TRANSPLANT INTERNATIONAL

1: NEW ASPECTS OF CELL METABOLISM DURING IRI

O/1 AUTOPHAGY IN ISCHEMIA REPERFUSION INJURY

J.P. Decuypere
Leuven, Belgium

O/2 MICROARRAY ANALYSIS AFTER PREOPERATIVE DIETARY RESTRICTION REVEALS POTENTIAL MECHANISMS INVOLVED IN THE PROTECTION AGAINST RENAL ISCHEMIA-REPERFUSION INJURY

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Background: Ischemia-reperfusion injury (IRI) is inevitable during kidney transplantation leading to oxidative stress. We previously reported that short-term preoperative dietary restriction (DR), 3-day fasting and protein-free diets protect against renal IRI while a fat-free or carbohydrate-free (CHO-free) diet do not. To understand underlying mechanisms, we performed microarray analysis and compared gene expressions of (non)-protective diets in search for pathways involved in the effect.
Methods: Male C57BL/6 mice were randomized to preoperative normal food or 2 weeks 30% DR, 3-day fasting, 3-day protein-free, 3-day CHO-free or 3-day fat-free. Kidneys were harvested after each diet. Gene expressions were analysed by Affymetrix and pathway analysis by Ingenuity. Cut-off for significance was set on fold change $\geq$1.5 and p-value$<$0.05.
Results: Compared to ad libitum fed, 2 weeks 30% DR resulted in 492 differentially expressed genes (DEG). Three-day fasting led to 2604 DEG and a protein-free diet to 391 DEG. The fat-free diet resulted in zero and a CHO-free diet in 1717 DEG. Seventy DEG overlapped in all protective diets, with an overlap of 30 with CHO-free. Ingenuity revealed involvement of metabolic processes like retinol biosynthesis as well as stress responses like Nrf2-pathway. Preliminary in depth analysis shows differences in these pathways between the protective diets and CHO-free, in which genes like *txnip* and *mrp1* may play a central role.
Conclusions: This microarray dataset of different dietary interventions points to the involvement of pathways related to oxidative stress resistance and retinol metabolism in the beneficial effects against renal IRI. Since the non-protective CHO-free diet also induced activation of these pathways, these findings need to be further explored. Collectively, these data suggest that a combined action of both metabolic and stress resistance pathways results in protection against IRI given by preoperative dietary interventions.

O/3 LIRAGLUTIDE PROTECTS β CELLS FROM PROINFLAMMATORY TISSUE FACTOR BEARING MICROPARTICLES IN AN *IN VITRO* MODEL OF ISCHEMIA REPERFUSION: INTEREST FOR PANCREATIC ISLETS TRANSPLANTATION

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Instant Blood Mediated Inflammatory Reaction (IBMIR) is associated to ischemia reperfusion events in pancreatic islets graft. IBMIR is characterized by cytokines release and the expression of the procoagulant protein Tissue Factor (TF). The active form of TF is conveyed by microparticles (MPs) that are plasma membrane fragments shed from stressed cells acting as cellular effectors. Recently Liraglutide (Lira), a GLP-1 analog, has been used in the treatment in type 2 diabetes for its cytoprotective effects on β cells.

We evaluated Lira effects on (i) β cells survival and function, (ii) MP release and (iii) TF-MPs signals delivered to target β cells in a MP-mediated cell cross talk-model.

Rat β cells, Rin-m5f, were stimulated by H_2O_2 or IL-1β + TNF-α. MPs generated by oxidative (MPox) and cytokine (MPcyt) stress were isolated and applied to naive Rin-m5f. Effects of Lira on insulin secretion, apoptosis, NF-κB pathway and on TF activity borne by MPs and target cells were assessed.

Direct protection by Lira is revealed by a significant decrease in oxidative stress-induced apoptosis (10% vs. 18%, $p < 0.0001$) and by restored insulin secretion. Indirect protection of β cell occurs through a reduction in MP shedding (oxidative: −25%, $p = 0.006$; cytokinic −18%, $p = 0.01$), through a major decrease in MP-induced apoptosis and NF-κB activation. Moreover, TF-activity at target cell surface and borne by MPs was limited significantly by the addition of Lira. Finally, Lira counteracted the decrease in insulin secretion induced by MPs (MPox: +70%, $p < 0.0001$; MPcyt: +22%, $p = 0.0002$). Pre-incubation of MPs with antibodies to TF restored insulin secretion.

In conclusion, endocrine MPs released in response to stress disseminate TF activity and proinflammatory signals. Lira counteracts noxious MP effects on beta-cell survival and function.Our data bring new hints on the cellular mechanisms prompted by ischemia reperfusion during IBMIR.

This work has received the financial support of Novo Nordisk.

O/4 METABOLOMICS ANALYSIS OF URINE AND KIDNEY SAMPLES AFTER RENAL ISCHEMIA/REPERFUSION INJURY IN MOUSE

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Ischemia/reperfusion (I/R) is the most common cause of acute kidney injury (AKI). Several cellular and tissular pathways have been implicated in renal I/R, including metabolism, ion transport, polarization, apoptosis, oxidative stress, and inflammation. Still, the pathophysiology of I/R-related AKI remains unclear, which confines the management of patients with AKI to supportive maneuvers. Metabolomics approach may help identify the metabolites involved in physiological and pathological changes of integrated living systems, as well as their dynamic fluctuations. In kidney diseases, metabolomics demonstrated enormous potential in the research on drug-induced nephrotoxicity, diabetic nephropathy, as well as AKI. Here, we performed a ^{1}H-Nuclear Magnetic Resonance (NMR) metabolomics analysis on urine and kidney samples from a 12-week-old C57BL/6J mouse model of renal 30-min ischemia followed by 48-h reperfusion ($n = 6$) in order to further investigate the metabolic changes in I/R-induced AKI. Sham-operated mice were used as controls ($n = 6$). The urine spectra were normalized to creatinine levels. Classical metabolomics post-treatment and non-supervised statistical analyses (i.e. Principal Component Analysis) significantly distinguished urine samples of animals with I/R-induced AKI from sham-operated mice. Additional investigations led to the identification of relevant changes in various metabolite levels including taurine, creatine, lactate, alanine, citrate and succinate. A similar discrimination was found after testing and processing kidney samples. Renal metabolite profile, including lactate, lipids, amino acids and taurine, was significantly affected by I/R injury. In conclusion, kidney I/R in mouse induces significant changes of metabolite profiles in both urine and renal parenchyma. Such investigations may help better understand the pathophysiology of I/R-related AKI, thereby leading to the identification of novel therapeutic targets.

O/5 INHIBITION OF STRESS KINASE-DEPENDENT PRO-OXIDANT SIGNALING PREVENTS ISCHEMIA/REPERFUSION INJURY (IRI)

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Background: While the excessive production of reactive oxygen species (ROS) during early reperfusion is causal for the development of IRI antioxidants proved ineffective in the setting of organ transplantation. He we propose approaches, which *a priori* prevent the increase in ROS. Reperfusion is characterized by the activation of stress kinases p38 and JNK. We recently showed that activated p38 increases mitochondrial ROS levels and cardiomyocyte death *in vitro*. Under these conditions also p66SHC translocates to the mitochondria to generate ROS and p66SHC ablation prevented IRI in the Langendorff-perfused heart. p66SHC activation requires phosphorylation. Here we studied both kinases as possible p66SHC activators. Moreover, we tested the benefit of inhibiting p38 *in vivo*.

Methods: We employed *in vitro* (cardiomyocytes, MEFs) and *in vivo* (rat kidney clamping and transplantation) models of ischemia/reperfusion (IR). Kinase function was blocked by small molecule inhibitors or siRNAs. Outcomes were monitored in terms of ROS-induced damage, cell death and organ function.
Results: Simulated IR (sIR) results in the activation of p38 and JNK, phosphorylation of p66SHC, and increased ROS production. However, only the inhibition of JNK blocked p66SHC phosphorylation and decreased ROS production, suggesting that p38 increases ROS independently of p66SHC. p38 activation also plays an important role *in vivo*. In the kidney clamping experiments p38 inhibition almost completely prevented severe functional impairment caused by IR. Using p38 inhibitor as a single agent a significant improvement was also observed in the setting of kidney transplantation. Histological and molecular analyses suggested that protection resulted from decreased redox stress and apoptotic cell death.
Conclusion: Interference with p38 and JNK/p66SHC signaling may provide a therapeutic approach for decreasing damage to cells and organs in the setting of IR.

O/6 FUNCTIONAL CONTRAST FREE MAGNETIC RESONANCE IMAGING (MRI) FOR EARLY DETECTION OF ISCHEMIA INDUCED ACUTE KIDNEY INJURY (AKI) AND ACUTE TRANSPLANT REJECTION

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Background: Acute kidney injury (AKI) is common after solid organ transplantation. The incidence of AKI after lung transplantation is 50–60% and causes increased morbidity and mortality. Detection of AKI oftentimes is delayed due limited sensitivity of diagnostic methods. In this study we present functional magnetic resonance imagining (MRI) imaging techniques to detect and to monitor early changes in the kidney due to experimental AKI such as decrease of renal perfusion and inflammation without contrast media.
Methods: Renal ischemia reperfusion injury (IRI) was induced in C57Bl/6 mice by transient unilateral clamping of the left renal pedicle for 35 and 45 min. MRI was performed prior to surgery and at different time points (d1, d7, d14, d21, d28) thereafter. Renal morphology, glomerular filtration rate (GFR), renal blood flow (RBF), expression of inflammatory cell infiltration and collagen expression was investigated. In a second model isogenic and allogenic kidney transplantation was performed and analyzed by multiparametric functional MRI.
Results: IRI induced renal perfusion impairment was clearly detectable by MRI at 24 h after injury and deteriorated until day 7 after IRI. Edema formation and changes in apparent diffusion coefficients (ADC) correlated with inflammation and fibrosis Furthermore, kidney volume reduced over time starting at d7 and continuing to day 28. Histologically dramatic increase infiltating cells and pro-fibrotic markers were observed. Reduction of MRI measured renal perfusion was verified by PAH clearance measurements to investigate renal blood flow (RBF). In the renal transplant model allogenic rejection caused severe blood flow impairment and edema formation.
Conclusion: Our study shows that contrast free MRI is a new and safe technique for early detection of IRI induced without contrast media. In addition, it is valuable for the detection of microcirculation impairment in allgenic kidney transplant rejection.

O/7 DESIGN OF NANOPARTICLE THERAPEUTICS FOR ISCHEMIA REPERFUSION INJURIES

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Reactive oxygen species (ROS) are known to play versatile roles on the occasion of many important events *in vivo*. However, excessive production of ROS causes significant adverse effect to living body. Such oxidative stress must be controlled appropriately. For example, ischemia-reperfusion (IR) injuries are caused by ROS, which is produced after a long ischemic period and can extend a damaged area. Low-molecular-weight (LMW) ROS scavengers can be applied to such IR injuries to suppress excessive generated ROS. However, such LMW antioxidants spread entire body and cause severe adverse effects because the LMW drug easily internalize inside of cell and disturb normal redox reactions in it. In order to suppress the adverse effects of LMW antioxidants, we have developed a novel redox nanoparticle (RNP), which possesses nitroxide radicals in its core. The covalently conjugate-nitroxide radicals catalytically scavenge ROS, which is excessively generated outside of cells. The RNP disintegrates under acidic environments such as tumor and ischemic tissues and expose nitroxide radicals outside of nanoparticle, which improves ROS scavenging activity. The preparation, physicochemical and biological characterization and antioxidant properties against renal cerebral and cardiovascular ischemia-reperfusion injuries, will be summarized in this paper.

2: IRI AND IMMUNOLOGY

O/8 COMPLEMENT AND ISCHEMY REPERFUSION INJURY

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O/9 ISCHEMIA REPERFUSION INJURY AND CHRONIC INJURY

O. Thaunat
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O/10 PROTECTION AGAINST RENAL ISCHEMIA REPERFUSION INJURY BY DIETARY RESTRICTION AND FASTING THROUGH DOWNREGULATION OF MANNAN-BINDING LECTIN

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Introduction: Ischemia-reperfusion injury (IRI) remains an important problem in transplantation. We recently showed that Mannan-binding lectin (MBL), the initiator of the lectin pathway of complement activation, plays a pivotal role. Preoperative dietary restriction (DR) offers robust protection against renal IRI in mice. However, the mechanism remains to be elucidated. Therefore, we investigated the impact of DR on MBL and the role of MBL in the protective mechanism of DR.
Materials and Methods: Male C57Bl/6 mice were fed *ad libitum* (AL) or underwent 72 h fasting (FA) or 2 weeks 30% DR (n = 8/group). Protein and functional activity of serum MBL-A and –C after DR were assessed as well as liver mRNA analysis. *In vivo*, IRI was induced by 37 min of bilateral clamping of the renal pedicles in all three groups. After clamping, mice were sutured and part of them was reconstituted via intra-peritoneal injection of 100 μg/ml of human MBL. All mice were subsequently observed for a period of 7 days.
Results: Assessment of mRNA showed a significant downregulation in liver MBL expression in both 30% DR and FA ($p \leq 0.004$) groups. In line with this, both MBL-A and MBL-C concentrations were significantly lower ($p \leq 0.001$) after 30% DR (A = 15.4 μg/ml; C = 89.4 μg/ml) and FA (A = 12.4 μg/ml; C = 49.5 μg/ml) compared to respectively 19.9 μg/ml MBL-A and 109.6 μg/ml MBL-C in the AL group. Furthermore, we observed a significant downregulation of lectin pathway activity in both groups. Importantly, reconstitution of MBL following reperfusion breaks DR-induced protection against renal IRI in the 30% DR group, but not in the FA group.
Conclusion: Dietary interventions downregulate the expression and presence of MBL. Reconstitution of MBL following reperfusion breaks DR-induced protection and strongly suggests that downregulation via the lectin pathway may be one of the mechanisms by which dietary interventions protect against renal IRI.

O/11 RECOMBINANT C1INH REDUCES ISCHEMIA REPERFUSION-INDUCED IMMUNE RESPONSE AND IMPROVES KIDNEY GRAFT OUTCOME

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Ischemia reperfusion (IR) induced immune response is a critical issue in transplantation (Tx). Complement and contact system activation are two of its key mechanisms.

We investigated the benefits of pre-reperfusion treatment with recombinant human C1INH (rhC1INH), inhibitor of both complement and contact activation, in a pig model of kidney autotransplantation in which the transplanted kidney was subjected to 60 min warm ischemia prior to 24 h static preservation in University of Wisconsin solution to maximize damage. This preclinical model (with contralateral nephrectomy after Tx) permits us to obtain clinically relevant functional and histological data on both the acute response and the chronic outcomes during a 3 months-follow up period.

ELISA and immunohistofluorescent analyses revealed an inhibition of both C5 and MASP2 in treated animals, indicating full inhibition of the complement system. Treated animals recovered kidney function quickly, as measured by serum creatinine, reaching pretransplant levels by Day 11 (≈100 μM) while Vehicle animals could never recover proper kidney function (≈300 μM, $p < 0.05$ AUC comparison). Urinary NGal measurements showed elevated levels that did not differ between groups, indicating that the treatment did not influence IR-mediated tubular cell necrosis.

With regards to chronic graft outcome, rhC1INH treatment prevented fibrosis development compared to vehicle (10% vs. 30%, $p < 0.05$), in association with improved function at months 1 and 3 ($p < 0.05$). Immunohistochemistry analyses showed a decreased number of invading macrophages within the graft and a reduction of epithelial to mesenchymal transition activation.

In this preclinical model of kidney transplantation, while tubular necrosis was similar between the groups the inhibition of complement activation by rhC1INH at reperfusion permitted the limitation of immune system activation, preserving graft integrity on the short and long term and significantly improving outcomes.

O/12 ENDOTHELIAL PROGENITOR CELLS IMPROVE THE VASCULARIZATION OF THE INTRAMUSCULAR SITE BEFORE ISLET IMPLANTATION IN THE MINIPIG

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Optimization of islet vascularization is of paramount importance in the field of islet transplantation. Endothelial progenitor cells (PECs) obtained from peripheral blood have been shown to be able to differentiate into vessels. These cells demonstrated their ability to improve the vascularization of cardiac muscle after heart attack in a rat model, and to improve clinical symptoms of chronic limb ischemia in humans.
Objective: The aim of our study was to determine if transplantation of PECs with islets would improve islet vascularization, and improve their survival.
Method: In our minipig model of intramuscular islet autotransplantation, we explored the outcome of autologous islet transplantation with or without PECs. For this purpose, we used 7 females minipigs (n = 7). Three hundred milliliters of blood was obtained from each animal by jugular puncture. PECs were isolated from blood and cultured. We next performed a caudal pancreatectomy and islet isolation on each animal, and islets were autotransplanted into the gracilius muscle using four different conditions: islets only, islet + PECs, islet + PECs (double dose), and PECs only. Two months later, grafts were explanted and animals were sacrificed. We used immunofluorescent staining for chromogranin A (red) to identify islets and immunofluorescence staining for von Willebrand Factor (vWF) (green) to assess the neovascularization of the graft. We measured the ratio between the green stained surface and the graft surface for each condition.
Results: The vascular density of the graft zone with PECs was significantly higher than the one without PECs (1.97 vs. 7.37; $p < 0.0015$).
Conclusion: These results suggest, in a clinically relevant preclinical model, that PECs can help to improve vascularization of the islet graft area.

3: IRI AND CELLS

O/13 ISCHEMIA REPERFUSION, INFLAMMATORY SIGNALLING AND DENDRITIC CELLS

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Tours, France

O/14

This lecture has been withdrawn.

O/15 DETERMINATION OF ISCHEMIA REPERFUSION MECHANISMS AT THE CELLULAR LEVEL: THE UNFOLDED PROTEIN RESPONSE

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Ischemia reperfusion injury (IRI) has critical consequences on graft outcome. Further definition of IRI mechanisms will uncover new targets for organ preservation improvement.

We studied the unfolded protein response (UPR) signaling misfolded proteins accumulation through 3 pathways: IRE1α-XBP1, regulating cell death, protein folding, red-ox metabolism and endoplasmic reticulum associated protein degradation (ERAD); PERK-eIF2α-ATF4, modulating apoptosis, red-ox and amino acids metabolism; ATF6, acting on survival, protein folding and ERAD.

We used *in vivo* (pig kidney preservation) and *in vitro* (endothelial cells preserved and reperfused) models to define the kinetics of UPR pathways activation and their involvement in cell survival.

We show that each pathway has a specific kinetic: PERK-eIF2α-ATF4 is activated in the first 12 h of preservation; ATF6 is activated between 18 and 24 h; IRE1α-XBP1 is activated at reperfusion.

Using specific compounds (STF083010 to inhibit IRE1α-XBP1 pathway; Salubrinal to activate PERK-eIF2α-ATF4 pathway and AEBSF to inhibit ATF6 pathway), we show the cell survival is improved by signal specific modulation. Further characterization using siRNA demonstrated that: IRE1α-XBP1 is activated at reperfusion and while splicing of XBP1 is pro-cell death, favoring the kinase activity of IRE1α and the presence of the unspliced version of XBP1 protein is pro-survival; PERK-eIF2α-ATF4 is activated early during ischemia, and if maintained this activation is protective against cell death; ATF6 is activated late during ischemia and its inhibition is protective.

To our knowledge, this is the first study detailing the involvement of the UPR in the physiopathology of IRI at the kinetic level. Our *in vitro* data on UPR modulation highlight several proteins which could be both therapeutic targets and biomarkers of cell resistance to IRI. In the current increased use of marginal organs, these data could improve the quality of graft preservation.

O/16 HEAT SHOCK PROTEIN 90 INHIBITION WITH AT13387 ABROGATES TLR4-MEDIATED NF-κB ACTIVITY AND PROTECTS FROM OXIDATIVE STRESS

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Background: Toll-like receptor 4 (TLR4) is critically involved in renal ischemia-reperfusion injury. Pre-treatment with the Heat Shock Protein 90 (Hsp90) inhibitor, 17-dimethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG) reduces renal ischemia-reperfusion injury but the mechanism of protection remains uncertain. AT13387 is a novel small molecule Hsp90 inhibitor with excellent translational potential. The hypothesis of this study was that AT13387 would lead to breakdown of IkappaB (IκB) kinase resulting in TLR4 mediated NF-kappaB (NF-κB) repression, reduced pro-inflammatory cytokine release and improved cell viability following oxidative stress.
Methods: Human embryonic kidney cells were stably co-transfected to express TLR4 and a NF-κB reporter. Cells were pre-treated with AT13387 or 17-DMAG and exposed to endotoxin-free hyaluronan to stimulate sterile TLR4-specific NF-κB activation. IκB kinase levels were then determined by Western blotting, NF-κB activity by secreted embryonic alkaline phosphatase assay, cytokine expression by array panel and cell viability following oxidative stress using a crystal violet assay. Controls were vehicle and medium only treated cells.
Results: AT13387-treatment resulted in complete breakdown of IκB kinase, which abolished TLR4-mediated NF-κB activation by hyaluronan (AT13387 vs. vehicle, $p < 0.001$), reduced pro-inflammatory cytokine release and increased cell viability following oxidative stress (AT13387 vs. vehicle, $p < 0.001$). In addition, AT13387 had inhibitory efficacy at lower doses than 17-DMAG (IC50 330 nM vs. 870 nM) and more effectively protected cell viability at equivalent doses following oxidative stress ($p < 0.05$).
Conclusions: AT13387 abrogates TLR4-mediated NF-κB activity. This is a novel finding that increases our current understanding of IRI and highlights a potential agent with potent anti-inflammatory effects that could be used for reducing IRI in renal transplantation.

O/17 INJURY OF PERIBILIARY GLANDS AND VASCULAR PLEXUS BEFORE LIVER TRANSPLANTATION PREDICTS FORMATION OF NON-ANASTOMOTIC BILIARY STRICTURES

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Peribiliary glands of large bile ducts have been identified as a niche of progenitor cells that contribute to regeneration of biliary epithelium after injury. It is unknown whether injury of the peribiliary glands is a risk factor for the development of non-anastomotic biliary strictures (NAS) after liver transplantation. Moreover, it is unknown whether pretransplant biliary injury is different in livers donated after brain death (DBD) or cardiac death (DCD). In 128 liver transplant procedures, biopsies were taken from the extrahepatic bile duct and injury was assessed using a systematic histological grading system. Histological injury was correlated with the occurrence of posttransplant biliary strictures and a comparison was made between DBD ($n = 97$) and DCD livers ($n = 29$). Biliary epithelial loss >50% was observed in 91.8% of the grafts before transplantation, yet NAS occurred in 16.4%. Periluminal peribiliary glands were more severely injured than the deep peribiliary glands located near the fibromuscular layer (>50% loss in 56.9% versus 17.5%, respectively; $p < 0.001$). Injury of deep peribiliary glands was more prevalent and more severe in livers that later developed NAS, compared to uncomplicated grafts (>50% loss in 50.0% versus 9.8%, respectively; $p = 0.004$). In parallel, injury of the peribiliary vascular plexus was more severe in livers that developed NAS, compared to uncomplicated grafts (>50% vascular changes in 57.1% versus 20.3%; $p = 0.006$). Comparison of DBD and DCD livers revealed significantly more vascular injury in the latter ($p = 0.005$). Conclusion: Injury of peribiliary glands and vascular plexus before transplantation is strongly associated with the occurrence of biliary strictures after transplantation. This suggests that insufficient regeneration due to loss of peribiliary glands and blood supply may explain the development of biliary strictures.

O/18 HO-1-DEPENDENT CD11B$^+$F4/80^{LO} RENAL CELLS CONFER RESISTANCE AGAINST RENAL I/R

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Introduction: Renal ischemia reperfusion injury (IRI) leads to major cellular damages, mainly driven by strong innate immune responses. The stress-response enzyme, heme oxygenase-1 (HO-1), generates cytoprotective byproducts from heme. Prior HO-1 induction is known to prevent renal IRI. However the role of renal cells expressing HO-1 such as the CD11b$^+$ cells is unknown. The aim of this study was to characterize their role during renal IRI.
Materials and methods: LysMcre HO-1 KO mice, expressing reduced HO-1 levels in myeloid cells, and wild-type (WT) C57/Bl6 mice underwent bilateral renal IRI. After 24 h, plasma and kidneys were harvested. WT mice were treated with hemin 5 mg/kg (i.p., HO-1 inducer) or saline 24 h prior renal IRI. Renal IRI was evaluated by plasma creatinine, KIM-1 and histology. HO-1 levels were assessed by ELISA. Renal inflammation, neutrophil influx and oxidative stress were assessed by PCR, immunostaining and nitrotyrosine levels respectively. HO-1 expression in renal leukocytes was assessed by FACS.
Results: LysMcre HO-1 KO mice displayed worse renal failure as compared to WT mice. In WT mice, hemin upregulated HO-1 expression in CD11b$^+$F4/80^{lo} renal cells specifically. Upon IRI, HO-1 was similarly induced (saline vs.

hemin) but hemin significantly increased the number of $CD11b^+F4/80^{lo}$ cells. Hemin pretreatment improved renal function (i.e. lower plasma creatinine and KIM-1 levels, and fewer tubular necrosis). It also increased plasma HO-1 and reduced transcription of proinflammatory genes (i.e. IL-6, IL-1β, TNF-α, MCP-1 and KC), oxidative stress and neutrophil influx.
Conclusion: Our data strongly suggest that HO-1-dependent $CD11b^+F4/80^{lo}$ renal cells are protective against renal IRI. These cells are specifically stimulated by hemin to confer protection. Interestingly, these $CD11b^+F4/80^{lo}$ exhibit shared characteristics to myeloid-derived suppressor cells. Their protective properties are under current investigations.

O/19 ISCHEMIA REPERFUSION INJURY INDUCED ACUTE KIDNEY INJURY AND LATER RENAL FIBROSIS ARE ATTENUATED BY COMPLEMENT DEFICIENCY

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Background: Ischemia reperfusion injury (IRI) causes acute kidney injury (AKI) and is a relevant complication in solid organ transplantation. AKI has an incidence of ~50–75% after lung- and heart transplantation. In addition, delayed graft function due to prolonged cold ischemia time can be up to 50%. AKI increases morbidity and mortality after organ transplantation and contributes to the progression to chronic kidney disease (CKD). Rapid activation of the complement cascade mediating myeloid cell activation and infiltration is a hallmark of IRI. In this study, we present the distinct role of complement factors and their receptors in an IRI mouse model.
Methods: Renal IRI was induced in mice deficient for C5aR, C3 and C5L2 and in wild type (WT; C57Bl/6) control mice by transient unilateral clipping of the right renal pedicle for 45 min. The renal morphology, the glomerular filtration rate (GFR), renal blood flow (RBF) and expression of pro-fibrotic and pro-inflammatory markers and infiltrating leukocytes were analysed 4 weeks after injury induction.
Results: Complement deficiency attenuated progressive tubulointerstitial fibrosis. Especially, C5L2 deficient mice had less fibrosis and collagen deposition compared to WT mice. C3 deficient mice also showed less tubular atrophy and fibrosis. In addition, inflammatory cell infiltration was significantly reduced compared to WT mice.
Conclusion: C3 and also C5L2 deficiency attenuated IRI and chronic kidney disease and complement inhibition might be a promising therapeutic target in solid organ transplantation.

O/20 ROLE OF INFLAMMATORY RESPONSE IN LUNG ISCHEMIA REPERFUSION INJURY IN A MODEL OF ORGAN-SPECIFIC CULTURE

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Objective: Warm ischemia period in pulmonary non heart-beating donors may increase the ischemia reperfusion (IR) injury. The aim of this study is to analyze the implication of inflammatory response in lung IR injury depending on the length of warm ischemia.
Materials and Methods: We have used a new hybrid model with in vivo lung warm ischemic period (Non heart beating donor) followed by *in vitro* lung slice culture (*in vitro* ex-vivo lung perfusion = *in vitro* EVLP) in Wistar rats. The warm ischemia length was 2 h and 4 h. The reoxygenation of the slices was in presence of lymphocytes (previously prepared from rat's total blood) (mimicking reperfusion) or without lymphocytes (mimicking EVLP) for 1 h, 2 h and 24 h. To assess the inflammatory lesions of IR, we measured the level of Tumor Necrosis Factor α (TNF α) and IL2 released by lymphocytes and lung resident cells. Histological analysis was performed. Statistical analysis has used Sigma stat Software (ANOVA, Tukey and Shapiro-Wilk tests).
Results: In the presence of lymphocytes during reoxygenation, the level of TNF α after 4 h of warm ischemia (127 ± 17 pg / mg protein) was significantly higher than after 2 h (24 ± 3.2 pg / mg protein) ($p < 0.05$), this level increased gradually with the reoxygenation time with a maximum at 24 h. The level of IL2 after 4 h of warm ischemia and 24 h of reoxygenation is significantly greater than after 2 h of warm ischemia ($p = 0.002$).

Histological features showed lesions of bronchiolar epithelium necrosis and desquamation after 2 h of warm ischemia in the presence of lymphocytes. After 4 h of warm ischemia and in the presence of lymphocytes we assessed alveolar epithelium necrosis.
Conclusion: In our hybrid model, the presence of lymphocyte increased significantly the inflammatory response at 4 h of ischemia. Reoxygenation after warm ischemia with lymphocytes is deleterious for the lung tissue, those findings suggest the benefit of EVLP without inflammatory cells for the postconditioning and the assessement of marginal grafts.

4: DONOR MANAGEMENT

O/21 FUTURE IN BRAIN DEATH DONOR MANAGEMENT

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O/22 USING PROTEOMICS AND METABOLOMICS AS NOVEL TOOLS TO IDENTIFY MITOCHONDRIAL DYSFUNCTION AND METABOLIC DYSREGULATION AS CRITICAL FACTORS IN BRAIN DEATH INDUCED KIDNEY INJURY

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Introduction: To gain better insight into patterns of injury and repair in kidneys from brain dead (BD) donors, we used next generation proteomics and metabolomics to identify the effect of BD on the protein signature of rodent renal samples.
Methods: BD was induced in ventilated Fischer rats (250–300 g) using a Fogarty balloon catheter inflated in the epidural space (n = 6) versus sham controls (n = 6). Following BD, ventilation was continued for 4 h. Cortical proteins were extracted using in-solution trypsin digestion and subjected to proteomic analysis using liquid chromatography mass spectrometry (LC-MS/MS). Proteins were identified based on having a > 2 peptide sequence homology and analysed using Progenesis and Ingenuity Pathway Analysis (IPA).

Samples were prepared for 1H-nuclear magnetic resonance (NMR) spectroscopy metabolomic analysis. Data was analysed as normalised intensity ratios and significantly differentially expressed metabolites determined ($p < 0.05$, Prism 6 Graphpad).
Results: Over 1400 proteins were identified, with 43 proteins being differentially expressed between BD and control samples (2 fold up or down regulated, $p < 0.05$). Mitochondrial proteins and proteins concerning small molecule biochemistry and metabolism were the top dysregulated canonical pathways (IPA).

Evaluation of the metabolome revealed significantly increased amounts of lactate ($p = 0.04$) in addition to alterations in other metabolites and intermediaries including increased isoleucine ($p = 0.002$) and decreased AMP ($p = 0.004$), TMAO ($p = 0.015$) and aspartate ($p = 0.015$). Creatinine, signifying renal dysfunction, was higher in the BD group in comparison to controls ($p = 0.009$).
Conclusion: This study underpins the importance of mitochondrial dysfunction and metabolic disturbances in the aetiology of BD induced kidney injury. To prevent kidney injury in the BD donor requires a multifaceted approach targeting protecting against mitochondrial damage and metabolic disturbance.

O/23 TRIPHASIC RESPONSE OF GLOMERULAR FILTRATION RATE AFTER EXPERIMENTAL BRAIN DEATH IN PIGS

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Introduction: Outcome after transplantation of kidneys derived from deceased brain death (DBD) donors is inferior compared to living donor kidneys. The aim of this study was to evaluate the effect of experimental brain death on immediate renal function. Secondarily, we tested the hypothesis that brain death results in acute renal inflammation and modulates renal metabolism.
Materials and Methods: Eight Danish landrace pigs served as DBD donors. After 4 h of brain death, kidneys were removed and stored for 19 h at 4°C in Custodiol® preservation solution, and then transplanted to eight recipients. Glomerular filtration rate (GFR) was defined as 51Cr-EDTA renal clearance. Systemic inflammation was studied by a plasma cytokine assay, while renal inflammation and changes in renal metabolism were measured by qRT-PCR.
Results: Directly following brain death GFR and urine output were reduced, while in the second hour hyperfiltration was observed. Subsequently, GFR and urine output decreased again. Free water clearance increased substantially following brain death indicating reduced vasopressin activity. No systemic inflammation was observed in the donors, while in recipients renal gene expression of IL-6, ICAM-1 and MCP-1, was increased 10 h post-reperfusion. Furthermore, DBD transplantation modulated renal gene expression of LDHA, PC and PCK-1 indicating changes in renal metabolism.
Discussion: In conclusion, brain death caused a triphasic response of GFR. No systemic inflammation in brain death donors was observed presumably due to active control of hemodynamics by fluid administration. I/R injury started to develop after reperfusion, as indicated by signs of mitochondrial dysfunction and renal inflammation, creating a window of opportunity for cytoprotective treatment early in the reperfusion phase.

O/24 A META-ANALYSIS AND META-REGRESSION OF OUTCOMES INCLUDING ISCHEMIC CHOLANGIOPATHY IN DONATION AFTER CARDIAC DEATH LIVER TRANSPLANTATION

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Background: Donation after cardiac death (DCD) liver transplantation is increasingly common but concerns exists over the development of biliary complications and ischemic cholangiopathy (IC). The aim of this study was to compare outcomes between DCD and donation after brain death (DBD) donor grafts.
Methods: Searching MEDLINE, EMBASE, and Cochrane library databases identified relevant articles. Studies reporting on post-transplantation outcomes after Maastricht category III and IV DCD liver transplantation were screened for inclusion. Odds ratios (OR) with 95% confidence intervals were produced using random effects models for the incidence of biliary complications and IC. Meta-regression was undertaken to identify between-study predictors of effect size. PROSPERO Record: CRD42012002113.
Results: Twenty-two studies with 9771 liver transplant recipients (DCD = 1145 and DBD = 8626) were included. In comparison to DBD liver transplantation, there was a significantly increased risk of biliary complications (19 studies, DCD = 990 and DBD = 6832; $I^2 = 32\%$; OR = 2.5 [2.0, 3.1]; $p < 0.00001$) and IC (16 studies, DCD = 982 and DBD = 6140; $I^2 = 77\%$; OR = 10.3 [5.4, 19.8]; $p < 0.00001$) following DCD liver transplantation. Nine factors including donor age, recipient age, model for end stage liver disease score and cold ischemic times for each group as well study year were entered into meta-regression models, but none explained between-trial effect size variability.
Conclusions: DCD liver transplantation is associated with an increased risk of biliary complications and IC. Significant unexplained differences in effect size exist between centres. Further research should identify factors that predict these complications.

O/25 α-MELANOCYTE STIMULATING HORMONE TREATMENT DOES NOT IMPROVE EARLY GRAFT FUNCTION IN PORCINE BRAIN DEAD KIDNEY TRANSPLANTATION

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Introduction: Delayed graft function and primary non-function are serious complications following transplantation of kidneys derived from deceased brain dead (DBD) donors. α-melanocyte stimulating hormone (α-MSH) is a pleiotropic neuropeptide and has been demonstrated to have renoprotective effects in models of acute kidney injury. We hypothesized that α-MSH treatment of the recipient improves early graft function and reduces inflammation following DBD kidney transplantation.
Material and Methods: Eight Danish landrace pigs served as DBD donors. After 4 h of brain death both kidneys were removed and stored for 18 h at 4°C in Custodiol® preservation solution. Sixteen recipients were randomized in a paired design into two treatment groups transplanted simultaneously. α-MSH or vehicle was administered at start of surgery, during reperfusion and 2 h post-reperfusion. The recipients were observed for 10 h following reperfusion. Blood, urine and kidney tissue samples were collected during- and at the end of follow-up. Glomerular filtration rate (GFR) was defined as 51Cr-EDTA renal clearance.
Results: α-MSH treatment reduced urine flow and impaired recovery of glomerular filtration rate (GFR) compared to controls. After each dose of α-MSH a trend towards reduced mean arterial blood pressure and increased heart rate was observed. α-MSH did not affect gene expression of inflammatory markers. Thirty minutes post-reperfusion α-MSH increased plasma aldosterone levels.
Discussion: Surprisingly, α-MSH impaired recovery of renal function in the first 10 h following DBD kidney transplantation. This is explained by activation of the renin-angiotensin-aldosterone system due to hemodynamic instability.

Thus, in a porcine experimental model α-MSH did not reduce renal inflammation and did not improve short-term graft function following DBD kidney transplantation.

O/26 SUDDEN ONSET OF BRAIN DEATH LEADS TO BETTER RAT KIDNEY FUNCTION THAN SLOW ONSET OF BRAIN DEATH

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Donor brain death is an independent risk factor for primary non- or delayed renal graft function. Brain death (BD) leads to a cascade of events including hemodynamic instability and a systemic state of inflammation. Sudden onset of BD can occur after traumatic injury whilst gradual onset of BD can result from intracranial bleeding. The impact of onset of BD on organ quality is not known nor studied in an experimental model.

BD was induced in 64 male Fisher rats by inflating a Fogarty catheter in the epidural space. Gradual onset BD was achieved by inflating at a speed of 0.015 ml/min until confirmation of brain death. Sudden onset of BD was achieved by inflating at a speed of 0.45 ml/min for 1 min. Blood pressure (BP) was maintained above 80 mmHg through the administration of plasma expanders or norepinephrine. Temperature, end tidal CO_2 and O_2 saturation were maintained at physiological levels. Organs, blood and urine were collected after 0.5, 1, 2 or 4 h of BD.

During the induction, gradual onset BD showed a consistent drop in BP to 40–60 mmHg and a subsequent increase, whereas sudden onset BD led to an increase above 200 mmHg with a subsequent decrease. We found an increased need of inotropic support in the first hour after sudden onset BD. Plasma creatinine values were significantly increased at all time points in rats subjected to slow onset brain death compared to sudden onset BD and control rats. Furthermore, lipid peroxidation, indicative for oxidative stress, was significantly increased in slow onset brain death rats compared to sudden BD and control rats.

Kidneys retrieved from gradual onset BD donors could lead to inferior renal transplantation outcomes. A possible explanation for inferior outcomes could be an increase of oxidative stress in these kidney grafts. Therefore, future research could be focused on anti-oxidative treatment in the gradual onset BD donor.

O/27 PRECLINICAL PORCINE MODEL OF ISCHEMIA-REPERFUSION INJURIES IN THE LUNG DURING CARDIOPULMONARY BYPASS

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Warm lung ischemia and reperfusion injuries in the lung are of great interest in both thoracic and cardiac surgery. During pulmonary transplantation, especially with non-heart beating donors, these lesions can lead to primary graft dysfunction. During cardiopulmonary bypass (CPB), lungs are vascularized only by the bronchial arteries: so lungs are in subtotal warm ischemia. We evaluated whether CPB is a pulmonary ischemia-reperfusion model by studying inflammation and ischemia-reperfusion injuries in the lungs of healthy swine.

We compared 3 groups of Large White swine (n = 6): the CPB group, in which CPB was established between the right atrium and the aorta; the sham group, animals underwent the same anesthetical and surgical procedure except CPB. The third group was a total cardio-circulatory arrest group. We evaluated an ischemic and a reperfusion time of 120 min. Hemodynamical datas were collected; blood and tissue samples were harvested for serical, proteomical and transcriptomical analyses.

Pulmonary arterial pressure was collapsed in the CPB group compared to sham group resulting in lung warm ischemia. IL-10 was greater in the sham group compared to CPB group at 120 min of reperfusion, and other markers of inflammation (TNFα, IL-6) were similar. We evaluated the HIF-1α pathway, specific of warm ischemia. In lung tissue sample, VEGF protein was increased significantly at 120 min of reperfusion after CPB and 120 min after total cardiac arrest.

That model is an accurate model of warm ischemia in the lungs. Results must be completed by further studies to assess the dynamical expression of genes of HIF-1α pathway. IL-10 may play a protective role in ischemia-reperfusion injuries in the lungs by counterbalancing the adverse effects of proinflammatory cytokines also expressed. That model may help to study warm ischemia during CPB or either during pulmonary transplantation with the development of non-heart beating donors programs.

5: ORGAN CONDITIONING (PRE-PER-POST)

O/28 UPDATE ON (VOLATILE) ANESTHETICS IN PREVENTING ISCHEMIA REPERFUSION INJURY

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O/29 REMOTE ISCHEMIC PRECONDITIONING IN NEUROLOGICAL DEATH ORGAN DONORS (RIPNOD) DECREASES LIVER REPERFUSION INJURY– PRELIMINARY DATA

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Body: Remote Ischemic Preconditioning (RIPC) studies in neurological death organ donors (NOD) have not been reported. In an on-going prospective, randomized, multi-center RIPNOD clinical trial (NCT01515072) to improve donor organ function and organ recovery, a substudy was nested to test the hypothesis that RIPC would decrease reperfusion injury and improve outcomes in liver recipients.
Methods: Over past 29 months, 234 donors were randomized 1:1 to receive RIPC or No RIPC. RIPC consists of 4 cycles of mid-thigh cuff inflation and deflation (5/5 min each) after brain death declaration and again at organ recovery. Donor data was collected prospectively and liver recipient data retrospectively from 3 collaborating centers. Primary outcome is a composite of early allograft dysfunction (EAD) (Total Bili 10 mg% or INR1.6 on Day 7 or AST/ALT2000 U/L in 1st week), prolonged ventilation (intubation>48 h/reintubation in 1st week) or acute kidney injury (2-fold rise in serum creatinine). Secondary outcomes include EAD, peak postoperative AST/ALT, creatinine, ventilator days, and length of stay. Continuous data is presented as medians (25th, 75th percentiles) and categorical as percents.
Results: Audited data is available for 106 donors. Except younger age in RIPC group other risk characteristics were similar between groups.

Characteristics	No RIPC (51)	RIPC (55)	p
Donor age, years	43 (31, 54)	32 (24, 48)	0.02
Donor Risk Index	1.6 (1.4, 1.9)	1.6 (1.4, 1.8)	0.92
Cold Ischemia (CIT), hours	6.3 (3.5, 7.2)	6.0 (5.1, 8.0)	0.18
Warm Ischemia, minutes	37 (31, 42)	40 (32, 43)	0.37
Recipient age	57 (54, 64)	56 (51, 61)	0.25
MELD	17 (13, 26)	21 (14, 29)	0.46
Primary outcome (%)	26 (51)	25 (45)	0.57

Overall the primary and secondary outcomes did not differ significantly between groups. Interestingly, in recipients with CIT $\geq$ 5 h RIPC has more marked effect.

CIT $\geq$ 5	No RIPC (32)	RIPC (43)	p
Primary outcome (%)	20 (63)	20 (47)	0.17
EAD (%)	15 (47)	12 (28)	0.09
Peak AST	1710 (728, 2678)	872 (486, 2196)	0.03
Peak ALT	960 (493, 1578)	529 (300, 1183)	0.02

Conclusions: Our data suggest that RIPC is safe, decreases liver reperfusion injury, and may improve clinical outcomes in recipients of NOD, especially in those with longer cold ischemia. Additional data are needed to substantiate these findings.

O/30 HEMO$_2$LIFE®, A NATURAL OXYGEN TRANSPORTER, IMPROVES DONOR HEART PRESERVATION DURING PROLONGED STORAGE

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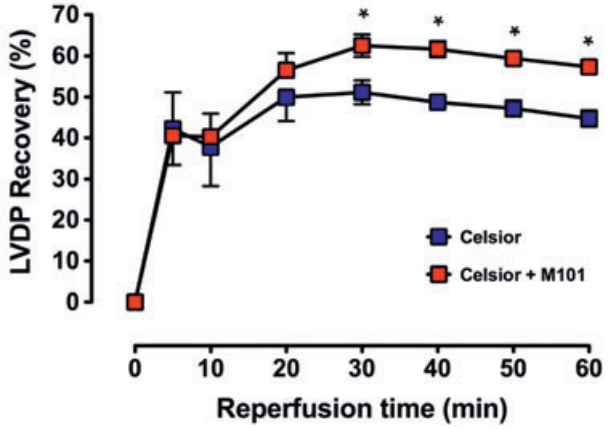
Background: Prior to heart transplantation, static storage of donor hearts is currently limited to 4–5 h, despite profound hypothermia (4–8°C). Because heart transplantation is an emergency procedure, improved protection to extend safe storage duration would be advantageous. We investigated whether the naturally respiratory pigment HEMO$_2$Life®, which is effective at hypothermia for the passive release of oxygen via oxygen gradient, could improve long-term preservation.
Methods: Isolated Langendorff-perfused rat hearts (n = 12/group) were equilibrated (20 min) and function (left ventricular developed pressure: LVDP) measured by intraventricular balloon before arrest with cold (7.5°C) Celsior® solution, either alone (control) or with the addition of HEMO$_2$Life® (Hemarina SA, France) at 1 g/L. Cold storage lasted 8 h prior to reperfusion (60 min) and recovery (as % of pre-ischemic function) was assessed. Hearts (minced and homogenized) were also assessed by TTC staining as a measure of viability and 2 hearts from each group were sliced and assessed by TTC staining for infarct size. Values are expressed as mean±SEM and analyzed by Student's *t*-test.
Results: Hearts recovered rapidly in both groups to a plateau by 20 min of reperfusion; control and HEMO$_2$Life® final recovery (60 min) was 45 $\pm$ 2% and 57 $\pm$ 1% ($p < 0.05$) respectively. Left ventricular end-diastolic pressure recovered to a similar extent in both groups (between 31 to 35 mmHg), as did heart rate (final recovery between 84 to 89% pre-ischemic value); however, coronary flow was significantly ($p < 0.05$) higher in HEMO$_2$Life® group (7.5 $\pm$ 0.7 ml/min) compared to control (5.4 $\pm$ 0.4 ml/min). Viability and infarct size measurements were similar between groups.
Conclusion: The addition of the natural oxygen releasing pigment HEMO$_2$Life® to Celsior® preservation solution significantly improved post-ischemic recovery of heart function. This additive may have major therapeutic potential for clinical heart transplantation.



O/31 ISCHEMIC POSTCONDITIONING OF THE LIVER GRAFT IN ADULT LIVER TRANSPLANTATION. EFFECTS ON ISCHEMIA-REPERFUSION INJURY AND LIVER FUNCTION

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Objective: To compare the results of liver transplantation (LT) performed with or without ischemic postconditioning of the graft (IPo).

Background: IPo has been shown to be beneficial in animal models, but no study has yet been carried out in the setting of clinical liver transplantation.
Methods: One hundred patients undergoing LT were prospectively included to receive IPo (IPo group, n = 50) or not (C group, n = 50). Immediately after arterial reperfusion, IPo consisted in three episodes of arterial occlusion for 1 min, interspersed with 1-min reperfusion pauses. The primary endpoint was postoperative peak values of transaminase (AST); early graft dysfunction (EGD) and liver ischemia-reperfusion (I/R) injury at pathological examination were secondary endpoints. Morbidity, mortality and 1-year graft and patient survival were also evaluated.
Results: Median postoperative AST peak values were slightly but not significantly lower in IPo group (426 vs. 463 IU/L, p = 0.21). Postoperative AST peak values were related to I/R injury that was significantly less severe in IPo group, with fewer graft presenting severe I/R injury (12% vs. 28%; p = 0.029) and severe centrilobular necrosis (10% vs. 26%, p = 0.024) at reperfusion biopsy. Factors independently associated with severe I/R injury were IPo (OR 0.20; 95% CI: 0.06–0.66; p = 0.008) and arterial warm ischemia (OR 1.05; 95% CI: 1.01–1.09; p = 0.008). EGD occurred in 20% of patients in IPo group vs. 26% in C group (p = 0.47), and was associated with severe I/R injury and cold ischemia. Specific and general morbidity, mortality and 1-year graft and patient survival were similar in both groups.
Conclusions: Compared to standard LT, IPo protocol was associated with better graft tolerance to I/R injury at pathological examination. This did not reflect a clear-cut difference in postoperative serum liver function tests. Further studies are now required to define the best protocol of IPo and clarify its clinical impact in LT.

O/32 ISCHAEMIC POSTCONDITIONING REDUCES RENAL WARM ISCHAEMIA REPERFUSION INJURY

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Aim: Ischaemic conditioning, using short repeated sequences of intermittent ischaemia, is a novel strategy that may ameliorate renal transplant ischaemia reperfusion injury. The aim of this study was to assess the effects of direct and remote ischaemic conditioning in a porcine model of renal warm ischaemia reperfusion injury.
Methods: Female landrace pigs weighing 50 kg underwent laparotomy and cross clamping of the left renal pedicle for 60 min. Animal were randomised into 3 groups. Untreated controls (n = 8), direct postconditioning involving 6 × 15 s cycles of clamping then releasing the left renal artery, performed immediately following the 60 min ischaemia (n = 7), or remote periconditioning involving 4 × 5 min cycles of clamping then releasing the left common iliac artery, performed 20 min after renal pedicle clamping (n = 8). Following left renal clamp release a right nephrectomy was performed and animals were recovered for 7 days.
Results: The area under the serum creatinine curve (1378 ± 157 vs. 2001 ± 1022 μM.day; p = 0.033) and peak creatinine levels (316 ± 46 vs. 501 ± 253 μM; p = 0.033) were significantly lower in the direct group compared to control. The peak creatinine was reached on day 1 in all animals in the direct and remote periconditioning groups compared to only 5/8 in the control group (p = 0.026). However, remote periconditioning did not improve renal function compared to control (p = 0.515). There was an increase in urinary protein/creatinine levels in the control and remote ischaemia group from days 1–7 (p < 0.05). In the direct conditioning group levels were recovered by day 5. There was no mortality in any of the groups and no complications directly related to either conditioning technique.
Conclusion: In this *in vivo* large animal model direct renal artery ischaemic postconditioning preserved renal function following warm ischaemic injury. This straightforward technique could easily be translated into clinical practice.

O/33 PREOPERATIVE FASTING PROTECTS AGED OBESE MICE AGAINST RENAL ISCHEMIA-REPERFUSION INJURY

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Introduction: Ischemia-reperfusion injury (IRI) is inevitable during kidney transplantation leading to oxidative stress and inflammation. We previously reported that preoperative fasting in young-lean male mice protects against IRI. Since patients are generally of older age and overweight, factors that may lead to a different response to fasting, we investigated the effects of preoperative fasting on renal IRI in aged overweight male and female mice.
Methods: Male and female F1-FVB/C57BL6-hybrid mice, average age 73 weeks weighing 47.2 grams, were randomized to preoperative ad libitum feeding or 3 days fasting, followed by renal IRI. Body weight, kidney function and survival of the animals were monitored until day 28 postoperatively. Histopathology was examined for all animals and scored for acute tubular necrosis and tubular regeneration. All experiments had the approval of the local Animal Experiments Committee of the National Institute of Public Health and the Environment, the Netherlands.
Results: Preoperative fasting significantly improved survival after renal IRI in both sexes compared with normal fed mice. Fasted groups had a better kidney function shown by lower serum urea levels after IRI. Histopathology showed less acute tubular necrosis and more regeneration in kidneys from fasted mice. Fasting resulted in a body weight loss of 14–17% in male and female mice. In the first week after IRI, body weight declined followed by recovery in the weeks thereafter. The normal fed mice showed a larger and faster weight loss after IRI.
Conclusions: Similar to young-lean, healthy male mice, preoperative fasting protects against renal IRI in aged overweight mice of both genders. These findings suggest a general protective response of fasting against renal IRI regardless of age, gender, body weight and genetic background. Therefore, fasting could be a non-invasive intervention inducing increased oxidative stress resistance in older and overweight patients as well.

O/34 RESUSCITATION OF WARM ISCHEMIA-DAMAGED PORCINE KIDNEY GRAFTS BY VENOUS SYSTEMIC OXYGEN PERSUFFLATION USING ECOSOL

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Introduction: The global shortage of organs for transplantation has necessitated the expansion of the organ pool through increased employment of less than ideal donors. Venous Systemic Oxygen Persufflation (VSOP) has previously demonstrated promising results in preservation of warm ischemia (WI) damaged kidney grafts.
Objectives: The aim of this study was to assess the efficacy of VSOP using the recently developed Ecosol preservation solution compared to cold storage (CS) using Ecosol or the widely used Histidine-Tryptophan-Ketoglutarate solution (HTK) for 24 h preservation of WI-damaged kidney grafts using the Isolated Perfused Porcine Kidney model. Kidneys cold stored for 24 h in HTK without WI served as controls.
Material and Methods: Before retrieval, the renal pedicle was clamped for 45′ in the VSOP-Ecosol, CS-Ecosol and CS-HTK groups. Consequently, kidneys (n = 5/group) were preserved for 24 h and thereafter reperfused for 60′ at 37°C with whole blood/Krebs-Henseleit Buffer medium (20/80%) for renal function assessment.
Results: At 60′ reperfusion, VSOP-Ecosol and CS-Ecosol showed significantly lower intravascular resistance and urine protein concentration compared to CS-HTK and not different from controls, mean±SEM: 0.6 ± 0.0 vs. 0.5 ± 0.1 vs. 2.8 ± 0.9 vs. 0.5 ± 0.1 mmHg/(ml/min)/100 g and 42 ± 11 vs. 106 ± 35 vs. 516 ± 108 vs. 72 ± 18 mg/dl respectively. Urine production and fractional sodium excretion were improved in VSOP-Ecosol compared to CS-HTK and not different from controls (304 ± 43 vs. 79 ± 42 vs. 461 ± 65 ml and 68 ± 2 vs. 90 ± 3 vs. 56 ± 7% resp.)
Conclusion: Both VSOP and CS using Ecosol preservation solution resulted in improved preservation quality compared to CS using HTK. Moreover, VSOP using Ecosol enabled resuscitation of extensively WI damaged porcine kidneys grafts.

6: ORGAN EX VIVO REPAIR

O/35 UPDATE ON RENAL *EX VIVO* WARM PERFUSION

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O/36 A NOVEL METHOD OF PROTEIN DELIVERY FOR EX-VIVO PROTECTION AND REPAIR OF ORGANS FOR TRANSPLANTATION

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In an attempt to increase the number of donor organs, one of the major strategies being employed is the use of extended criteria donor (ECD) organs. Unfortunately, ECD organs are more susceptible to ischemia and reperfusion injury (IRI). Induction of HO-1 expression under conditions of cellular stress protects against IRI but delivery of HO-1 has proven difficult.

The aim of this study is to introduce active and functional HO-1 conjugated to a cell penetrating peptide (CPP) directly *in vitro* to hepatocytes, and *ex vivo* to hepatocytes, endothelial cells, and Kupffer cells in hypothermic and anoxic conditions. This novel strategy may also be used for the *ex vivo* delivery of other protective proteins in hypothermic perfusion systems.

We have been able to consistently produce a functional HO-1 protein fused to a CPP. The ability of our fusion protein to cross cell membranes was demonstrated *in vitro* by incubating rodent (McA-RH7777) and human (Hep G2) hepatocytes with HO-1-CPP. Staining with anti-HO-1 and anti-His antibodies followed by fluorescent secondary antibodies revealed succesful intracellular penetration of HO-1 with localization to the endoplasmic reticulum. Further evidence of the cell permeability of HO-1-CPP has been studied in *ex vivo* perfusion experiments using a Langendorff perfusion apparatus. HO-1-CPP perfused livers sections were paraffin embedded, sectioned and stained with an anti-HO-1 antibody which revealed intracellular localization of HO-1-CPP in hepatic zones 1–3.

Our ability to successfully deliver an active protein conjugated to a CPP to cells of a whole organ in an *ex vivo* hypothermic and hypoxic perfusion model holds great potential for future repair and protection of organs for transplantation. Future studies to determine the ability of HO-1-CPP to modulate the response to ischemia reperfusion injury (IRI) in our *in vitro* and *ex vivo* models as well as conjugating other protective proteins to our CPP are planned.

O/37 AN *EX VIVO* PERFUSION SYSTEM TO ACHIEVE RECOVERY OF HEARTS SOURCED FROM MARGINAL DONORS

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Introduction: Widening the donor pool for heart transplantation can be achieved by sourcing hearts from marginal donors including hearts from donors after circulatory death (DCD). *Ex vivo* perfusion (EVP) is a method of resuscitating such hearts and provides a platform for functional assessment prior to transplant. We developed a novel EVP system to reanimate firstly porcine DCD hearts and subsequently a human heart from a marginal brainstem-dead donor.
Method: In the first stage 23 porcine hearts were procured following circulatory death. All hearts were subjected to a period of primary warm ischaemia followed by 2 h of cold preservation. The period of cold preservation was initially static cold storage (SCS) then machine perfusion with oxygen and finally a combination of static cold storage and retrograde oxygen persufflation. Hearts were subsequently perfused with a warm, oxygenated blood-based solution in our Langendorff system. This same system was then used to perfuse a human heart from a marginal brainstem-dead donor. This donor did not meet criteria for consideration of heart donation.
Results: 15 of the 23 (65.2%) DCD porcine hearts reanimated following reperfusion on the EVP system. Reanimation was achieved with 63.6% (7/11) in the SCS group; 33.3% (2/6) in the machine perfusion group and 100% (6/6) in the persufflation group. The human heart was placed in the rig after a cold ischaemic period of 7 h 4 min and perfused with a mid-thermic temperature solution for a further 2 h 40 min to allow for correction of hyperkalaemia before warming. The heart started to work after a further hour and then maintained until the experiment was terminated after a further 2 h.
Conclusion: The EVP system used has the potential to resuscitate marginal hearts including hearts from DCD. The rig devised in this study can also be used to functionally assess marginal human hearts. This form of viability testing could determine suitability for transplant.

O/38 MIMIKING NON-HEART BEATING LUNG DONORS WITH HYBRID MODEL OF *IN VIVO* ISCHEMIA FOLLOWED BY *IN VITRO* EX-VIVO LUNG PERFUSION (*IN VITRO* EVLP)

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Objective: Non Heart Beating donor (NHBD) in lung transplantation is considered as solution for organ shortage, but is characterized by warm ischemic period, which could be involved in severe ischemia-reperfusion (IR) lesion with early graft dysfunction. Existing *in vitro* models of NHBD are lacking in relevance as they useone type of lung cell with different degrees of resistance to ischemia. Different *in vitro* and *ex-vivo* models have been used to study lung viability in NHBD after various ischemic times. The aim of our work was to describe a new hybrid model combining *in vivo* ischemia followed by *in vitro* reoxygenation using organ-specific culture.
Material and methods: A hybrid model using *in vivo* ischemic period followed by *in vitro* lung slice reoxygenation was set up in a rat model to mimic NHBD in lung transplantation with EVLP. Different markers (bioenergetics, stress oxidant assays and histology) were measured to evaluate the viability of lung tissue after different ischemic times (I0, I1, I2, I4, I 15 h) and reoxygenation times (R0, R1, R4, R24 hours).
Results: No differences were found in Alamar Blue, ATP concentrations, extracellular LDH assays and histology, witnessing extensive viability of up to 4 h of lung to warm ischemia, significantly different compared to other organs (heart, liver and kidney). Statistical difference in lung viability was found at I 15. Oxidative stress assays showed significant differences for [Mn-SOD] with different ischemic times while [GPx] differences were for different reoxygenation times. Histologic features showed difference in this model of ischemia-reoxygenation between bronchial epithelium and lung parenchymal cells.
Conclusion: Our results suggest extensive lung viability of up to 4 h ischemia in this *in vitro* EVLP model, which is in adequacy with current preclinical and clinical publications. This model could be an interesting tool to study different reconditioning techniques using EVLP for marginal grafts.

O/39 MICROVESICLES ABROGATE THE EFFECTS OF RENAL ISCHEMIA REPERFUSION INJURY ON KIDNEY FUNCTION

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Background: Ischaemia reperfusion injury (IRI) causes allograft damage and results in long-term transplant dysfunction. The increasing use of organs from marginal donors makes the development of protective / restorative therapies to treat IRI a priority.

Cell based treatments for IRI may work via paracrine-mediated repair, but the exact mechanism remains undetermined. We have previously demonstrated that a novel pancreas derived repair initiator cell type, termed Pathfinder cells, are a potential therapeutic for damaged kidneys, whose paracrine mediated repair activity is generated via inter cellular transfer of microvesicles (MVs).
Methods: In a novel rat model of renal IRI, the left renal artery is clamped. The left kidney was perfused with 1 ml saline containing Pathfinder cell MVs (treatment group) or vehicle (control group) via an intra-renal artery injection. Clamps are removed to provide 120 mins of warm ischaemia.

Animals are recovered for either 2 or 6 weeks before undergoing terminal GFR studies by inulin clearance, measuring GFR of each kidney via separate cannulation of each ureter. Groups are then compared for GFR and renal histology.
Results: Intra-arterial MV therapy preserves GFR after renal injury at 2 wks (0.105 vs. 0.060 ml/min/100 g, $p = 0.02$) and at 6 wks (0.116 vs. 0.085 ml/min/100 g, $p = 0.0093$).

Light microscopy of renal sections revealed fewer epithelial cell breaks ($p = 0.019$) and fewer hyaline casts ($p = 0.012$) in MV treated kidneys.
Conclusion: In a novel rat model of severe renal IRI, intra-arterial MV therapy significantly improves renal function and reduces histological damage.

Intra-renal artery therapy mimics the route most likely employed in clinical transplantation when the renal artery is easily accessible.

Intra-arterial MV therapy has the potential to reduce peri-transplant injury and stimulate cellular regeneration, improving long-term allograft function. It also offers the major advantage of enabling allogeneic therapy.

O/40 CRITERIA FOR VIABILITY ASSESSMENT OF DISCARDED HUMAN DONOR LIVERS DURING *EX-VIVO* NORMOTHERMIC MACHINE PERFUSION

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Although normothermic machine perfusion of donor livers may allow assessment of graft viability prior to transplantation, there is currently no data on what would be a good parameter of graft viability. To determine whether bile production is a suitable biomarker that can be used to discriminate viable from non-viable livers we have studied functional performance as well as biochemical and histological evidence of hepatobiliary injury during *ex vivo* normothermic machine perfusion of human donor livers. After a median duration of cold storage of 6.5 h, twelve extended criteria human livers that were declined for transplantation were *ex vivo* perfused for 6 h at 37°C with an oxygenated solution based on red blood cells and plasma, using pressure controlled pulsatile perfusion of the hepatic artery and continuous portal perfusion. During perfusion, two patterns of bile flow could be identified: (i) steadily increasing bile production, resulting in a cumulative output of ≥30 g after 6 h (high bile output group), and (ii) a cumulative bile production <20 g in 6 h (low bile output group). Concentrations of transaminases and potassium in the perfusion fluid were significantly higher in the low bile output group, compared to the high bile output group. Biliary concentrations of bilirubin and bicarbonate were 4-times and 2-times higher in the high bile output group. Livers in the low bile output group displayed more signs of hepatic necrosis and venous congestion, compared to the high bile output group.

In conclusion, bile production is an easy assessable biomarker of hepatic viability during *ex vivo* machine perfusion of human donor livers. It could potentially be used to identify extended criteria livers that are suitable for transplantation.

O/41 ANTI-APOPTOTIC EFFECTS OF 3,3′,5-TRIIODO-L-THYRONINE IN THE LIVER OF BRAIN-DEAD RATS

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Donor organs retrieved from Brain death (BD) are the main source of organs for liver transplantation. However, BD negatively affects organ quality resulting in impaired graft function and patient outcome. BD causes hemodynamic instability, hormonal impairment and a cascade of inflammatory events resulting in poorer transplantation outcomes. T_3 preconditioning has shown anti-apoptotic and pro-mitotic effects in liver ischemia/reperfusion injury. Therefore our aim is to study the effects of T_3 on liver tissue of brain dead animals.

Male fisher rats were used. BD was induced by inflating a Fogarty catheter in the epidural space. T_3 (0.1 mg/kg) or vehicle were administered intraperitoneally 2 h prior to BD induction (n = 7 per group). Sham operated animals were used as a control. Animals were kept mechanically ventilated and blood pressure above 80 mmHg for 4 h after which organs were harvested. Temperature, end tidal CO_2, and oxygen saturation was controlled and kept at normal values. Gene and protein expression was measured with real-time reverse transcriptase–polymerase chain reaction and immunohistochemistry.

T_3 administration reduced plasma AST and ALT levels in brain dead animals. BD resulted in an increased gene expression of IL-6, MCP-1 and IL-1β and a decreased expression of TNF-α, which was not altered by T_3 – administration. The BAX/Bcl-2 ratio increased in brain dead animals and decreased after T_3-treatment. Caspase-3 was also reduced after T_3-treatment. No difference was found in Cyclin D expression nor Ki-67 presence in BD animals treated with T_3.

T_3 displays protective preconditioning effects in the liver of brain dead rats resulting in decreased liver injury and apoptosis. So far, we have found no anti-inflammatory or regenerative mechanism responsible for the protective effects of T_3 pretreatment. Further research will need to be performed to gain insight into the protective effects of T_3 preconditioning and its implication into the clinical practice.

7: MACHINE PERFUSION: WHY AND HOW?

O/42 MACHINE PERFUSION OF THE LIVER, WHERE ARE WE GOING?

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O/43 METABOLOMIC PROFILE OF PERFUSATE AND BILE COMPARING MACHINE PERFUSION WITH A NEW CELL FREE OXYGEN CARRIER SOLUTION AND COLD STORAGE PRESERVATION IN A PORCINE MODEL OF LIVER TRANSPLANTATION

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Background: This is the first series of experiments in a pre-clinical large animal model using MP with a newly developed cell free oxygen carrier solution (OCS) under subnormothermic conditions (21°C).
Methods: Two groups of 6 pigs underwent orthotopic liver transplantation after a cold ischemia time of 9 h. The control group had the livers preserved with cold storage (CS) and the study group with MP. Prospective data was obtained from peripheral blood, tissues, perfusate and bile before, during and after liver preservation. Additional transcritptomic, metabolomic and inflammatory mediator analyses were performed.
Results: The MP had significantly better survival (100% vs. 33%, $p < 0.05$) when compared to CS. Sixteen additional parameters analyzed showed a significantly ($p < 0.004$) better graft function in the MP group. Gene expression was driven towards reanimation and resuscitation pathways in the MP group. Genes expressed in metabolic, cellular, and cell communication processes were significantly upregulated by MP. MP donwregulated IFN-α and IFN-γ transcription factors while minimizing inflammation and apoptosis. IL-RA and IL-12/IL-23p40 levels were significantly higher in the MP group. CS livers showed significantly higher levels of adenosine monophosphate (AMP), nucleosides adenosine, guanosine, inosine and hypoxanthine. MP livers cleared lactate, produced urea and sustained gluconeogenesis. Bile production was significantly higher during the first 24 h and throughout the study in the MP group. Bile acid release during perfusion was suppressed while sterol synthesis was increased in the MP group after transplant. MP livers showed greater levels of cholesterol, lathosterol, and campesterol in the bile after reperfusion.
Conclusions: Effective ex-vivo oxygenation provided by MP with an OCS has a clear beneficial impact, in gene regulation, modulation of inflammatory mediators and down-stream metabolic functions of liver allografts preserved with this new technology.

O/44 METABOLOMIC ANALYSIS OF PERFUSATE FROM CADAVERIC KIDNEYS STORED BY HYPOTHERMIC MACHINE PERFUSION

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Background: Hypothermic Machine Perfusion (HMP) provides an opportunity for assessment of cadaveric kidneys prior to transplantation. The aim of this study was to use Nuclear Magnetic Resonance (NMR) spectroscopy to assess the metabolomic profile of perfusate from cadaveric kidneys with both immediate and delayed graft function (IGF/DGF).
Methods: Perfusate was sampled at 45 min and 4 h of HMP. 1-D NMR spectroscopy was used for sample analysis. Resultant NMR spectra were examined using Chenomx profiling to identify metabolites and their concentrations. Clinical parameters were recorded to correlate with the profiles. Data were analysed using IBM SPSS 19 (IBM Corp. Armonk, NY).
Results: Samples were analysed from the perfusate of 29 cadaveric kidneys. Glucose concentrations were significantly lower in DGF kidneys compared to those with IGF at both 45 min (8.045 v 9.829 mM, $p = 0.006$) and 4 h (8.219 vs. 10.626 mM, $p = 0.003$). Concentrations of inosine and leucine were significantly different between DGF and IGF kidneys at 45 mins (0.002 v 0.013 mM, $p = 0.009$ and 0.010 v 0.006 mM, $p = 0.036$) and gluconate levels were also significantly different at 4 h (51.152 v 57.258 mM $p = 0.009$).
Discussion: NMR spectroscopy can identify differences in the metabolic profile between DGF and IGF kidneys. Glucose metabolism may be an important pathway in the development of post-transplant DGF. This type of analysis may help to identify markers to indicate the quality of kidneys prior to transplant.

O/45 MEASUREMENT OF OXYGEN LEVELS IN HUMAN LIVERS UNDERGOING HYPOTHERMIC OXYGENATED MACHINE PERFUSION

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Oxygenation of the perfusate in HMP remains a highly debatable issues, many researchers believe it is unnecessary to add oxygen to the perfusate, but this view has been challenged recently.

We have been successful in measuring the partial oxygen pressure in livers undergoing hypothermic oxygenated machine perfusion (HOMP), both inside the liver and in the perfusate in real time.

Rejected human livers were used. The livers were initially stored in a cold storage box for transport. On arrival to our center, the portal vein was cannulated and perfusion achieved using the LifePort machine (Organ Recovery System, Zaventem, Belgium).

Fiber-optic oxygen sensors were used to measure PaO_2 inside the liver and in the perfusate outside.

Oxygen was supplied using 100% oxygen cylinder (flow of 0.5 l/min).

Initially, the circuit was allowed to run unoxygenated. Oxygen was then started and continued until a steady state was reached, before switching the oxygen off. PaO_2 measurements were recorded until the oxygen levels went down to pre-oxygenation levels.

Our study showed that it is possible to measure the oxygen levels inside the organ in real time. The PaO_2 in the perfusate was faster to reach a steady state after starting oxygen flow, compared to tissue sensors. Tissue sensors had a slower increase in oxygen levels and in addition, a lag period before the oxygen levels started to increase. A possible explanation for these observations would be that the tissues were utilising most of the oxygen supplied, leading to an oxygen debt during the non-oxygenated phase. Maximum PaO_2 in the perfusate was higher than that in the tissues too

On stopping oxygen flow, time taken for PaO_2 levels to drop to pre-perfusion levels was shorter in tissues compared to perfusate.This could be due to the fact that oxygen was still being consumed by the tissues after the oxygen supply was stopped.

We conclude it is possible to measure the kinetics of increase/decrease and oxygen levels in HOMP.

O/46 MACHINE PERFUSION FOLLOWING STATIC COLD STORAGE IN KIDNEY TRANSPLANTATION DECREASES THE DURATION OF DELAYED GRAFT FUNCTION AND TIME TO HOSPITAL DISCHARGE

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In Brazil the incidence of DGF is very high (60–70%) mainly due to an inadequate care of the donors and long cold ischemia time. This high incidence of DGF is associated to a longer hospitalization and poorer long term graft survival. The objective of this work is to analyze the incidence of DGF, its duration and the time of hospitalization after transplantation in patients who received a kidney preserved in the machine perfusion (MP Group) after a long time of cold storage. We report the data from forty kidneys from DD preserved in the MP transplanted from 2/2013 to 12/2013 and compare their evolution to 136 kidney transplants preserved by Cold storage (Control Group), realized from 11/2008 to 5/2012 at our hospital. Results: The mean total ischemia time was 32.8 $\pm$ 6.5 (22.7 $\pm$ 5.1 CS plus 10 $\pm$ 2.1 MP) for MP and 22.7 $\pm$ 4.8 for Control Group ($p < 0.0001$). Donor age (46.5 $\times$ 43 Y), Creatinine (1.35 $\times$ 1.30) and death by stroke (57.1% $\times$ 50%) were not significantly different between MP and Control group patients. DGF incidence was 62.5% for MP compared to 76% in the control group ($p = 0.14$). However, DGF duration (days on dialysis) was 3.8 in the MP compared to 10 days in the control group ($p = 0.021$). The hospital discharge was 13.8 days after transplantation for the MP and 19 days for the control group (($p = 0.011$) and

the mean creatinine at discharge was 2.14 for MP and 2.6 for the control group. In conclusion, in a group of patients with a high incidence of DGF the use of MP after cold storage did not decrease DGF but contributed to a faster recovery of renal function and to a shorter time of hospitalization.

O/47 ARE PRE AND POST-TRANSPLANT RENAL HEMODYNAMICS MEASURE WITH THE PERFUSSION MACHINE AND DOPPLER ULTRASOUND STUDIES ABLE TO PREDICT ACUTE TUBULAR NECROSIS?

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Objective: To evaluate the utility of pre-transplantation Renal Resistance (RR) - as measured with Hypothermic Machine Perfusion (HMP) - and Resistance Index (RI) measured with Doppler-ultrasound in the 24 h post-transplantation period.
Methods: Retrospective analysis of 42 kidneys from Donors after Cardiac Death (DCD) Type II and 24 kidneys from Expanded Criteria Donors after Brain Death (DBD), transplanted from 2009 through 2012, all preserved with HMP. Grafts with clinical criteria and proven biopsy for Acute Tubular Necrosis (ATN) were analyzed.
Results: DBD kidneys had a lower RR than DCD kidneys (0.22 vs. 0.29 mmHg/ml/min; $p = 0.02$). However, no differences were found among these groups regarding RI (0.78 vs. 0.73; $p = 0.12$). A higher number of clinical ATN cases was found in the DCD group in comparison to the DBD group (77% vs. 31%: $p < 0.001$). In only 5 cases, an elevated RI was associated with histological confirmed ATN (sensitivity 18%, specificity 57%). Just 2 cases with ATN and venous thrombosis required transplantectomy. Simple linear regression failed to prove a significant degree of correlation between pre-transplant RR and post-transplant RI ($p = 0.76$).
Conclusions: RR and RI are used to evaluate the hemodynamic situation of the graft. However, they are neither correlated nor able to predict ATN in this study. Ex vivo hemodynamic evaluation would only reflect the preexisting organ damage and vasoconstriction related with ischemic damage. On the other side, *in vivo* assessment of RR with US can be affected by many clinical variables and could be difficult to predict ATN. Perhaps other markers of ischaemic damage can be more efficient to predict ATN than hemodynamic approach.

O/48 NORMOTHERMIC MACHINE PERFUSION REDUCES BILE DUCT INJURY AND IMPROVES BILIARY EPITHELIAL FUNCTION IN RAT DONOR LIVERS

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Background and Aims: Biliary injury may occur during liver procurement and transplantation, especially in livers donated after cardiac death (DCD). Normothermic machine perfusion (NMP) has been shown to reduce hepatic injury, compared to static cold storage (SCS). However, it is unknown whether NMP provides better preservation of bile ducts. The aim of this study was to determine the impact of NMP on bile duct preservation in DCD and non-DCD livers.
Methods: DCD and non-DCD livers obtained from Lewis rats were preserved for 3 h using either SCS or NMP, followed by 2 h *ex-vivo* reperfusion. Biliary epithelial cell injury and function were assessed by quantification of biochemical markers and immunohistochemistry. Morphology of biliary epithelium was analyzed by scanning and transmission electron microscopy.
Results: Biomarkers of biliary injury (gamma-GT and LDH in bile) were lower in NMP preserved livers, compared to SCS preserved livers. Biliary bicarbonate concentration was higher in NMP preserved livers ($p < 0.01$). In parallel, pH of bile was significantly higher in NMP preserved livers (7.63 ± 0.02 and 7.74 ± 0.05, for non-DCD and DCD livers, respectively), compared with SCS (7.46 ± 0.02 and 7.49 ± 0.04, for non-DCD and DCD livers, respectively). Eelectron microscopy of extrahepatic bile ducts demonstrated significantly decreased injury of the biliary epithelium of NMP preserved donor livers. Differences between NMP and SCS were most prominent in DCD livers.
Conclusion: Compared to conventional SCS, NMP provides superior preservation of biliary epithelial cell function and morphology, especially in DCD donor livers. By reducing biliary injury, NMP could have an important impact on the utilization of DCD livers and outcome after transplantation.

8: BIOMARKERS

O/49 BIOMARKERS IN ISCHEMIA REPERFUSION INJURY

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O/50 NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN, BUT NOT KIDNEY INJURY MARKER-1, CORRELATES WITH DURATION OF DELAYED GRAFT FUNCTION

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In kidney transplantation, no specific biomarker is available for evaluating kidney damage. Both neutrophil gelatinase associated lipocalin (NGAL) and Kidney injury marker 1 (KIM-1) have shown to increase after oxidative kidney injury. We evaluated their role as potential biomarker for delayed graft function (DGF).

Twenty recipients of a donation after circulatory death (DCD) kidney transplantation were included. Recipient serum creatinine, eGFR, C-reactive protein, as well as incidence and duration of DGF were monitored. Graft perfusate was collected at the end of cold ischemia time. Serum samples were collected before and after transplantation, and 1, 4 and 7 days after transplantation. NGAL and KIM-1 were measured by ELISA.

Seventeen patients experienced DGF (85%). NGAL in perfusate correlated with donor age ($p = 0.01$) and serum creatinine ($p = 0.05$), both risk factors for DGF. Perfusate NGAL levels were higher in kidneys from donors with a cardiac cause of death ($p = 0.03$). Serum NGAL levels at day one post-transplantation were significantly higher in patients with DGF compared to immediate graft function (IGF) (730 ng/ml [490–1655] vs. 417 ng/ml [232–481] $p = 0.01$). This was not seen at the other time points. Serum NGAL levels correlated with duration of DGF at 1, 4 and 7 days after transplantation. KIM-1 was not detectable in perfusate and serum until day 4 after transplantation in most cases (80%). No correlations could be found.

NGAL is detectable in perfusate and correlates with known risk factors for DGF. Serum NGAL levels at day one can discriminate between DGF and IGF. Furthermore, serum levels at day 1, but also day 4 and day 7 correlate with duration of DGF. Serum NGAL appears to be a valuable biomarker for (the duration of) DGF. Furthermore, NGAL levels in perfusate may reflect graft quality. More studies are needed to determine the clinical potential for this biomarker. No role for early serum KIM-1 levels could be found.

O/51 ROLE OF LIPOCALIN-2 IN A MURINE KIDNEY TRANSPLANTATION MODEL OF ISCHEMIA/ REPERFUSION INJURY (IRI) AND ALLOGRAFT REJECTION

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Background: Organ transplantation is invariably linked to the development of IRI which is known to negatively influence allograft function in terms of allograft rejection. Lipocalin-2 (Lcn2/NGAL), rapidly produced by injured nephron epithelia, is one of the most promising markers of renal damage and DGF. Discrepancy exists about the role of Lcn2 in ameliorating or deteriorating IRI. Determination of Lcn2 function during IRI and acute allograft rejection could provide new therapeutic options for their treatment.

Methods: Murinekidney transplantation in syngenic (C57Bl/6 wild-type [wt] and $Lcn2^{-/-}$) and allogenic (BALB/c to C57Bl/6 [wt and $Lcn2^{-/-}$]) was employed to understand the role of Lcn2 in renal IRI and allograft rejection. Tissue sections were stained with HE and PAS stains and evaluated according to the Banff classification. To estimate kidney function serum creatinine and urea were measured and serum NGAL concentrations were determined by ELISA. Expression of Lcn2, cytokines and immune cell surface markers in the renal graft was measured by RT-qPCR and IHC analysis.
Results: Three hours of ischemia followed by 24 h of reperfusion resulted in a substantial upregulation of Lcn2 and in a severe damage to the renal graft, resulting in reduced kidney function. At post transplant day-7 histomorphology showed moderate to severe tubulitis, interstitial infiltrate and periarterial lymphocytic aggregates, associated with a significant increase in serum creatinine and urea level. Lcn2, TNFα, IFN-γ, IL6, IL1β, ICAM1 and CD3 were strongly upregulated in the rejecting allografts. Lcn2 treatment of the recipient perioperatively resulted in functional and morphological amelioration of the allograft, though not significantly different between C57Bl/6 wt and $Lcn2^{-/-}$ recipients. Daily immunosuppression with CsA could prevent severe acute allograft rejection.
Conclusion: Lcn-2 could be a potential therapeutic target for the treatment of IRI and acute allograft rejection.

O/52 HYPOTHERMIC MACHINE PERFUSION OF THE PANCREAS: A PROMISING PRESERVATION METHOD FOR ISLET ISOLATION

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Introduction: Transplantation of islets of Langerhans is a well-known treatment for type 1 diabetes. Because of high susceptibility of islets to hypoxic damage, the traditional method of organ preservation is not sufficient to completely prevent ischemic damage. Due to the impaired quality of the islets isolated from a donor pancreas, it often requires two or three donor pancreases to treat one patient. For kidneys and livers, hypothermic machine perfusion (HMP) has demonstrated to have beneficial effects on ischemic damage. With our study we aim towards the development of a system for HMP of the human pancreas, to ensure a better preservation of islets of Langerhans.
Methods: For optimal perfusion of the pancreas, a dual arterial perfusion system was developed. Human donor pancreases that could not be used for transplantation were perfused for 6 h with oxygenated Belzer-Machine Perfusion solution. Perfusion fluid was supplemented with a fluorescent dye. Tissue samples of pre- and post-perfused pancreases were taken for histological analysis and ATP-content. Perfusate samples were taken every hour to analyse amylase, lipase and LDH levels.
Results: The uniform fluorescent staining in the tissue samples demonstrated adequate perfusion of the entire pancreas. After perfusion, the architecture of the pancreatic tissue was intact. No signs of apoptosis or inflammation were observed. ATP-content in the tissue increased significantly after perfusion ($p < 0.01$). Analysis of the perfusate showed an increase in amylase, lipase and LDH levels during HMP, likely due to the washout of these enzymes.
Conclusion: In this study, we developed a system for HMP of the pancreas. With our system an uniform perfusion of the pancreas could be realized, whereby integrity of the tissue was preserved. ATP-content increased significantly after 6 h of HMP. In the near future, the effect of HMP on the quantity and quality of isolated islets of Langerhans will be examined.