9: POSTERS

P/1 THE UNFOLDED PROTEIN RESPONSE (UPR) CAN PARTICIPATE TO THE LIVER ISCHEMIC POSTCONDITIONING PROTECTION AGAINST ISCHEMIA/REPERFUSION (I/R) INJURY VIA THE MODULATION OF NF-KB/CHOP/IL-1b SIGNALING PATHWAY

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In the clinical setting of human liver transplantation we found a decrease of necrosis and inflammation in the grafts treated by ischemic postconditioning. Electron microscopy analysis of these grafts showed a more marked dilation of the endoplasmic reticulum (ER) mirroring the unfolded protein response (UPR). Indeed, URP or ER stress response and its interplay with other cellular organelles play an important role in ischemia/reperfusion (I/R) injury in secretory cells rich in ER, such as hepatocytes.

Moreover, in liver tissues submitted to I/R, we observed a modulation of inducible NO synthase (iNOS) and C/EBP homology protein (CHOP) expressions that are different in normal and steatotic livers.

In an immortalized hepatocyte cell line (HHL16) submitted to ER stress, we are studying the UPR response and in particular how its activation modulates CHOP/ IL-1b signaling pathway. We have shown the role of IKKB / NF-kB activation that is influenced by NO levels especially in steatotic liver cells. The modulation of NF-kB/CHOP/IL-1b pathway participates to necrosis/apoptosis switch via autophagy activation. The UPR response through the regulation of NF-kB/CHOP/IL-1b may partly explain the protective effect of post-conditioning.

P/2 ACTIVATION OF THE CALCIUM-SENSING RECEPTOR BEFORE RENAL ISCHEMIA/REPERFUSION EXACERBATES KIDNEY INJURY IN MOUSE

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Background: The calcium-sensing receptor (CaSR) belongs to the family C of G-protein coupled receptors. In cardiomyocytes, hepatocytes and neurons, CaSR activation at the time of ischemia/reperfusion (I/R) promotes cell death. Its role in renal I/R is unknown.
Method: We used a 12-week-old C57BL/6J mouse model of renal 30-min ischemia followed by 48-h reperfusion. Prior to I/R, mice were administered daily with the CaSR agonist, R-568 (250 l g, i.p.), or with vehicle (4% DMSO) for 48 h.
Results: Blood analyses (serum blood urea nitrogen (BUN) levels), examination of kidney histology (using Jablonski's score) and comparative metabolomics by nuclear magnetic resonance on urine and kidney samples showed that R-568 treatment was not associated with significant nephrotoxicity in comparison to vehicle (n = 2 9 8 mice). Still, serum Ca^{2+} level was significantly decreased in R-568-treated animals (2.37 □ 0.27 vs. 2.66 □ 0.21 mM, $p < 0.05$). Following kidney I/R, blood analyses indicated that serum BUN levels increased higher in R-568-treated animals than in controls (4.54 □ 0.82 vs. 0.78 □ 0.21 g/l, $p < 0.05$). Jablonski's score was higher in R-568-treated kidneys than in controls (3 □ 1 vs. 1 □ 1, $p < 0.05$). Immunodetection and quantification of PCNA (proliferating cell nuclear antigen) and ApopTag expression revealed that R-568-treated kidneys were characterized by a significantly higher rate of cell proliferation and apoptosis in comparison to controls.
Conclusions: The activation of CaSR before renal I/R increases the structural and functional damage in mouse. Modulating CaSR activity might serve as a novel pharmacological approach to prevent I/R-associated kidney injury.
Disclosure: The R-568 compound was provided by AMGEN Company (Thousand Oaks, CA, USA) under the agreements MMFA 2012578383 and RPA 2012578387.

P/3 N-OCTANOYL DOPAMINE CONDITIONING HAS AN IMMEDIATE EFFECT ON THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN

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Introduction: N-octanoyl dopamine (NOD) is a lipophilic non-hemodynamic dopamine derivative with potent anti-inflammatory properties. We showed that NOD reduces acute rejection in a rat kidney transplantation model and protects cardiomyocytes from cold ischemia/reperfusion (CI/R) injury in vivo. For both inflammation and CI/R injury, mitochondria in target organs have been identified as key players.Some lipophilic substances as ceramides, N-acyleth-anolamines and others have been described to decrease state 3 respiration (oxygen consumption of the ATP-synthase under the influence of ADP), with a subsequent decrease of the respiratory control ratio (RCR; state 3/state 4 [inhibition of the ATP-Synthase]).The aim of this study is to show that NOD may also reduce the RCR, possibly by the induction of a hypo-metabolism and might limit the production of reactive oxygen species.
Methods: Liver and kidney mitochondria were isolated from Dark Agouti rats. With a Clark-Electrode the oxygen consumption was measured with (1 l M/ 100 l M) or without NOD, followed by stimulation with the typical substrates and inhibitors to investigate the function of each complex. Membrane potential and ROS production were determined with fluorescence microscopy in cultured cytokine-stimulated primary hepatocytes and kidney cells +/□ NOD.
Results: Liver and kidney mitochondria both showed a dose dependent reduction of oxygen consumption in the presence of NOD after stimulation with ADP and a subsequent reduction of the RCR (liver: state3 respiration/RCR - control versus NOD 100 l M, $p = 0.0172/p = 0.001$; kidney: state 3 respiration/ RCR- control versus NOD 100 l M, $p = 0.0121/p = 0.0259$). The other respiratory states showed no differences. We hope to also soon be able to present our fluorescence microscopy data.
Conclusion: NOD inhibits the state 3 respiration in mitochondria. This might be the result of an induced hypo-metabolism and could make mitochondria more resilient.

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P/4 SOLUTION SCOT15 IMPROVES EARLY SURVIVAL COMPARED TO THE UW SOLUTION FOLLOWING RAT LIVER TRANSPLANTATION IN A STRONG ALLOGENIC COMBINATION

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The new preservation solution SCOT15 contains 15 g/l polyethylene glycol (PEG) 20 kDa. Experimental studies and results in clinical transplant suggested that PEG could decrease immunological recognition of foreign antigens and consequently rejection.

Aim of this study was to test this assumption in a rat model of of allogeneic liver transplantation.
Methods: Liver from DA rats (240 □ 1 g) were rinsed either through the portal vein (SOLUTION_pv) or through the aorta (SOLUTION_ao), preserved for 1.4 □ 0.4 h at 4□C in SCOT15 or UW (University of Wisconsin) solutions and then orthotopically transplanted without arterial reconstruction in Lewis rats (285 □ 2 g).
Results: The median days of survival were higher in the 2 SCOT groups than in the 2 UW groups (LogRank): SCOT_pv: 12.4 [10.5□13.3] UW_pv: 10.1. [0.9□ 11.8] (n = 21 and 25, $p = 0.017$). Rinsing the graft through the aorta allowed perfect discoloration of the grafts but decreased survival, especially in group UW: SCOT_ao: 11.2 [10.0□15.3] and UW_ao: 0.2 [0.1□13.4] (n = 23 and 10, $p = 0.086$). At postoperative day 3, 5, 7, 9 there was no difference observed between each group for plasma AST, ALT, CK, LDH, bilirubin, alkaline phosphatase, gGT (n = 7 to 14 per group) levels, but creatinine levels decreased to 14 □ 2 l M at postoperative day 7 in the SCOT_pv group and was between 20 and 21 l M in the other groups (ANOVA, $p = 0.047$). At day 5, histologic examination of sacrificed rats showed a similar inflammatory infiltration in all the groups.
Conclusion: Objective and similar signs of rejection were present in the UW and SCOT groups. With both solutions, flushing the graft through the aorta improved the quality of the rinse but impaired early survival and did not change late survival. However, the SCOT15 solution improved early survival in

comparison to UW. This effect seemed to be related to a reduction in ischemia-reperfusion injuries rather than to a decrease in rejection.

P/5 HISTOLOGICAL INJURY DETECTED IN BIOPSIES OF EXTRAHEPATIC BILE DUCT OF DONOR LIVERS REPRESENTS INJURY IN THE REST OF THE BILIARY TREE, INCLUDING THE INTRAHEPATIC BILE DUCTS

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Histological examinations of distal end of extrahepatic bile duct of donor livers at time of transplantation have revealed signs of severe injury, characterized by a loss of biliary epithelium, mural stroma necrosis and injury of the peribiliary vasculature. It is, however, unknown whether injury of extrahepatic bile duct is representative for the injury in the rest of the biliary tree, including the intrahepatic bile ducts. Aim of this study was to examine whether the degree of histological injury of the distal extrahepatic bile duct is representative for the rest of the biliary tree. Ten donor livers that were not used for transplantation were included after obtaining informed consent from the relatives. After a median of 6 h of cold ischemia, biopsies were taken from extrahepatic and two different levels of intrahepatic bile ducts: sectoral ducts and segmental ducts. Histological injury was assessed using a systematic histological grading system. Biliary epithelial loss of >50% of the biliary lumen was observed in all the levels of the biliary tree. Minimal injury of peribiliary vascular plexus (<50% vascular changes) was observed in 91.9% of all the biopsies, and there were no significant differences between extrahepatic and intrahepatic bile ducts. There were no signs of microthrombi in the peribiliary vasculature at any level. Mural stroma necrosis was not different in extrahepatic and different levels of intrahepatic bile duct. Minimal intramural bleeding (<50% of the bile duct wall) was found in only 5% of all the biopsies. The degree of injury of periluminal and deep peribiliary glands was similar at all levels of the biliary tree. Injury of periluminal peribiliary glands, however, was more severe than injury of deep peribiliary glands (>50% loss observed in 43% and 6.25%, resp.; p = 0.002). Histological examinations of bile ducts of donor livers after cold preservation reveal extensive biliary injury. The degree of injury detected in extrahepatic bile duct of donor livers is representative for the rest of the biliary tree. Biopsies of extrahepatic bile duct of donor livers are a valuable tool for research focusing on bile duct injury in liver transplantation.

P/6 CARDIOPROTECTIVE EFFECT OF FONDAPARINUX IN A RAT MODEL OF MYOCARDIAL ISCHEMIA-REPERFUSION

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Introduction: Fondaparinux (FDX) was shown to be cardioprotective in a rat model of myocardial ischemia-reperfusion. It is able to reduce infarct size after 2 h of reperfusion, involving the activation of the survival pathway SAFE. Our aim was to study if this cardioprotection could be explained by anti-inflammatory mechanisms and a protective effect on the endothelium.
Methods: Wistar male rats were submitted to 40 min of myocardial ischemia followed by 30 min or 2 h of reperfusion. Rats were randomized in 4 groups: control 30 min (n = 7), FDX 30 min (n = 7), control 2 h (n = 7), FDX 2 h (n = 7). FDX groups received a 10 mg/kg intraperitoneal injection of FDX, 10 min before the beginning of reperfusion. Hearts were collected at the end of reperfusion. We studied the expression of mRNA of endothelial markers (P-Selectin, thrombomodulin, EPCR, tissue factor) and pro-inflammatory markers (IL-1b, IL-6 and ICAM-1). We also studied the proteic expression of ICAM-1, tissue factor and pro-inflammatory signalling proteins (NFj B, Ij B and JNK). Leucocyte infiltration was assessed by histochemistry (hematoxylin-eosin stain).
Results: After 30 min of reperfusion, there is a significant increase of the expression of endothelial markers in the FDX group. This difference is not evidenced any more after 2 h of reperfusion, except for the expression of P-Selectin. Regarding the pro-inflammatory markers, there is a significant increase of their expression after 30 min of reperfusion in the FDX group. The same difference was observed after 2 h of reperfusion except for the expression of IL-1b. After 2 h of reperfusion, there is no effect of FDX on the expression of the pro-inflammatory signalling proteins, tissue factor and on leucocyte infiltration in the myocardium.
Conclusion: At early stage of reperfusion, FDX induced cardioprotection was not mediated by an anti-inflammatory effect. Our work suggests that FDX might have a protective effect on the endothelium at 30 min of reperfusion.

P/7 CINACALCET TREATMENT AT THE TIME OF TRANSPLANTATION IS ASSOCIATED WITH A SIGNIFICANT RISK OF DELAYED GRAFT FUNCTION IN KIDNEY TRANSPLANT RECIPIENTS

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The calcium-sensing receptor (CaSR) has been implicated in the ischemia/reperfusion (I/R) cascade in heart, liver and brain. Renal I/R occurs at the time of transplantation (Tx), with a deleterious impact on early graft function. Here, we retrospectively investigated if the use of cinacalcet, a CaSR agonist, in kidney transplant recipients (KTR) influences early graft recovery. All KTR from 2007 to 2012 in our Academic Hospital were prospectively included in a database. Patients actively treated with cinacalcet on the day of Tx were retrospectively identified from this database and matched with controls on (i) type of donor (living [LD], deceased after brain or circulatory death [DCD]); (ii) cold ischemic time (CIT) ± 1 h; (iii) residual diuresis (± 500 ml); and (iv) donor age (± 5 years). Delayed graft function (DGF) was defined as dialysis requirement after Tx. Baseline characteristics were compared between groups with student's t-test or Chi-2 as appropriate. The endpoint was the percentage of DGF in both groups. Among 337 KTR, 36 (10.7%) were treated with cinacalcet at Tx. Control group included 61 patients. Characteristics of patients and donors are summarized in the table. DGF occurred in 42 and 23% of cinacalcet-treated and control groups, respectively (p = 0.05). These retrospective observations suggest that CaSR activation at the time of Tx impairs early graft recovery.

	Cinacalcet (n = 36)	Controls (n = 61)	p
Recipient			
Age at Tx (years)	50.2 ± 10.3	49 ± 13.5	0.92
Sex ratio (% female)	47	41	0.55
Dialysis vintage (years)	3.7 ± 2.1	3.3 ± 3.8	0.57
Resting diuresis (ml)	430 ± 655	444 ± 541	0.91
Multi-organ Tx (%)	5.6	1.7	0.28
Donor			
Age (years)	46.8 ± 11.4	47 ± 11.4	0.93
Sex ratio (% female)	42	46	0.67
LD (%)	2.8	1.6	0.70
DCD (%)	30.6	21.3	0.31
Transplantation			
CIT (min)	779 ± 297	825 ± 255	0.43
HLA mismatches			
A	0.8 ± 0.5	0.9 ± 0.5	0.75
B	1.2 ± 0.7	1 ± 0.5	0.08
DR	0.8 ± 0.4	0.8 ± 0.3	0.99

P/8 INCREASED INFLAMMATION AND FIBROSIS CAUSED BY HYPEROXALURIA IN AN EXPERIMENTAL MODEL OF RENAL ISCHEMIA AND REPERFUSION

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Introduction: Acute kidney injury (AKI) is defined as a rapid loss of renal function due to damage to the organ, resulting in the retention of products of metabolism and uremic toxins that are normally excreted by the kidney. AKI caused by ischemia and reperfusion (I/R) induces renal dysfunction associated with specific markers of inflammation such as TNF-a, interleukins and interferons. On the other hand, the injury I/R may contribute to crystal deposition of calcium oxalate (CaOx) renal tubules, causing additional damage in tubular epithelial cells, inducing necrosis and leading to progressive tubular atrophy and interstitial fibrosis.
Objective: The objective was to assess whether the deposition of calcium oxalate crystals increase renal damage in rats with acute kidney injury and to analyze how animals exposed to ischemia and reperfusion evolve when subjected to an overload of CaOx.

Methods and Results: Male rats received a solution with 0.8% ethylene glycol (EG) and 1% ammonium chloride (NH4Cl) in drinking water, for a period of 4 weeks. Then, they were submitted to 60 min of renal ischemia. The reperfusion injury were analyzed 24 h after the re-establishment of renal blood flow. Serum creatinine, urea and renal tissue histology were evaluated. Addition of EG increased urine volume and led to reduced urine pH. Serum creatinine and urea levels in animals subjected to renal ischemia and reperfusion increased compared to control group. EG treatment showed a further increase in these levels, with a significant increase compared to group I/R. EG treatment also induced higher gene and protein expression of inflammatory cytokines, like CINC2, CINC3, TNF-a, IL-6 and IFN-c, with subsequent higher collagen and a-SMA expression, glomerular alteration and increased crystals presence in tubules after I/R, a characteristic of calcium oxalate deposition.
Conclusions: Renal ischemia and reperfusion injury is increased after crystals deposition in renal tubule, leading to increased inflammation and facilitating renal fibrosis.

P/9 VASCULOTIDE ADMINISTRATION IN A BRAIN DEATH RAT MODEL

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Kidneys derived from deceased brain dead (DBD) donors have inferior outcomes after transplantation compared to kidneys from living donors. Also, DBD donors suffer from bacterial translocation and endotoxemia. A link between Angiopoietin 1 (Ang1), Angiopoietin 2 (Ang2) and endotoxemia has been established. Ang1 and Ang2 are antagonictic ligands that bind to the Tie2 receptor. We aimed to modify the Ang1 levels in our brain death rat model by administrating vasculotide (VT), a synthetic Tie2 agonist, to clarify whether exogenous administration of VT has a protective role and may be of therapeutical value to improve outcome after DBD transplantation.

We administrated 3 l g/kg VT or PBS 30 min before brain death (BD) induction. BD was induced by inflating a subdurally placed balloon catheter in rats. A group of sham operated animals was injected with PBS (n = 7 for all groups). The animals were monitored for 4 h. Just before sacrificing the animals blood was collected and lungs and kidneys were harvested for histology and PCR.

Plasma levels of ALT, AST, creatinine, LDH and urea were equal in both BD groups. The fold induction of ICAM-1, IL-6 and Tie2 in the kidney and lung is not infuenced by VT administration in the BD groups. Tie2 fold induction of both kidney and lung decreased significantly in the BD + PBS group (mean fold induction kidney 1.1 and lung 0.27) compared to the sham + PBS group (mean fold induction kidney 2.54 and lung 10.83).

Functional and inflammatory markers were increased in the BD groups compared to the sham + PBS group and not affected by this dosage of VT. These results show a remarkable effect of brain death on Tie2 fold induction. This reduction suggests a functional role for Tie2 in BD which could not be compensated by administrating VT.

P/10 THE PROPER ISCHEMIC PRECONDITIONING ATTENUATES FUNCTIONAL. METABOLIC. MORPHOLOGIC INJURY FROM RENAL ISCHEMIA-REPERFUSION INJURY IN THE MOUSE

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Purpose Ischemic preconditioning (IPC) has been shown to ameliorate injury due to subsequent ischemia in the kidney. The underlying mechanisms are not completely understood. Heat shock proteins (HSP) play an important role in protecting cells against stress and have been discussed as mediators in preconditioning. In this mouse model study, we tested the hypothesis that ischemia preconditioning increase the expression of HSP resulting in attenuation of renal ischemia-reperfusion injury. Methods Male C57BL/6 mice were randomized to control (non-IPC), 3 min (group I), 5 min (group II), 7 min (group III) ischemia and 10 min reperfusion before 23 min ischemia time after right nephrectomy. Renal HSP70 expression was determined by Western blotting and kidney function was assessed by serum creatinine. Renal cross sections were microscopically examined for tubular necrosis, exfoliation of tubular epithelial cells, cast formation, and monocyte infiltration. Results IPC increased intra-renal HSP expression ($p = 0.031$). Among the three groups, there was significant difference in serum creatinine ($p = 0.042$). Mean serum creatinine level was 1.7 ± 0.3 mg/dl (non-IPC), 1.5 ± 0.4 mg/dl (group I), 0.5 ± 0.2 mg/dl (group II), 1.6 ± 0.4 mg/dl (group III). In group II, tubulointerstitial abnormalities were clearly slighter compared with the other groups ($p < 0.001$). Conclusion Our experiments suggest that (i) IPC could induce HSP expression, but the correlation of HSP expression and I/R injury was not clear (ii) Only proper IPC (5 min/10 min) could relieve renal I/R injury in the mouse model.

P/11 GRADUAL WARMING-UP OF KIDNEYS REDUCES INJURY COMPARED TO IMMEDIATE REPERFUSION

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Background: Reperfusion injury after cold storage (CS) of organs is an inevitable consequence of a transplant procedure. We hypothesize that the sudden warm reperfusion after CS is detrimental for the graft. We therefore evaluated different warming-up temperatures of the organ before reperfusion in order to improve organ quality.
Methods: Rat left kidneys were retrieved and stored in University of Wisconsin solution for 24 h at 4°C followed by immediate reperfusion at 38 °C or gradually warming-up (WU) to 10°C, 25°C or 38°C, using isolated perfused kidney machine (IPK). Renal function and renal injury was assessed by IPK with an oxygenated modified Williams Medium E solution for a total of 90 min.
Results: The increases in biochemical markers of injury such as AST and LDH in perfusate in the control group was significantly greater compared with WU groups ($p < 0.05$). In the 10°C and 38°C WU groups NAG was significantly lower than the control group. Glomerular filtration rate (GFR) at the end of the reperfusion was similar in all the groups while ultra filtrate output was significantly higher in the control group. Sodium re-absorption improved in the WU groups and it was significantly greater in the 25°C WU group. Parallel to this, level of total protein was significantly lower in all the WU groups after 90 min reperfusion. KIM-1 and HSP-70 gene expression was reduced in the 10°C and 25°C WU groups compared to the control group. There were no differences in renal blood flow (RBF) and intra renal resistance (IRR) between the four groups after 90 min of reperfusion. ATP level did not change in all the four groups. Histological evaluation did not show significant differences between the groups.
Conclusion: Gradually warming up is associated with less renal injury and better renal function indicating that reperfusion injury might be reduced.

P/12 N-OCTANOYL DOPAMINE AMELIORATES LUNG FUNCTION IN THE ACUTE PHASE AFTER LUNG TRANSPLANTATION

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Introduction: In contrast to other solid organ transplantations, the 5 year graft survival of lung transplants remains inferior. Brain death leads to a systemic inflammatory release of pro-inflammatory mediators with a consecutive loss in barrier function in the lung, worsened by cold ischemia reperfusion injury after transplantation. Pre-conditioning therapy with dopamine in the brain dead donor has been successfully employed for prolonged graft survival in kidney and heart transplantation. To prevent undesired side effects of dopamine, the non-hemodynamic derivative N-octanoyl dopamine (NOD) was developed. Since NOD showed anti-inflammatory properties and a benefit in acute kidney injury, the aim of this study was to investigate if NOD can decrease acute inflammation and improve lung function in a model of brain dead donor lung transplantation.
Materials and Methods: Twenty four Fischer rats were randomly assigned into three donor groups for isogenic left lung transplantation: (i) untreated control group, (ii) acute traumatic brain death (BD) and (iii) brain death with continuous NOD treatment. All animals were stabilized by fluid resuscitation and ventilated for 4 h before lung harvest. The recipient animals received a single lung transplantation and were ventilated for 6 h after transplantation. Gas-exchange and respiratory system mechanics were analyzed.
Results: Before lung transplantation there were no substantial differences between the three donor groups. However, after transplantation the oxygenation index is better in the control group compared to the brain death groups both 5 min and 6 h. after reperfusion. Interestingly, the NOD treated lungs were the only ones that improved during the 6 h. of ventilation. Soon we hope to be able to show also an anti-inflammatory effect in the NOD treated lungs.
Conclusion: In contrast to untreated donors, NOD therapy in the brain dead donor improves respiratory function in the transplanted lung.

P/13 EARLY ALLOGRAFT DYSFUNCTION DECREASES PATIENT SURVIVAL AFTER LIVER TRANSPLANTATION

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Background: Early Allograft Dysfunction (EAD), the clinical manifestation of Ischemia Reperfusion Injury (IRI), is characterized by a poor graft function immediately after Liver Transplantation (LTx). EAD is defined as peak AST/ALT > 2000 IU/l during first 7 days post-LTx, or total bilirubin >10 mg/dl on day 7, or INR >1.6 on day 7 post-LTx. We studied (i) risk factors of EAD and (ii) its impact on patient survival after LTx in our center.
Methods: Donor/recipient demographics, procurement/preservation/LTx data and patient/graft survival from 552 LTx recipients (01/2000-12/2010) were retrospectively analyzed. Uni- and multivariate models were built for EAD; Kaplan-Meier curves were constructed with log-rank test to compare survival.
Results: One hundred and fifty patients (27%) developed EAD. Procurement team (locally procured versus imported, OR = 0.51, 95% CI = 0.31–0.84), cold ischemia time (OR = 1.16, 95% CI = 1.04–1.32), preservation solution (UW versus HTK, OR = 0.53, 95% CI = 0.31–0.84), Lab MELD (OR = 1.06, 95% CI = 1–1.12) and length of surgery (OR 1.22, 95% CI 1.04–1.42) were independent risk factors for EAD in multivariate analysis. Extended criteria donors & donation after circulatory death were not a risk factor for EAD. ICU and hospital stay were longer in EAD; $p < 0.01$. One and 5-year patient survival was lower in recipients with EAD compared to those without EAD (81.8% vs. 94.5% at 1 year, $p < 0.01$; and 66.3% vs. 81.9% at 5 years, $p < 0.01$, respectively).
Conclusion: EAD decreases recipient survival and strategies to reduce EAD are therefore urgently needed. They should primarily focus on: improving procurement strategies, re-questioning HTK preservation, reducing cold ischemia time and length of intervention. In addition, optimizing preservation techniques (e.g. machine perfusion) and pharmacological modulation of IRI in recipient need to be studied.

P/14 DONOR PRE-TREATMENT WITH THE GERANYLGERANYLACETONE DERIVATE, NYK9354, REDUCES BRAIN DEATH-ASSOCIATED INFLAMMATION IN THE KIDNEY AT ORGAN RETRIEVAL

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Brain dead-derived kidney grafts have inferior transplantation outcomes compared to living donated kidneys. This is due to increased inflammation during the brain death (BD) period. Our previous studies showed that cytoprotective heat shock proteins (HSP) become upregulated at the end of the BD period, suggesting a role for HSP in preventing kidney damage. To reduce the BD-related kidney injury, we aim to increase the HSP expression at the start of BD. Geranylgeranylacetone (GGA) is a drug known to induce HSP expression, however the use of this drug is limited due to its poor pharmacokinetic properties. We investigated whether intravenous treatment (iv) with NYK9354, a GGA derivate with improved physical-chemical properties compared to GGA, can reduce pro-inflammatory changes, and increase HSP expression in an in vivo brain death rat model.

Male F344 rats (275–300 g, n = 34) underwent slow induction of brain death and were ventilated for 4 h. We administered NYK9354 (0.5 mg/kg iv), GGA (0.5 mg/kg iv) or a vehicle 20 h and 0 h prior to brain death induction. Left kidneys were collected after the 4 h BD period.

Renal mRNA expression of the adhesion molecules E-selectin and ICAM-1 were significantly lower in NYK9354- (0.58 ± 0.08 and 0.48 ± 0.07) (p-value <0.05) compared to saline- (1.11 ± 0.13 and 0.76 ± 0.07), or GGA-treated groups (0.89 ± 0.02 and 0.71 ± 0.06). In addition, renal mRNA IL-6 expression was lower in NYK9354- (1.08 ± 0.46) compared to saline- (2.49 ± 0.67), and GGA-treated groups (1.84 ± 0.39). Also, renal neutrophil infiltration was significantly lower in NYK9354- (2.93 ± 0.63) (p-value<0.05) compared to saline-treated groups (4.64 ± 1.13). Renal Hsp72 protein expression was increased in NYK9354- (2.14 ± 0.54) compared to saline- (0.99 ± 0.08), and GGA-treated groups (1.05 ± 0.14).

These results show that NYK9354 reduces pro-inflammation during the BD period in contrast to GGA, indicating NYK9354 as a candidate drug to improve kidney transplantation outcomes in brain dead donors.

P/15 FLUSHING PORCINE DCD LIVERS WITH CYCLO-DEXTRIN COMPLEXED CURCUMIN DOES NOT REDUCE ISCHEMIA REPERFUSION INJURY

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Introduction: Curcumin is a pleiothrophic polyphenol with antioxidant and anti-inflammatory activity. In renal ischemia-reperfusion injury (IRI) transplant models, curcumin improves outcome.
Aim: To investigate whether adding curcumin – as a water soluble Cyclo-Dextrin Curmumin Complex [CDC, Novobion (Espoo, Finland)] – to the flush solution of liver grafts reduces IRI in a model of liver transplantation (LTx) donated after circulatory death (DCD).
Methods: Pig livers were exposed to 15–warm ischemia, flushed with warm (25–C) Ringers enriched with 60 l M CDC and Histidine-Tryptophane-Ketoglutarate (HTK) enriched with 60 l M CDC through aorta and portal vein. Grafts were cold stored (4 h) in HTK and transplanted (n = 6) and compared to controls without CDC (n = 6). Animals were sacrificed at day 4. End-points measured: log AUC_{AST} within the first 3 h post-reperfusion and TNF-a (hepatic IRI), lactate and bile production (liver function) and graft/recipient survival.
Results: LogAUC_{AST} within the first 3 h post-reperfusion was similar in CDC group versus controls (7.15 – 0.57 vs. 7.55 – 0.66, p = 0.28). TNF-a was higher in the CDC group versus controls (275 – 57 vs. 135 – 67 pg/ml, p = 0.003 at 2 h; and 274 – 100 pg/ml vs. 148.5 – 61.53 pg/ml at 3 h, p = 0.025). Lactatewas lower in the CDC group versus controls (3.78 – 2 vs. 8.83 – 4.9 mM at 3 h, p = 0.045). Similar amounts of bile were produced at 3 h post-LTx in the CDC group versus controls: 25 – 19 vs. 15 – 5 ml, p = 0.39). 3/6 recipients were alive at day 4 in both the CDC group and controls.
Conclusion: Despite improved liver function, hepatic IRI, graft/recipient survival was not improved by adding 60 l M CDC to the flush solution in a well validated pig DCD LTx model. The increase of TNF-a by adding an anti-inflammatory compound was not expected. These findings deserve further investigation, especially in light of encouraging results in kidney IRI.

P/16 VIABILITY ASSESSMENT OF DISCARDED HUMAN KIDNEYS USING EX-VIVO NORMOTHERMIC PERFUSION

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Background: Ex-vivo normothermic perfusion (EVNP) is a new technique of preservation that involves circulating an oxygenated blood based solution through the kidney at normal body temperature. Kidney function is regained and therefore it may be a valuable device on which to assess the viability of marginal donor kidneys prior to transplantation.
Methods: From October 2012 to December 2013, 81 discarded human kidneys underwent 60 min of EVNP. Kidneys were perfused with an oxygenated red blood cell based solution at a normal body temperature. Renal blood flow and urine output were the primary functional parameters. Receiver operating characteristic (ROC) curves were used to identify thresholds of these variables for kidney viability. These thresholds, along with macroscopic appearance, were incorporated into a viability score (renal blood flow <63 ml/min = 1; urine output <50 ml/h = 1; macroscopic assessment of perfusion: good = 1, patchy = 2, poor = 3), with a possible total score of 1 to 5.
Results: Forty one kidneys were graded (1–2), 26 (3–4) and 14 grade (5). Grade 1–2 kidneys performed significantly better across all of the perfusion parameters compared to grade 3–4 and grade 5 kidneys. They had a significantly higher renal blood flow ([1–2] 95 – 25, [3–4] 50 – 16, [5] 32 – 13 ml/min/100 g; $p < 0.0001$), higher level of oxygen consumption ([1–2] 66 – 20, [3–4] 36 – 16, [5] 22 – 11 ml/min/g; $p < 0.0001$), higher urine output ([1–2] 124 – 75, [3–4] 55 – 76, [5] 13 – 12 ml; $p < 0.0001$) and higher creatinine clearance ([1–2] 2.2 – 1.9, [3–4] 1.1 – 0.9, [5] 0.1 – 0.1 ml/min/100 g; $p < 0.0001$). Grade 3–4 kidneys performed significantly better than grade 5 kidneys ($p < 0.05$).
Conclusion: EVNP may potentially allow us to quality-assure kidneys prior to transplantation. Based on appearance and functional parameters we consider that grade 1–2 and 3–4 kidneys would be suitable for transplantation.

P/17 INITIAL EXPERIENCE OF SEQUENT "IN SITU/EX VIVO" NORMOTHERMIC EXTRACORPOREAL PERFUSION FOR RESUSCITATION KIDNEY FROM UNCONTROLLED DONORS

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Background: The organ shortage could lead to use of kidney from donors with sudden irreversible cardiac arrest, or uncontrolled donors after cardiac death (UDCD). Inevitable ischemic injury of organs from UDCDs remains the crucial problem. This obstacle caused the refusing from wide clinical acceptance of such kind of kidney. We present first experience of sequent normothermic in situ/ex vivo perfusion in comparison with hypothermic machine perfusion (HMP).
Methods: In 2012, two kidney grafts were procured from a UDCD. Donor was a 40 -year-old man with a traumatic brain injury. The death was declared after irreversible cardiac arrest and failed 30-min advanced CPR in ICU. The warm ischemia time (WIT, 45 min) was defined as the gap between finishing CPR and cannulation of femoral vessels for ECMO. The duration of ECMO with leukocyte depletion "in situ" continued 130 min. Left kidney after explantation was placed in the device for HMP. Right kidney graft was placed in the apparatus for normothermic perfusion with leukocyte-free, modified donor's blood. The time of "ex vivo" perfusion by donor's blood was 340 min., HMP was 450 min. The grafts were transplanted to 48 and 56 years recipients.
Results: There were delayed graft functions for both recipients, which demanded 3 hemodialysis procedures for "normothermic" graft and 7 procedures for "hypothermic" kidney. There weren't the episodes of acute rejection. The 1 year follow-up creatinine levels were 0.105 mM, and 0.135 mM, correspondently.
Conclusions: The cold storage stage perhaps should be eliminated from preservation practice for kidney obtained from UDCDs. Our initial experience shows the necessity of the further investigation for definition of perspectives for wide clinical adoption of sequent "in situ/ex vivo" normothermic extracorporeal perfusion procedures in context of expanding pool of the organ donors.

P/18 NEW PERFUSION APPARATUS TO IMPROVE CARDIAC GRAFT VIABILITY DURING HYPOTHERMIC TRANSPORT

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One of the main problems encountered in heart transplantation is the limited preservation time of the donor heart, commonly less than 6 h. Long-term preservation would allow transport for long distance and adequate time for transplant assessment.

In order to increase this preservation time, a simple, portable and reliable equipment for graft perfusion has been conceived and patented. This system allows pulsatile transplant microperfusion at 4°C during which the heart tissue is simultaneously perfused (during the imitated diastolic phase) and massed (during the imitated systolic phase). To evaluate functionality of this system, feasibility tests were performed on isolated pig heart (n = 12). After harvesting, heart were preserved 20 h in cold cardioplegic medium (St. Thomas' Hospital solution) either by simple storage (control group) or pulsatile microperfusion. Hypothermic heart preservation was followed by reperfusion, performed on an ex-vivo functional testing system at the end of the cold period.

Preliminary investigations demonstrated good viability and functionality of the hearts preserved with this new hypothermic perfusion apparatus. Moreover, hearts preserved with the new perfusion system exhibited an improved mitochondrial function (a better respiration and a lesser mitochondrial permeability transition pore opening) suggesting lesser apoptosis. These encouraging preclinical data allow envisaging a future clinical use, after confirmation of the present results.

P/19 MACHINE PERFUSION PRESERVATION USING ECOSOL ATTENUATES WARM ISCHEMIC DAMAGE IN PORCINE KIDNEY GRAFTS

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Introduction: Expansion of the donor organ pool by increased use of warm ischemically (WI) damaged organs obtained from deceased after cardiac death donors is considered a necessity to combat the universal organ shortage. Hypothermic machine perfusion (MP) has proven to be highly beneficial in preservation of WI damaged organs.
Objectives: The aim of this study was to assess the efficacy of MP using the recently developed colloid-based 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 retrieved without WI, cold stored for 24 h in HTK were employed as controls.
Material and Methods: Before retrieval, the renal pedicle was clamped for 45" in the MP-Ecosol, CS-Ecosol and CS-HTK groups. After washout, kidneys (n = 5/group) were preserved for 24 h by MP or CS using their respective solutions and thereafter reperfused for 1 h at 37°C with whole blood/Krebs-Henseleit Buffer medium (20/80%) for renal function assessment.
Results: At 1 h reperfusion, MP-Ecosol showed significantly higher urine output compared to CS-HTK and not different from CS-Ecosol and controls, mean ± SEM: 332 ± 54 vs. 86 ± 41 vs. 197 ± 27 vs. 443 ± 58 ml. Urine protein concentration and intravascular resistance were lower in MP-Ecosol and CS-Ecosol compared to CS-HTK and did not differ from controls (98 ± 22 vs. 94 ± 34 vs. 439 ± 119 vs. 70 ± 19 mg/dl and 1.4 ± 0.3 vs. 0.5 ± 0.1 vs. 3.0 ± 0.8 vs. 0.6 ± 0.1 mmHg/[ml/min]/100 g resp.).
Conclusion: MP using Ecosol solution was able to attenuate extensive WI damage in porcine kidney grafts and demonstrated improved preservation quality compared to CS using HTK.

P/20 INFLUENCE OF AGING PROCESS ON STRUCTURE AND FUNCTION OF RAT LIVERS SUBJECTED TO ISCHEMIA/REPERFUSION

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Introduction: Ischemia/reperfusion (IR) is considered to be one of the main causes of liver damage after transplantation. Many age-related changes in hepatic structure and function were described. The aim of the study was to evaluate the effect of aging process on rat liver subjected to IR.
Methods: Rats were divided into two groups (C2, IR2) of young rats (2–4 months old) and another two groups (C12, IR12) of old rats (12–14 months old). Rats belonging to group IR2 and IR12 were subjected to 60 min of partial (70%) ischemia achieved by occlusion of branches of the portal vein and the hepatic artery (left lateral and median lobes) followed by 4 h of global reperfusion. Blood samples were obtained before surgery and during reperfusion (0, 30 and 240 min) to estimate the activity of aminotransferases. When the experiment was terminated livers were harvested for histological examination on light microscopy.
Results: Initial activity of ALT was comparable in all groups of rats regardless of age, but initial activity of AST was significantly higher in old rats than in young ones (IR2 vs. IR12, $p < 0.001$). At all points of reperfusion the increase in activity of amiontransferases in ischemic groups was observed and was greater in mature animals than in young ones. No significant differences in hepatic structure were seen for both the sham-operated young and mature non-ischemic animals (C2, C12). Livers from those groups displayed normal hepatic architecture, and only slight degree of steatosis. In ischemic groups (IR2, IR12) the increase in percentage of necrosis associated with intense neutrophil recruitment was observed regardless of age. Adult ischemic rats (IR12) showed the most pronounced changes in hepatic architecture including increased micro- and macrosteatosis and parenchymal cell destruction.
Conclusions: We concluded that the function and structure of mature livers are slightly worsened and under IR conditions such differences are more noticeable.

P/21 INFLUENCE OF AGING PROCESS ON SELECTED PARAMETERS OF OXIDATIVE STRESS IN RAT LIVER SUBJECTED TO ISCHEMIA/REPERFUSION

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Introduction: Ischemia/reperfusion (IR) is considered to be the main cause of cellular damage of various organs. The process occurs, among others, during a procedure of liver transplantation. The aging process is characterized by the loss of adaptive responses to conditions of physiological stress, resulting in an increased susceptibility to diseases and death. We evaluated effect of aging process on selected parameters of oxidative stress in rat liver subjected to IR.
Methods: Animals were divided into C2 and IR2 group of young rats (2–4 months old) and C12 and IR12 group of old rats (12–14 months old). Livers belonged to IR2 and IR12 group were subjected to partial ischemia by occlusion of branches of the portal vein and the hepatic artery (60 min, left lateral and median lobes) followed by global reperfusion (4 h). Upon the completion of the reperfusion livers were isolated and weighted. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx) were determined in liver homogenates.

Results: Changes of SOD, GSH and PGx activity depended on age. Under IR conditions they decreased in young animals and increased in mature animals. In case of SOD activity such changes were not significant. However, in case of GSH level difference between young and old ischemic rats was on the border of significance (IR2 vs. IR12, $p = 0.07$) and in case of GPx activity the difference between young and old animals was significant in both non-ischemic and ischemic groups of animals (C2 vs. C12, and IR2 vs. IR12, $p < 0.05$ in both comparisons). Concentration of MDA depended on age and increased significantly in both ischemic and non-ischemic groups (C2 vs. C12, and IR2 vs. IR12, $p < 0.01$ in both comparisons). Only the negligible increase in MDA level under IR conditions was observed.
Conclusions: We concluded that the changes in oxidative stress parameters are dependent on age and are more evident in young livers.

P/22 IMPACT OF ISCHAEMIA-REPERFUSION INJURY AND STEATOSIS IN PRE- AND POST-REPERFUSION BIOPSIES ON LIVER TRANSPLANT OUTCOMES

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Aims: The aim of the study was to assess the effect of ischaemia-reperfusion injury (IRI) and steatosis on post liver transplant outcomes.
Methods: Pre and post reperfusion liver transplant biopsies from 64 patients (48 donation after brain death or DBD and 16 donation after cardiac death or DCD) were assessed blindly for (i) Presence of hepatocellular injury, based on the extent of neutrophil infiltration and hepatocyte necrosis, (ii) large droplet steatosis and (iii) small droplet steatosis. IRI was said to be present when there was significant increase in hepatocellular injury on the post-reperfusion biopsies. The impact of these factors on graft function, rejection, cholangiopathy as well as in-hospital and ITU stay was evaluated.
Results: There was significantly higher IRI in DCD compared to DBD livers ($p < 0.001$). There was no difference in the degree of large or small droplet steatosis between DCD and DBD livers. Interestingly, surgeon's macroscopic assessment of steatosis had no correlation with the biopsy grading of steatosis or patient outcomes. Presence of more than 30% small droplet steatosis in post-reperfusion biopsies was independently associated with the occurrence of cholangiopathy post transplantation ($p < 0.005$). Though IRI was associated with higher peak AST levels post transplantation ($p = 0.006$), it was not associated with cholangiopathy. Higher grade of hepatocellular injury on post reperfusion biopsies was associated with a higher bilirubin level on day 5 ($p = 0.017$) and a higher peak AST level post transplantation ($p = 0.001$). None of these biopsy factors influenced graft rejection or in-hospital and ITU stay.
Conclusion: Small droplet steatosis may be an important predictor for the development of ischaemic cholangiopathy post transplantation, independent of IRI. Though IRI leads to a slower recovery of graft function, it has no impact on long term graft function. Neither small droplet steatosis nor IRI impact graft rejection or in-hospital and ITU stay.

P/23 NOVEL RODENT HYBRID MODEL OF RENAL ISCHEMIA REPERFUSION INJURY/AUTOTRANSPLANTATION

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Introduction: Ischaemia reperfusion injury (IRI) causes acute kidney injury and allograft dysfunction. No evidence-based treatments are in widespread clinical use. Current in-vivo models cannot mimic the severe IRI observed clinically, without excessive deaths from acute renal failure. Stem cell-based therapies may protect against IRI and aid regeneration of damaged tissues but systemic delivery leads to side-effects that limit dosing and produce sub-optimal drug concentrations in the key tissues. Serum markers commonly tested lack the sensitivity to track changes in renal function in response to IRI +/- therapeutic intervention.
Methods: In a novel rat model of severe left renal IRI the left renal artery is clamped and transected. The kidney is perfused via intra-renal artery infusion of therapeutic agents or vehicle. After arterial anastomosis, clamps are removed to provide 120 mins of warm ischaemia. The right kidney remains to prevent death from acute renal failure. Animals are recovered for a 2 or 6 week period before terminal GFR studies by inulin clearance. Each ureter is cannulated to allow the GFR of kidney to be calculated separately. Kidneys are analysed for histology, and molecular markers of damage.
Results: Compared to sham operations, saline treated animals suffer a long-term reduction in GFR of ~ 40%. Anastomotic patency rates approach 100%. Despite the injury severity, post-operative animal losses are <5%.
Conclusion: Using this model, we inflict a severe, consistent renal injury. Intra-renal artery infusion mimics the route most likely employed in clinical transplantation, where the renal artery is accessible. Inulin clearance most accurately characterizes GFR, allowing full assessment of therapeutic intervention. This model is a useful tool for screening potential therapeutic agents prior to testing a full transplant model. This reduces animal numbers needed to test drugs for clinical transplantation and allows refinement of dosing schedules.

P/24 NATURAL HISTORY OF KIDNEY WARM ISCHEMIC DAMAGE IN RAT MODEL

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One of main problems in transplantology is limitation in donor organs quantity. That is why now transplantologists focus on the usage of suboptimal donor organs and ways of revitalization of these organs. Main factors of damage of transplant organ during transplantation are ischemia occurring after the death of donor and subsequent reperfusion in a body of recipient. In order to understand important damaging pathways influencing on the organ in dead body we performed study of natural history of postmortal kidney as if it were before transplantologists' assistance and initiation of conditioning procedures. We focused on the determination of so called the window of preservation of kidney in the body after the death and understanding main processes which had been initiated under ischemia condition in different time points in rat as a model organism (0, 15, 30, 45, 60, 120, 180 min and 15 h as an end-point post mortem). Specifically we analyzed in homogenized kidneys the level of caspases 3/7 activity as a mark of apoptosis, reduced and oxidized forms of glutathione as a characteristic of oxidative stress, ADP/ATP ratio as a marker of energetic state of the tissue. Also we performed quantitative analysis of gene expression in a time-scaled manner in a panel of genes involving in different pathogenic processes such as apoptosis, necroptosis, oxidative stress response, transcription factors involved in various pathways, components of immune response which could play an important role in subsequent damage of kidney. That allowed us to find out the window of possibilities for resuscitation organ procedure in which damaging processes were not irreversible. Moreover, we determined processes and genes which could be the targets for targeted therapy for prevention, or the minimizing the negative consequences of warm ischemic injuries of rat kidney.

P/25 A MURINE MODEL OF PULMONARY ISCHEMIA AND REPERFUSION INJURY: TRICKS OF THE TRADE

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Pulmonary ischemia-reperfusion injury (IRI) is responsible for considerable post-operative morbidity in patients undergoing lung transplantation, isolated lung perfusion and cardiopulmonary bypass. It may lead to potentially lethal pathologies such as the adult respiratory distress syndrome (ARDS). A variety of animal models of pulmonary IRI are available in the literature, but the procedure itself is difficult to perform without previous experience or guidance. Mice are a popular species to perform surgical procedures due to the availability of knock-out models and their relatively small size. However, the small size also causes surgical procedures to be more complex and limits the involvement of less experienced surgeons. Currently, there is no specific protocol available in the literature. We present a detailed and illustrated protocol to study unilateral pulmonary IRI in a murine model through clamping of the left hilum. We pinpoint possible pitfalls and discuss the tricks of the trade, learned through experience. Finally, we offer relevant solutions to commonly encountered problems. This protocol may help fellow investigators to establish their own, reliable and controlled, murine model of pulmonary IRI.