischemia reperfusion injury contributes to the initial poor function or primary non-function after transplantation. This is due to the cold preservation, rewarming and reperfusion, respectively. We evaluated the benefits of using a new rinse solution for flushing liver grafts before reperfusion.
Experimental: Sprague-Dawley rats (180-200 g; n= 6 for each group), were classified as follows: Group 1 (controls) = Livers preserved in UW solution (24 hours; 4 C) were flushed with Ringer lactate solution (at room temperature)and then subjected to 2 h-reperfusion at 37 C using an isolated perfused liver model; Group 2 (washout solution) = Same as 1 but the liver grafts were flushed (at room temperature) with the new rinse solution composed by CaCl2.2H2O (1.3 mM), KH2PO4 (5 mM), NaH2PO4 (20 mM), MgSO4.7H2O (5 mM), lactobionate (100 mM) and rafinose (30 mM) at pH=7.4; Group 3 = Same as 2 but with polyethyleneglycol-35 (PEG35) addition at 5g/L and Group 4 = Same as 3 but with PEG35 addition at 1g/L. Liver injury (AST/ALT) and function (Bile,%BSP, vascular resistance) were measured and correlated with activated adenosine monophosphate protein kinase (AMPK) and heme-oxygenase-1 (HO-1) and HSP70 activities, oxidative stress (MDA) and nitric oxide (NO).
Results: The use of this graft washout solution prevented liver injury (AST/ALT) and ameliorated hepatic function (bile production, vascular resistance) when compared to those washed with RLS, only. This was accompanied by decreases in GLDH levels (mitochondrial lesion) and oxidative stress. Graft washout benefits were associated with increases in NO (e-NOS activation); as well as the induction of cytoprotective factors such as AMPK and HO-1 and HSP70, respectively.
Conclusion: This new "washout" solution containing PEG35 protects the liver grafts against reperfusion injury.

RO-077 HYPOTERMIC MACHINE PERFUSION (HMP) VERSUS STATIC COLD STORAGE (CS) IN KIDNEY ALLOGRAFT PRESERVATION. PROSPECTIVE CASE-CONTROL TRIAL

Annalisa Amaduzzi, Fausto Catena, Giulia Montori, Matteo Ravaioli, Antonio Pinna. *Multiorgan Transplant Centre, Sant'Orsola Hospital, Bologna, Italy*

Background: The shortage of organ availability for transplant has led to an increased use of expanded criteria donors grafts to enlarge the donors pool. It has been suggested that HMP may improve early outcome after transplantation of kidneys donated after cardiac death, but no prospective case control trial have been reported in brain death donor. Aim of the present trial is to identify the most effective preserving method comparing in a randomized case-control trial hypothermic machine perfusion with the current standard of static cold storage preservation.
Methods: From October 2008 to February 2011, 59 pairs of kidney from consecutive 18 to 79 years old donors were included in the present trial. One kidney was randomly assigned to HMP and the contralateral kidney to CS. Among the 59 kidneys enrolled in HMP group, 11 have been excluded for technical/logistic issues or renal artery unavailability. Primary endpoint was delayed graft function (DGF), secondary endpoints were DGF lenght, primary non function (PNF), serum creatinine level and clearance, acute rejection, acute tubular necrosis, length of hospital stay and allograft and patient survival.
Results: No statistically significant difference was found between graft preserved by machine perfusion and cold storage in terms of DGF rate (37,8% vs 30%, respectively $p>0.05$). No significant differences were observed for the other secondary end points.
Conclusion: The effectiveness of preservation with HMP in brain death donors, both ideal and marginal, appear controversial; more data need to be collected in selected donors.

RO-078 GASEOUS HYDROGEN SULFIDE (H_2S) IS PROTECTIVE DURING CARDIAC ISCHEMIA/REPERFUSION

Pauline M. Snijder[1,2], Rudolf A. de Boer[3], Eelke M. Bos[1,2], Joost C. van den Born[1], Willem-Peter T. Ruifrok[3], Inge Vreeswijk-Baudoin[3], Marcory C.F. van Dijk[1], Henri G.D. Leuvenink[2], Harry van Goor[1]. *[1]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [2]Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [3]Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands*

H_2S can reversibly induce a hypometabolic state in mice, and has anti-apoptotic, anti-inflammatory and ROS scavenging properties. We investigated whether gaseous administration of H_2S is protective in cardiac IRI and whether a state of hypometabolism is required for a beneficial effect.
Male C57BL/6 mice were assigned to one of three different treatment regimens receiving 0 (control), 10 ppm, or 100 ppm H_2S starting 30 minutes pre-ischemia until 5 min pre-reperfusion. IRI was inflicted by temporary ligation of the left coronary artery for 30 minutes. Core body temperature was maintained at 37°C. CO_2-production during H_2S treatment was measured by respirometry. Cardiac damage and fibrosis were determined in haematoxylin-eosin (1 d) and Masson (7d) stained sections. To investigate granulocyte influx, sections were stained for Ly-6G.
CO_2-production of mice treated with 100 ppm H_2S rapidly declined to ~60% of basal levels. Treatment with 10 ppm had no effect on CO_2-production. IRI caused significant damage in controls compared to sham-operated animals after 1d and 7d ($p<0.01$). No effects of 10 ppm H_2S on relative infarct size was observed at 1d, while treatment with 100 ppm H_2S reduced infarct size by 62% ($p<0.05$). At 7d, both 10 ppm and 100 ppm H_2S showed a reduction in fibrosis compared to control animals (relative fibrotic area: sham 2.0%; control 17.2% ($p<0.001$ vs sham); 10 ppm 7.0%; 100 ppm 7.4% (both $p<0.01$ vs control)). The influx of granulocytes was reduced by 46% after treatment with 100 ppm H_2S ($p<0.05$) but was not affected by 10 ppm H_2S.
We conclude that gaseous administration of H_2S is a promising treatment for reducing cardiac IRI. Since IRI is a frequent and important cause of myocardial damage during cardiac transplantation, H_2S may be used in these settings to salvage myocardial function.

RO-079 IDENTIFICATION AND QUANTIFICATION OF CIRCULATING ENDOTHELIAL CELLS IN PORCINE MODELS

Ivo C.J.H. Post, Robert Weenink, Albert C.W.A. van Wijk, Thomas M. van Gulik. *Department of Surgery, Surgical Laboratory, AMC, Amsterdam, Netherlands*

Introduction: Endothelial cell damage is regarded as a crucial step in the pathogenesis of several vascular disorders. In humans, the amount of circulating endothelial cells (CECs) correlates to disease intensity and plasma markers as von Willebrand factor and E-selectin in ANCA-associated vasculitis, kid-