POSTERS SESSION

P1 ELECTRON SPIN RESONANCE IS A POWERFUL TOOL TO DETECT OXIDATIVE AND NITROSATIVE STRESS DURING ISCHEMIA-REPERFUSION INJURY

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Objective: Oxidative and nitrosative stress is caused by free radicals such as superoxide and nitric oxide. An unpaired electron on their outer electron shell causes them to be highly reactive and play a major role in the inflammatroy cascade that is ischemia-reperfusion injury (IRI). Electron spin resonance (ESR) is a technique based on the magnetical properties of molecules with unpaired electrons. With it we can detect free radicals, fingerprint them, and quantify them.
Materials and Methods: An ESR device consists of a large magnet that alligns the unpaired free radical electrons. A microwave power source excites these electrons, causing them to absorb energy. This absorption spectrum can be made visible by specialised software. Free radicals can be fixated with spin traps and spin probes, increasing their half-life and detection range. Because every free radical has its own specific absorption spectrum, we can identify and quantify different free radicals that are generated during IRI.
Results: With the correct protocols, researchers are able to detect and quantify superoxide (indicative of reactive oxygen species, ROS), nitric oxide (reactive nitrogen species, RNS), ascorbyl radical and hydroxyl radical. With a correct protocol, this procedure can be used to detect ROS and RNS in isolated cells, macrophages, blood and tissue. The technique is limited by the amount of experience of the operator and the device itself, which can be quite expensive.
Conclusion: Based on our own results and the results presented in the literature, ESR is a useful technique to detect ROS and RNS in blood, isolated cells and tissue when specific needs are met: an experienced operator, the right device and, most important, the correct protocols. It is an incentive to collaborate more closely with colleagues active in the field.

P2 STIMULATION OF P2Y11 PURINERGIC RECEPTOR REDUCES REJECTION LESIONS AND INCREASES SURVIVAL TIME OF HEART ALLOGRAFT IN A MOUSE MODEL

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Introduction: Graft rejection is the main complication after heart transplantation. We recently demonstrated in vitro that the stimulation of P2Y11 receptor reduces ischemia/reperfusion lesions in human cardiomyocytes and is responsible for dendritic cells maturation, down-modulating the inflammatory response. The objective of this in vivo study was to specify the effect of P2Y11R stimulation on heart graft rejection lesions and its role in the maturation of dendritic cells.
Materials: Hearts from BalbC mice were transplanted intraabdominally into allogenic C57BL6 mice (n = 60). Mice were injected in the retro-orbital sinus with P2Y11R agonist (NF546). Mice in the sham group were injected with saline solution. In the control group, hearts from C57BL6 were transplanted into syngenic C57BL6 mice. Rejection was defined by cessation of palpable heartbeat and confirmed by echocardiography. Rejection lesions were investigated using histology and immunohistochemistry (CD3, CD11c, CD45) in allografts at days 3, 5 and 7 after transplantation. To quantify apoptosis, activity of caspase 1, 3 and 9 was measured. Maturation of dendritic cells was investigated by studying expression of markers CD83, CD25, CCR7, CXCR4, and production of cytokines IL6, IL10, IL12, IFNγ.
Results: Cardiac allograft survival was significantly prolonged after stimulation of P2Y11R by its agonist (9.6 ± 1.9 vs. 8.2 ± 1.4 days; $p = 0.04$). Rejection lesions, classified according to ISHLT guidelines and quantified using the mean number of inflammatory cells per field, were significantly reduced in the treated group. At day 5 after transplantation, P2Y11R agonist pretreated allografts also demonstrated less apoptotic lesions.
Conclusions: Stimulation of P2Y11 receptor reduces rejection lesions observed after allogenic heart transplantation. Our previous results suggest that this protective role may imply dentritic cells maturation toward an antiinflammatory profile, depending on P2Y11 signaling pathway.

P3 THE EFFECT OF PICROSIDE II ON ISCHEMIA REPERFUSION INJURY IN MYOCART STREPTOZOTOSIN INDUCED DIABETIC RATS

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The scientific studies for the treatment of reperfusion injury after perfusion of ischemic tissue have been increasing day by day. The aim of this study was to determine the effect of picroside II on myocardial ischemia reperfusion injury in STZ induced diabetic rats .

In the experiments, 30 Wistar-Albino, 6–8 week old male (210–300 g) rats were used. The subjects have been divided into five groups as; Sham, Sham Diabetes Mellitus (DM), DM-Picroside II group, DM- Ischemia group and DM-Ischemia Picroside II group. In diabetics groups diabetes was incuded by STZ. In order to I/R injury, we have performed 60 min ischemia and followed by 2 h reperfusion to descending branch of the left coronary artery. Picroside II was injected intraperitoneally to Picroside II groups before ischemia process. After homogenization of samples extracted from myocardial tissue total antioxidant status (TAS), total oxidant status (TOS) work was done.

In total antioxidant capacity and total oxidative stress of Picroside II use was found to have a statistically significant effect ($p = 0.026$).

Streptozotocin induced diabetic rats in the myocardial ischemia reperfusion injury piicroside II over the protective effect was found.

P4 METABOLIC EFFECTS OF PHARMACOLOGICAL STIMULATION OF THE HYPOXIA INDUCIBLE FACTOR PATHWAY DURING MACHINE PERFUSION OF PORCINE KIDNEYS.

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Background: The hypoxia inducible factor (HIF) pathway is of great scientific interest with activation triggering wide ranging cellular responses including the up-regulation of glycolytic enzymes. It has previously been shown in a murine model that pharmacological HIF activation of donor animals led to a favorable outcomes post transplant. The aim of this study was to determine if the HIF pathway could be manipulated during hypothermic machine perfusion (HMP) conditions to promote glycolytic activity.
Methods: Following organ harvest, porcine kidneys (n = 8) were flushed and perfused using a lifeport kidney transporter machine. Kidneys were perfused with a KPS-1 based perfusion fluid containing 10 mM of isotopic $U^{13}C$ glucose, with or without the non-selective PHD inhibitor desferrioxamine. Kidneys were perfused for 24 h with perfusate and tissue sampled. The metabolic fate of this labelled glucose was determined using 2D nuclear magnetic resonance spectroscopy (HSQC) and our in house analytical software Metabolab.
Results: ^{13}C Labelled metabolic derivatives were consistently seen in both perfusate and extracted tissue samples at all time points including both lactate and alanine. These molecules indicate glycolytic activity within this hypoxic hypothermic environment. There was no difference identified in the proportionate labeling patterns between the control and HIF stimulated kidneys.
Conclusion: Introduction of the non-selective PHD inhibitor, desferrioxamine did not appear to alter the rate of *de novo* glycolysis during HMP in this large animal DCD transplant model. The reasons for this require further study but a possible explanation may be that the HIF mechanism is maximally stimulated during HMP conditions.

P5 CYTOPROTECTIVE ACTION OF SOLUBLE HEME OXYGENASE-1-CELL PENETRATING PEPTIDE (SHO-1-CPP) OBSERVED IN *IN VITRO* HEPATIC ISCHEMIA-REPERFUSION INJURY (IRI) MODELS

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Background: Experimental models of *in vitro* ischemia-reperfusion injury (IRI) that simulate *in vivo* studies are powerful tools to examine cytoprotective strategies. This *in vitro* IRI model using McA-RH7777 cells, Clone 9 cells, primary hepatocytes and Kupffer cells attempts to determine the cytoprotection exerted by the recombinant protein soluble heme oxygenase-1-cell penetrating peptide (sHO-1-CPP).
Methods: *In vitro* ischemia-reperfusion was achieved by placing the cells in a hypoxic chamber followed by transfer of cells to a normally oxygenated incubator and observed for cell death using flow cytometry, cell and nuclear integrity by transmission electron microscope (TEM) and quantification of mitochondrial DNA using PCR at various time points.
Results: *In vitro* ischemia and reperfusion resulted in increased damage to cells and treatment with sHO-1-CPP showed significant cytoprotection. Following eight hours of warm ischemia and two hours of reperfusion, sHO-1-CPP treated cells showed significant decrease in cell death ($p < 0.05$) as determined by flow cytometry analysis of propidium iodide/annexin, a significant decrease in the number of mitochondria and mitochondrial genes (COX-3 and Cytochrome b) and a marked increase in cell and nuclear integrity when compared to cells with IRI not treated with any protein and cells with IRI treated with sHO-1 protein without CPP.
Conclusions: These findings show that the recombinant protein sHO-1-CPP offers cytoprotection to cells from IRI *in vitro* and also provides information on the concentration of sHO-1-CPP that will be required to observe cytoprotection. Based on the results from this *in vitro* IRI study future experiments are planned to confirm these data in *in vivo* models of IRI.

P6 HYPOTHERMIC MACHINE PERFUSION OXIGENATION. A REAL STEP FORWARD? TISSUE AND PERFUSATE MRNA EXPRESSION

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Introduction: The lack of oxygen combined with hypothermia during preservation leads to the reduction of aerobic metabolism to protect the organ.

Deceased after cardiac death donors (DCDs) represent a valuable source of organs; however, preventing poor outcome is difficult, even with the use of machine perfusion (MP). Addition of oxygen during MP could allow the graft to maintain a low metabolic aerobic respiration and ATP levels which permitted a delay of injury process.

miRNAs can be secreted to body fluids mostly in exosomes, in a GTP and calcium-dependent manner. Our group has demonstrated the feasibility of miRNAs detection in preservation solution during MP. Tissue miRNAs in pre and post perfusion biopsies could translate transcriptional changes at cellular level induced by oxygenation.
Objetive: To determine the potential benefit of low flow aerobic machine preservation through miRNAs expression.
Material and Methods: A porcine orthotopic transplantation model mimicking type III DCD conditions was developed. Cold preservation was performed by conventional non-oxygenated MP in Life-Port™ device or oxygenated MP by a continuous oxygenation flow ($PO2 > 500$ mmHg). Evaluation included miRNAs in preservation solution and in pre and post MP biopsies.

miRNAs were determined by qRT-PCR after RNA extraction and results were expressed as differential of DCTs.
Results: Nine female commercial farm pigs 3–6 months were randomized. Oxygenated and non-oxygenated grafts exhibited similar miRNAs expression levels in kidney biopsies. On the contrary, oxygenated grafts showed higher levels of miRNAs expression in preservation solutions, suggesting that oxygenation could allow more efficient secretion of miRNAs.
Conclusions: Preservation solution oxygenation did not seem to modify kidney tissue miRNAs expression, however could modulate miRNAs secretion to preservation solution, most probably trough alterations in energetic kidney status and intracellular calcium.

P7 OBJECTIVE OF 20 HOURS OF LIVER PRESERVATION: DEVELOPMENT OF A PORCINE EXPERIMENTAL MODEL

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Introduction: The success of hepatic transplantation increases the need of liver graft. Currently the dynamic preservation, i.e. with machines of perfusion (MP), upsets the traditional technique (static and hypothermic). But, MP are expensive materials developed to be used at the human. Therefore, to explore the various possibilities of MP and to validate them an experimental model as near as possible to the human is needed. In the pig, we experienced rate of survival (≥7 days) of 4/5 animals following 4 h of hypothermic static preservation and liver transplantation. In this model, to our knowledge no survival has been reported beyond 12 h of static conservation.To develop in a large animal an experimental model with extensive time of preservation using MP.
Material and Methods: Livers of pigs large-white (35–40 kg) were harvested during the afternoon, cooled and stored with the solution SCOT15® at 4°C, then divided and perfused on a LiverAssist® MP with an acellular medium (MPS®, 3 l.) oxygenated (FiO2 60%) at 20°C for 18 h before being orthotopically transplanted in a pig the next morning.
Results: 4 pig livers (665 ± 36 g, mean ± SEM) were transplanted. During the machine perfusion, the mean of arterial pressures and flows were of 42 ± 7 mmHg and 342 ± 112 ml/mn, and the mean of portal pressure and flow were of 5 ± 1 mmHg and 394 ± 140 ml/mn. During the perfusion, the concentration of LDH in the perfusate increased gradually with time from 155 ± 133 to 282 ± 71 UI/l, urea from 0.16 ± 0.09 to 1.33 ± 0.6 mmol/l. The biliary flows were quite absent. Two pigs died 2 h and 9 h after the reperfusion. Two pigs survived 43 days and 89 days respectively, until the sacrifice for protocoled reasons.
Conclusion: Survivals following 20 h of preservation using a MP are possible and make possible to explore the borders of ischaemia-reperfusion in a large animal model.

P8 TYPE 4 DIPEPTIDYL-PEPTIDASE (DPP-4) EXPRESSION IS DECREASED AT BOTH MRNA AND PROTEIN LEVELS FOLLOWING RENAL ISCHEMIA/REPERFUSION IN RAT AND MAN

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Type 4 dipeptidyl-peptidase 4 (DPP-4) is a serine protease expressed at the surface of most epithelia, including renal proximal tubules (PT). Since DPP-4 participates to inflammation, recruitment of immune cells and apoptosis, we investigated its expression and distribution in case of renal ischemia/reperfusion (I/R).

Renal ischemia was induced in Wistars rats by unilaterally clamping the left kidney for 60 min. The right kidney was simultaneously excised and used as comparator. First group ($n = 6$) had no reperfusion (NR) and the kidney was removed straight after the hour of ischemia. For the other groups, renal reperfusion was allowed for 6 ($n = 6$), 24 ($n = 6$) or 48 ($n = 6$) hours. Kidneys were snap-frozen and lysed for mRNA and protein extraction. In parallel, the expression and distribution of DPP-4 was studied by immunohistochemistry on 10 biopsies of human kidneys with non-toxic acute tubular necrosis (ATN).

In rat kidneys, mRNA abundance of DPP-4 was significantly decreased following I/R in all group: NR (2.07-fold, $p < 0.001$), 6 h (8.12-fold, $p < 0.001$), 24 h (12.5-fold, $p < 0.001$) and 48 h (12.9-fold, $p < 0.001$) in comparison to the controls. Similarly, immunoblotting analyses showed a 2.14-fold in the 6 h group ($p < 0.05$) and a 2.3-fold at both 24 h ($p < 0.05$) and 48 h ($p < 0.05$) *post* reperfusion. In human kidneys with ATN, the abundance of DPP-4 appeared reduced at the PT cells in comparison to healthy controls. No DPP-4 internalization into PT cells was evidenced.

In conclusion, renal I/R is associated with reduced expression of DPP-4 in rat and human kidneys at both mRNA and Protein levels, which may be caused by PT tubulorrhexis and/or DPP-4 shedding into the urine.

P9 CELL ATP AND VIABILITY ALTERATIONS INDUCED BY ISCHEMIA-REPERFUSION IN THE RENAL CORTEX: AN AGENT-BASED COMPUTER MODEL

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Introduction: In renal preservation-transplantation, ischemia-reperfusion (IR) causes graft inflammation and fibrosis, dysfunction and loss. Events involved in IR injury (IRI) grow identified, but their intricacy hampers prediction and therapeutics. We develop a computer model of renal response to IRI at cell/tissue level. Using our previous dynamic model of cortical oxygenation (DYN), we 1) adapt it to a (O_2-)steady-state model (STE), 2) couple epithelial (EPI) and peritubular capillary (PTC) cells energetics to O_2 level, and 3) couple cell agents health to their ATP level, and 4) explore cell fate under ischemia and hypoxemia (37°C).
Methods: Multi-agent modeling tool NetLogo© is used. Model: 10 μ-thick cortex slice (300 × 300μ^2). Structure/function reference values from bibliography (REF° RBF = 5.3 ml/min/g, PO_2 = 48 mmHg). In DYN and STE, mean tissue O_2 (tPO_2 mmHg) is calculated by solving perfusion, diffusion and consumption (model accuracy 1.0–2.0 mmHg).
Results: (1): At REF° DYN & STE oxygenation models yield tPO_2 42.1 & 42.0 mmHg; from REF° normo-to anoxemia and from RBF° to total ischemia, DYN and STE give similar tPO_2 within 0.3 ± 1.1 mmHg (n = 14). (2): In STE, ATP modules were added in EPI and PTC, for production (Oxphos, Glycolysis) and consumption (Na-transport, House-keeping). Model adjustment was performed: 1) REF° levels: EPI exhibit ATP° 2.5 mM (vs. 2.5 ± 1.0, from 7 ref.); 2) 80% Oxphos-sensitive ATP in EPI (PTC 39%). Ischemia causes ATP to vanish in 40 min. (3): Cell survival as a function of time and ATP fitted from Lieberthal et al. 1998: ATP <20%, cells die by necrosis; 20% < ATP < 70%, cells die by apoptosis; ATP <2%, 70% of cells die within 2 h (Glauman et al. 1975).
Conclusion: Our model reproduces oxygen-dependent ATP and cell viability as observed experimentally. This construct will allow to progressively address, in experimentals and in human clinics, renal IR inflammatory/fibrogenic responses and therapeutics.

P10 HYPERLIPIDEMIA INDUCED BY HIGH-FAT DIET PROMOTES RENAL VASCULAR REMODELING IN A PORCINE RENAL AUTOTRANSPLANTION MODEL

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Introduction: The number of recipients with a higher prevalence of comorbidity factors such as dyslipidemia increases. The ischemia-reperfusion injury in the transplant procedure induces vascular lesions limited by induction of regeneration processes. The goal of our study was to characterize the role of a diet-induced increase in plasma oxidized LDL on the vascular remodeling in the cortex kidney graft.
Methods: We used three-month-old pigs following a kidney auto transplantation: left kidneys were removed and cold stored for 24 h at 4°C in the University of Wisconsin solution and autotransplanted. A contralateral nephrectomy was performed to mimic renal mass in clinical situations. Two experimental groups were studied: kidney graft removed 3 months after surgery either from animals exposed to a standard diet (Normal diet, n = 5) or from animals fed a high-fat diet started immediately after weaning (High-fat diet, n = 5). We characterized the cortical microvasculature by microcomputed tomography and histological injury analysis 3 months post-surgery.
Results: Increased plasma oxidized LDL levels at three months promoted concomitant microvascular rarefaction for small vascular segments with diameter inferior to 40 μm particularly in the middle cortex (vascular density as a percentage of vessels expressed as mean ± SEM: 0.46 ± 0.21 in high-fat diet vs. 1.61 ± 0.45 in normal-diet pigs) and a decrease of vascular segment diameter average (52.11 ± 4.05 vs. 85.63 ± 5.58 μm in outer cortex, 69.75 ± 4.69 vs. 117.79 ± 11.21 μm in middle cortex, 74.70 ± 2.73 vs. 128.76 ± 13.96 μm in inner cortex). These results were associated with an increase of monocyte infiltration and interstitial fibrosis/tubular atrophy in high-fat diet group.
Conclusion: These results highlight that high fat-diet leads to a microvascular rarefaction which worsens the vascular remodeling induced by ischemia-reperfusion and suggest controlling hypercholesterolemia in recipients at the early stage of renal transplantation.

P11 RENAL PRESERVATION WITH SCOT 15® SOLUTION COULD REDUCE THE INCIDENCE OF ACUTE REJECTION IN RENAL TRANSPLANTATION

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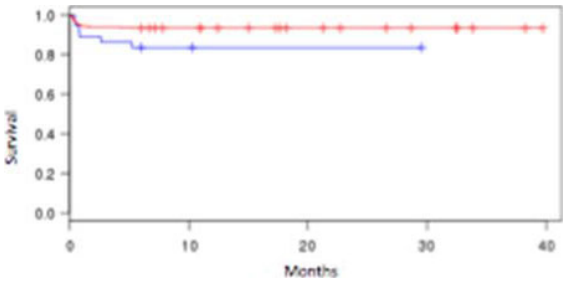
Introduction: SCOT 15® solution has been developped by an academic team specialized in immunocamouflage. The solution is able to extend allograft's survival of pancreatic islets in mouse as well as allograft's survival of renal allograft in pig
The aim of the study was to evaluate the rejection free survival at 6 months after preservation with SCOT 15 for renal transplantation.
Methods: We retrospectively reviewed renal transplantation in one center between 2009 and 2014. We retrieved 445 renal transplantations (12% living donnors, 71% deceased donnor brain dead and 17% deceased donnor by cardiac death MII). 59% of the kidney's storage were static and 41% were dynamic on Lifeport® system. Preservation solution were regrouped in 5 groups : *Non S* : grafts never perfused with Scot 15® + static storage (n = 36); *S* : grafts initially perfused with Scot15® + static storage (n = 155); SKS : grafts initially perfused with Scot15® + dynamic storage with KPS solution then rinsed with SCOT15® before transplantation(n = 133); A*S* : grafts initially perfused without Scot15® + static storage (n = 73); *AKS* : grafts initially perfused without Scot15® + dynamic storage with KPS solution then rinsed with SCOT15® before transplantation. (n = 50). The last 4 groups were combined in group *S* (i.e. grafts rinsed with Scot15® before transplantation, regardless solution initially used) All rejection (cellular or humoral) have been proven by biopsy. Biopsy were realised in case of graft dysfonction.
Résults: Rejection free survival at 6 months was significatively improved in group *S* (red curve) compared to group *Non S* (blue curve) (p = 0.03) .



Conclusion: This study suggest that SCOT 15® properties in immunocamouflage (demonstrated in animal models) could have a protective role in human renal transplantation. Further and multicentric studies are necessary to confirm these results.

P12 BRONCHOALVEOLAR LAVAGE FLUID (BALF) ANALYSIS WITH HIGH RESOLUTION NMR SPECTROSCOPY IN A PRECLINICAL MODEL OF LUNG ALLOTRANSPLANTATION

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Introduction: Evaluation of preservation protocols for organ transplantation is of primary importance regarding donor pool heterogeneity. We previously present NMR metabolomic analysis of preservation solution in the case of kidney. In the same manner, we propose a NMR analysis of bronchoalveolar lavage fluid (BALF) after an ischemia reperfusion sequence.
Materials and methods: Bronchoalveolar lavage fluid (BALF) was collected after 5 h of reperfusion; explantation of the graft and an upper lobectomy was performed. Washing was done directly by instillation of 40 cc of physiological saline in the left upper bronchus. Samples were centrifuged and supernatant kept at −80°C until high resolution NMR acquisition on an Avance 500SB Spectrometer (Bruker) equipped with a 5 mm broadband inverse probe. Three groups of lung preservation protocol were studied: static preservation with Perfadex solution alone, static preservation with Perfadex solution with addition of an oxygen carrier and sham operated lung (N = 5 in each group).
Results: In the control lung, the BALF appears to be clean and we found only few metabolites in low concentrations as lactate. We also found in the NMR spectra metabolites from cell release as choline compounds. BALF from lungs preserved with Perfadex alone showed spectra more complex with higher concentrations of lactate, addition of some metabolites aminoacids (valine, alanine) or sugar body (probably dextran-40 from prefadex solution). The last group with lung preserved with perfadex solution with addition of an oxygen carrier showed intermediate NMR profiles with intermediate concentration. The oxygen carrier seems to have a real impact on BALF composition.
Conclusion: We proposed descriptive and preliminary results on BALF analysis by NMR with a comparison of two lung preservation protocols vs. healthy lung.

P13 INFLUENCE OF COLD ISCHEMIA TIME ON OXIDATIVE STRESS AND INNATE IMMUNITY ACTIVATION

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Introduction: Recent studies have shown that the duration of cold ischemia (CI) impact the outcome of kidney grafts and seems to have an impact on the risk of death. This peri-transplantation period, however, is an ideal time for a variety of protocoles to improve graft quality. The aim of this work was to study the influence of CI time on the kidney grafts outcome in an autotransplant model in pigs in the early phase and after 3-month follow-up.
Materials and Methods: Three groups were studied with progressive duration of IF: Group 1 (n = 6) time of IF 2:30 h, group 2 (n = 6) time of IF 24 h, group 3 (n = 6) time IF 48 h. Renal function recovery, the markers of tissue damage and oxidative stress were studied. The impact of IF on the expression of markers of innate immunity was also analyzed. At 3 months, the impact ofIF was studied on interstitial fibrosis expression and tissue inflammation.
Results: The time duration of CI proportionally impacted function of transplanted kidneys. This duration significantly negatively impacted the level of oxidative stress, and inflammatory markers during the first week after reperfusion. CI duration showed an effect on chronic lesions expression such as fibrosis or cellular infiltration to 3 months.
Conclusion: CI time is a pivotal factor affecting graft function and different pathways. It is of utmost importance to have tools to characterize these mechanisms in depth and evaluate new treatments to prevent organ tolerance to CI injury. This model seems to be a valuable tool to allow validation of new concepts and propose peri-transplantation management protocols providing clinicians with innovative resources.

P14 THE NORMOTHERMIC REGIONAL PERFUSION: A TIME FOR ASSESSMENT AND CONDITIONING OF ORGAN

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Introduction: The normothermic regional perfusion (NRP) is a technique for organ conditioning from deceased donors after cardiac arrest. This is a time useful for organs quality evaluation and for protective treatment application. The aim of this study was to develop a porcine model of NRP fiting the specifications of the Biomedicine French Agency
Materials and Methods: A model of NRP was established in the large white pig to mimic the situation in the clinic situation. The animals weighed between 38 and 45 kg. Death was caused by lethal injection of potassium and the circuit was installed. NRP was evaluated for 4 h, a hemodynamic monitoring was performed, and laboratory monitoring of renal function and electrolyte was implemented. During this monitoring, urine collections were made with dieresis evaluation and renal function evaluation. Markers of injury and inflammation were determined and NMR spectroscopy analysis was performed.
Results: The various specific markers allowed determining a persistent renal function. Electrolyte changes showed an increased level of potassium. It was observed an increase of LDH which were relatively variable. In the urine, sodium excretion remained relatively stable without increase of natriuresis. NMR spectroscopy enabled to determine tubular injury and metabolic status.
Conclusion: The NRP is a key time in the care of the donor and the organs. This technique enables to measure level of function and functional quality of organs (particularly the kidney). It is probably a time window for validation of markers and also a time for organ conditioning

P15 IDENTIFICATION OF PROTEINS INTERACTING WITH CYTOPLASMIC HIGH-MOBILITY GROUP BOX 1 (HMGB1) DURING THE HEPATOCELLULAR RESPONSE TO ISCHEMIA REPERFUSION INJURY

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Introduction: Ischemia/reperfusion injury (IRI) occurs in liver transplantations and major resections, resulting in liver dysfunction. HMGB1 locates in the nucleus of normal hepatocytes, but translocates to the cytoplasm and to the extracellular space during IRI. The functions of nuclear and extracellular HMGB1 are well explored, but the role of cytoplasmic HMGB1 in hepatic IRI still remains elusive. We hypothesize that cytoplasmic HMGB1 interacts with partner proteins involved in hepatocellular response to IRI. The aim of this study is to identify cytoplasmic binding proteins of HMGB1.
Material and Method: Normal and warm ischemia reperfusion (WI/R) liver tissues were used for cytoplasmic protein extraction. The protein extracts were subjected to enrich HMGB1-protein complexes using co-immunoprecipitation. To separate and identify the immunoprecipitated proteins in eluates, 2DE and MS detection were performed. The identified partner proteins were verified using immune western blotting. Information regarding the biological function of the binding proteins was retrieved using PubMed and KEGG pathway analysis.
Result: Three binding proteins were identified and verified, betaine–homocysteine S-methyltransferase 1 (BHMT), cystathionine gamma-lyase (CTH) and ATP synthase beta subunit (ATP5B). There is substantial evidence that all three candidates are involved in homocysteine metabolic pathways as well as autophagic pathways.
Conclusion: Our results demonstrate that cytoplasmic HMGB1 binds to BHMT, CTH and ATP5B during hepatic WI/R. Since all binding proteins are involved in homocysteine metabolic pathways and autophagy, we can speculate that the cytoplasmic HMGB1-binding protein complex may take part in hepatocellular response through these pathways and related molecules.

P16 ROLE OF VEGF ON RENAL GRAFT INTÉGITY WHEN ADDED DURING MACHINE PRESERVATION

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Introduction: The integrity of the vascular system is pivotal for tissue integrity during ischemia reperfsion (IR). Previously, we have shown the importance of the role of VEGF and its isoform 121 during the static conservation. Using a deceased donor after cardiac arrest model we studied the interest of VEGF 121 during machine pefusion.
Methods: Two groups were studied: Group VEGF 121, n = 6, left kidney after 1 h of warm ischemia was removed, flushed and then perfused at 4°C with the machine infusion LifePort 1.1® with KPS-1® solution containing VEGF 121 25 micrograms per liter of solution. The control group was made with the KPS-1® solution without addition of VEGF. The machine perfusion time was 24 h. The evaluation was made in a model of renal autotransplantation in Large White pigs with 3 months follow up and compared to a control group and nephrectomized group. Recovery of renal function and inflammatory response (determination of pro-inflammatory markers) and lesions markers tubulaires were determined. At three months, the animals were sacrificed. The expression of HIF-1α; VEGF and TGF-β and evaluation of tubulo interstitial fibrosis was performed.
Results: Renal functions recovery was improved in the group with KPS-1® + VEGF 121 and during follow-up. Tubular functions have also been significantly improved. Use of VEGF 121 limited significantly expression of proinflammatory markers. Urinary excretion and plasma NGAL determination was significantly reduced in the group treated with VEGF 121. Three months after transplantation, interstitial fibrosis and tubular atrophy were reduced more significantly in the group Viaspan + VEGF 121.
Conclusion: This work highlights the importance of the vascular lesions and emphasizes the role of endothelial cell as a pivotel target which could have consequences for other structures of the kidney.

P17 QUALITY ASSESSMENT OF DISCARDED HUMAN KIDNEYS: A COMPARISON OF HYPOTHERMIC MACHINE PERFUSION AND EX-VIVO NORMOTHERMIC PERFUSION TECHNIQUES

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Introduction: In renal transplantation accurate means of viability and quality assessment are essential to ensure the efficient use and allocation of organs. The aim of this study was to compare hypothermic machine perfusion (HMP) and ex-vivo normothermic perfusion (EVNP) assessment techniques.
Methods: Ethical approval was granted for the study by the national research ethics commission in the UK. Ten human kidneys rejected for transplantation underwent 60 min of HMP (Lifeport Kidney Transporter) with KPS-1 solution at 4°C. At 60 min the renal resistance (RR) was recorded. After HMP, kidneys underwent 60 min of EVNP with an oxygenated packed red cell based solution at 36°C. Functional parameters were measured after 60 min.
Results: The mean donor age was 54 ± 9 year and the cold ischaemic time 43.9 ± 11.6 h. During HMP the RR fell in all kidneys but remained above 0.3 mmHg/ml/min in 9/10 kidneys at 60 min (mean 0.6 ± 0.4 mmHg/ml/min).
During EVNP the mean renal blood flow (RBF) was 94 ± 33 ml/min/100 g and intra renal resistance (IRR) 0.4 ± 2.5 mmHg/ml. Seven out of ten kidneys produced a significant quantity of urine (range 100–330 ml) and all kidneys appeared evenly perfused. The mean oxygen consumption was 74 ± 23 ml/min/g. The RR after HMP did not correlate with any of the perfusion parameters during EVNP ($p > 0.05$).
Conclusion: There was no association between the parameters measured during HMP and EVNP. The level of RR during HMP was indicative of a high level of injury. However, the majority of kidneys during EVNP demonstrated a good level of recovery and function. Restoring function using EVNP allows a more comprehensive assessment of the kidney prior to transplantation.

P18 PROLONGED COLD ISCHEMIA TIME ENHANCES RENAL PERFUSION IMPAIRMENT AND LOCAL INFARCTION AFTER KIDNEY TRANSPLANTATION IN MICE

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Background: Kidney transplantation (ktx) in mice is challenging but offers good translational models to study mechanisms of disease. In this study allogenic and isogenic ktx in combination with different cold ischemia times (CIT) was investigated by functional magnetic resonance imaging (fMRI) and histology.
Methods: For the model of CIT induced allograft damage C57Bl/6 male donor kidneys were transplanted to Balb/C recipients. CIT was 60 min and warm ischemia time was 30 min. In a second model Balb/C donor kidneys were transplanted to C57Bl/6 recipients with 30 min cold and warm ischemia time. After 3 weeks in the acute rejection model and 7 weeks in the chronic rejection model FACS analysis and histology for inflammation and fibraosis evaluated and immunostaining was done. fMRI was performed repetitively to quantify renal perfusion impairment.
Results: Isogenic ktx histology revealed normal renal morphology and stable renal blood flow in functional MRI. Allogenic ktx with 60 min CIT resulted in acute rejection (Banff IIA) with severe inflammation. 75% of the allografts had local infarctions mainly in areas of enhanced inflammation. Infarcted areas without any leukocytes were suggestive for surgical complications. fMRI showed declining renal perfusion over time with ongoing rejection also when excluding areas of perfusion disturbances. Perfusion defects occurred mainly after allogenic ktx. Prolonged CIT enhanced inflammation and the incidence of infarction (75% with 60 min CIT, 25% with 30 min CIT). In the vicinity of occluded vessels fibrosis was markedly enhanced compared to the rest of the tissue.
Conclusion: The duration of cold ischemia time enhances the risk for local perfusion disturbances and accelerates rejection. Renal perfusion measurement by functional MRI correlate with rejection and offers a non-invasive technique to monitor inflammation and to assess graft pathology.

P19 FREE HEME AGGRAVATES RENAL ISCHEMIA REPERFUSION INJURY (IRI) AND CONTRIBUTES TO ACUTE KIDNEY INJURY (AKI)

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Acute kidney injury (AKI) is a frequent complication after solid organ transplantation. Especially, lung- and heart transplantation are associated with blood loss and the need of packed red blood cell (pRBC) transfusions. Although pRBC are beneficial adverse effects have been described. Therefore, in this study, the effect of free hb / heme on renal ischemia reperfusion injury (IRI) was investigated in a mouse model.
IRI was induced by 15 min renal pedicle clamping in mice. Sham surgery was done in controls. Afterwards, free hb / heme or vehicle was infused iv. Clinical chemistry for renal function parameters and functional magnetic resonance imaging (fMRI) was done to quantify creatinine elevation, renal perfusion impairment and tissue edema. qPCR for cytokine expression, histology and immunohistochemistry for acute kidney injury and inflammation were done.
Free hb / heme infusion resulted in marked aggravation of AKI with elevated s-creatinine and BUN whereas vehicle treatment did not cause a relevant impairment in renal function. By fMRI significant decrease of renal perfusion was measured due to heme injection after IRI but not after sham surgery. In addition, increase of T2 relaxation time in the outer medulla because of capillary leakage and edema formation was detectable. Inflammation, acute tubular injury, increase in NGAL were more prominent in mice after free hb or free heme infusion than in those given vehicle. Tissue levels of pro-inflammatory cytokines (IL-6, MCP-1, TNF-alpha and PAI-1) were significantly higher in free hb/heme-treated mice than in vehicle treated animals.
Conclusion: Transfusion of aged pRBC is pro-inflammatory and aggravates AKI in an experimental renal IRI mouse model. Clinical studies are needed to evaluate the risk of post-surgical AKI if aged pRBC had been transfused during major surgery.

P20 EARLY ANTI-HYPERTENSIVE THERAPY CAN BE DELETERIOUS IN EARLY RENAL ISCHEMIA REPERFUSION INJURY

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Background: Many patients undergoing kidney transplantation are on hypertensive medication. After kidney transplantation ischemic allograft injury is present in 5–25% of cases and contributes to the delayed graft function. In this experimental study we investigated whether antihypertensive treatment has an effect on the severity of renal ischemia reperfusion injury (IRI).
Methods: In a CD1 mouse model we performed unilateral renal pedicle clamping to induce IRI and treated the mice with an ACE inhibitor or with the soluble epoxyhydroxylase (sEH) inhibitor TPPU and compared the results to vehicle treatment alone. Functional MRI to measure renal perfusion and histology work up was done.
Results: IRI increased systolic blood pressure by +20 mmHg in the vehicle group. ACE inhibitor and sEH inhibitor treatment successfully attenuated blood pressure elevation and inhibited glomerulosclerosis. However, functional MRI using arterial spin labelling showed aggravated renal perfusion impairment by antihypertensive treatment at d1 after IRI. By immunohistochemistry we could show that the treatments had no effect on later fibrosis and tubular atrophy, which developed in the treatment groups and in the vehicle group within 14 days.
Discussion: Antihypertensive treatment with an ACE inhibitor and with the sEH-inhibitor TPPU after IRI was potent in attenuating blood pressure elevation and glomerulosclerosis. However, tubulo-interstitial fibrosis and inflammation was not influenced by antihypertensive therapy. Importantly, early renal perfusion was negatively affected by lowering systemic blood pressure.
Conclusion: The timing, type and dose of antihypertensive treatment for kidney transplant recipients needs careful consideration in order to avoid worsening of renal perfusion impairment. Individual therapy decisions need to be made taking the existing co-morbidities (e.g.cardiovascular conditions) and duration of cold ischemia time, which aggravates IRI into account.

P21 TRANSPLANTATION OF GRAFT FROM OLDER DONORS: DOES AGE STILL MATTER?

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Improved outcome after Liver Transplantation (LT) created a gap between organ request and availability. Hence the donor pool has expanded including elderly donors (>70 year) but concerns remain whether donor age might worsen outcome post-LT. Donor and recipient demographics, transplant and outcome data of grafts >70 year ($D > 70$) and <70 year ($D < 70$) were compared in 643 consecutive LT (01/2003–12/2014). The influence of older donors on survival was tested at a multivariable analysis. Median (IQR) is given.
98/643 (15%) LT were performed with grafts $D > 70$. $D > 70$ [71–91 year] were all brain dead, peak AST/ALT was lower, most were

locally procured, had shorter extraction time [30 min (24–36) vs. 38 min (28.5–50) $p < 0.0001$] and higher DRI [2.6 (2.4–2.8) vs. 2.1 (1.7–2.4) $p < 0.0001$]. Recipients of D > 70 were older [62 year (56–68) vs. 57 year (48–64) $p < 0.0001$] with equal MELD but had more frequently HCC (44.9% vs. 28.3% $p = 0.002$). Cold ischemia (CIT) [7.24 h (6–9.5) vs. 7.98 h (6.2–9.5) p = ns] and anastomotic time [80 min (67–91) vs. 78 min (67–90) p = ns] was similar. Peak AST post-LT was lower in D > 70 [527 IU/L (298–1071) vs. 721 IU/L (383–1386) $p = 0.025$]. The incidence of early allograft dysfunction (D > 70 26.3% vs. D < 70 28.9%, p = ns) or acute kidney injury (D > 70 22.7% vs. D < 70 22.7%, p = ns) was similar. Non-anastomotic biliary strictures were less frequent in D > 70 (6.2% vs. 13.4%, $p = 0.045$). One and 5 year graft survival were similar between D > 70 and D < 70 (89.7% vs. 87.7%, 73.5% vs. 70.6% respectively p = ns); likewise 1 and 5 year patient survival did not differ. At a multivariable Cox analysis D > 70 did not influence patient nor graft survival (HR:0.95, 95%CI:0.55–1.63 and HR:0.78, 95%CI:0.46–1.33) in contrast to CIT which was independent risk factor of recipient death (HR: 1.11, 95%CI:1.04–1.2, p:0.004). In our experience, LT of grafts >70 year did not increase morbidity and achieved survival comparable to that one of younger grafts. Careful selection of graft >70 year to be procured by local team seems essential to transplant elderly grafts

P22 CHANGES IN MITOCHONDRIAL ELECTRON TRANSPORT CHAIN COMPLEX EXPRESSION IN ISCHAEMIA AND REPERFUSION.

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Background: Due to the defecit of donor kidneys, there is currently an effort to utilise suboptimal organs for transplantation.These organs frequently experience warm ischaemia and so are vulnerable to ischaemia-reperfuion injury (IRI) which causes delayed graft function and graft loss. Mitochondria have been implicated in this process through the generation of reactive oxygen species (ROS) and isofluorane has been shown to have a protective effect through unkown mitochondrial mechanisms. Therefore mitochondria may be an important target and isofluorane a potential therapy to ameliorate IRI and increase graft survival.
Aims: To determine whether renal IRI affects mitochondrial electron transport chain expression and function, and to determine the effect of isofluorane pre-conditioning.
Methods: A murine surgical model induced ischaemia and reperfusion through renal pedicle clamping. A range of ischaemic and reperfusion times were used to identiy mitochondrial changes. Isofluorane pre-treatment in a group of mice allowed analysis n of its effect. After harvest, the kidneys were snap frozen and processed for Western Blot, Blue Native PAGE and in gel activity analysis to analyse expression and function of mitochondrial electron transport chain proteins.
Results: Ischaemia increased the expression of Complexes III, IV and V and decreased complex II activity. These results were more severe with longer ischaemic times and persisted in early reperfusion. Isofluorane pre-conditioning prevented these changes.
Conclusion: The increase in Complex III levels and the reduction of Complex II activity indicate mitochondrial involvement in IRI and could contribute to cellular damage through ROS production upon reperfusion. Isofluorane prevented mitochondrial changes and therefore has potential to be a therapeutic agent for IRI in transplantation.

P23 INFLAMMATORY MONOCYTES ARE THE MAIN PRODUCERS OF TNFα, A KEY CYTOKINE IN HEPATIC IR INJURY.

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TNFα production is a key driver of hepatic Ischaemia Reperfusion (IR) injury. Inhibition of TNFα has been shown to reduce IR injury. The source of the TNFα is unclear and was investigated in this study.
Methods: A mouse model of lobar liver IR injury was established using male Bl6 mice aged 8–12 weeks. At laparotomy, 45 min of warm hepatic IR injury was induced by vascular inflow occlusion to the left and middle lobe (n = 6). Following 2 h reperfusion the animal was terminated, the liver parenchyma was immediately harvested and intrahepatic lymphocytes (IHL) were isolated. IHLs were cultured in Brefeldin A for 4 h, to block cytokines being secreted, stained and analysed via multichannel flow cytometry. T cells (CD3+, CD4+ and CD3+, CD8+), Macrophages (CD3-, F4/80+), inflammatory monocytes (CD3-, CD11b+, Ly6c+, Ly6 g-) and Neutrophils (CD3-, Ly6c-, Ly6 g+) were isolated and their intracellular production of TNFα was measured per cell type following IR injury. A control group (n = 6) consisted of a 3 h sham laparotomy and the liver was similarly processed.
Results: Total intracellular TNFα production in the liver was significantly upregulated following IR injury (16.3% vs. 8.1%, $p = 0.026$). Inflammatory monocytes produced the highest levels of TNFα (32%) vs. macrophages (15%) vs. CD4+ T cells (10%) vs. CD8 T cells (9%). Production of TNFα by inflammatory monocytes was significantly increased following IR injury ($p = 0.047$) whilst TNFα production by all other cell types was unchanged following IR injury.
Conclusions: Inflammatory monocytes are the main producers of TNFα in the early phase of liver IR injury and targeting agents should be developed.

P24 THE EFFECT OF REMOTE ISCHAEMIC PRECONDITIONING PRIOR TO HEPATIC ISCHAEMIA REPERFUSION INJURY ON CD4+ T CELL CYTOKINE PRODUCTION

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The lack of Ischaemia Reperfusion (IR)injury susceptibility in mice lacking CD4+ T cells identifies them as a key driver of this pathological process. Remote Ischaemic Preconditioning (RIPC) has been shown to ameliorate liver warm IR injury. The mechanism remains unclear. Whether RIPC alters CD4+ T cell cytokine function in the early period following IR injury remains to be elucidated.
Methods: An established mouse liver lobar warm IR model was used with limb RIPC using 24 male Bl6 mice which were divided into four groups.
1: sham laparotomy (3 h)
2: 3 cycles of 5 min of RIPC (left femoral pedicle) followed by sham laparotomy (3 h)
3: 45 min warm hepatic IR injury (left and middle lobes) followed by 2 h reperfusion
4: 3 cycles of 5 min of RIPC (left femoral pedicle) followed by 45 min warm hepatic IR injury (left and middle lobes) followed by 2 h reperfusion
At the end of the experiment the animals were terminated, the livers were immediately harvested and intrahepatic lymphocytes were isolated and cultured in Brefeldin A for 4 h prior to analysis by flow cytometery. CD4 T cell production of the pro inflammatory cytokines IL-6, IL-17A, IFNγ, and TNFα were measured by intra-cellular staining and flow cytometery and was compared between the groups.
Results: IFNγ production by CD4+ T cells was significantly raised following IR injury (groups 1 vs. 3, $p = 0.02$) however RIPC did not significantly reduce IFNγ production by CD4+ T cells (groups 3 vs. 4, $p = 0.57$). Although TNFα production increased significantly in other cell types, TNFα production by CD4+ T cells was not significantly raised following IR injury (groups 1 vs. 3, $p = 0.2$). IL-6 and Il-17A production was minimal in all groups.
Conclusions: IFNγ is the primary cytokine released by CD4+ T cells following IR injury. RIPC does not alter CD4+ T cells cytokine production following hepatic IR injury.

P25 ACCELERATION OF RENAL FIBROGENESIS FOLLOWING ACUTE KIDNEY INJURY: INSIGHTS FROM THE TRANSCRIPTOME OF PROXIMAL TUBULES ISOLATED *EX VIVO*

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Understanding the link between ischemia reperfusion injury and the progression of chronic kidney diseases (CKD) is a new challenge. “Maladaptive repair” was coined to name the pro-fibrotic epithelial changes after acute kidney injury (AKI). So far, models studying maladaptive repair were characterized by an unresolved AKI (unilateral ureteral obstruction or prolonged ischemia). In human, a reversible AKI increases the risk of CKD. Our aim was to interrogate the cellular program of tubular epithelial cells with a history of reversible AKI.
Adult C57BL6/J wild-type mice were subjected to a left nephrectomy, plus (AKI group) or minus (sham group) the clamping of the right kidney pedicle during 20 min. At day 2, AKI mice displayed acute tubular necrosis at the cortico-medullary junction, and impaired renal function (plasma creatinine 19.9 ± 18.4 μmol/l vs. 12.1 ± 11.0 in sham group). At day 28, histological and functional recovery were complete and indistinguishable from sham mice. At this time point, all mice were subjected to angiotensin 2 continuously administered via subcutaneous pumps (1 μg/kg/min) to accelerate fibrogenesis. A similar increase in arterial blood pressure was observed in both groups. At day 56, mice with a previous history of AKI displayed significantly more renal fibrosis. *Ex vivo* isolation of proximal tubules cells (expressing prominin, an antigen from the brush border) at day 0, 28 and 56 allowed mRNA high-throughput sequencing. Principal component analysis showed a high consistency within experimental groups. Pairwise comparison showed a number of genes differentially regulated at day 56. Genes encoding

enzymes involved in metabolic pathways (oxydative phosphorylation, fatty acid metabolism, glycolysis, PPAR signaling pathway) were upregulated in mice with a previous history of AKI.

This suggests that a resolving episode of AKI influences the capacity of proximal tubular epithelium to later produce energy. This is a clue to establish a bridge from AKI to CKD.

P26 QUALITY OF DONOR LUNG GRAFTS: A COMPARATIVE STUDY BETWEEN FAST AND SLOW BRAIN DEATH INDUCTION MODELS IN RATS

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Despite the fact that brain death (BD) negatively affects graft quality and transplantation outcome, brain-dead donors remain the major source for transplantation. This study is designed to test if fast or gradual increase in intracranial pressure, ultimately leading to brain death, differentially affects quality of donor lungs.

Fisher rats were randomly assigned into three donor groups: 1) ventilated animals, no other interventions and immediately sacrificed, 2) fast - and 3) gradual BD induction. For the latter two modalities animals were sacrificed at 30 min, 1 h, 2 h and 4 h after BD induction. BD animals were hemodynamically stabilized (MAP >80 mmHg) by HAES/noradrenaline and ventilated with a Tidal Volume of 6.5 ml/kg of body weight and a PEEP of 3 cmH_2O. Hemodynamics and pulmonary inspiratory pressure were monitored. Lungs (n = 8/group; excluding lost animals n = 6) were analyzed with a histological scoring system and for pro-inflammatory changes in gene expression with RT-PCR.

During slow induction severe hypotension occurred in contrast to severe hypertension during fast BD induction. After BD induction MAP was maintained above the target value however in the fast model higher inotropic support was required. In both groups patho-histological changes were found, albeit that parenchyma injury was more pronounced in the fast model. No difference in the expression of proinflammatory genes was observed between both models.

The results of this study suggest that the time course of intracranial pressure increase leading to BD is critical for the quality of the potential donor lungs.

P27 TREATMENT WITH LITHIUM REDUCE ISCHEMIA-REPERFUSION INJURY IN AGED AND STEATOTIC LIVER IN RATS

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Background: Lithium has been widely used in the treatment of mental illness. It acts on many stress and survival pathways especially on autophagy pathways. Recent studies showed that treatment with lithium can reduce ischemia-reperfusion (I/R) injury in liver via a modulation of MAPK and GSK3b pathways. In this study, we aimed to evaluate the effects of lithium in selective warm I/R model of aged and steatotic liver in rats.
Methods: Steatosis rats (induced by feeding a high fat and methionine-choline reduced diet for 14 days) and aged rats (2 years) received lithium (2 mmol/kg/day, 3 days before and after ischemia). Selective warm ischemia/reperfusion was induced by clamping the hepatoduodenal ligament of the left lateral and median lobe for 60 min. Animals were observed for 30 min, 6 h, 24 h and 48 h (n = 6/group). Read-out parameters consisted of serum liver enzyme levels, HMGB1 translocation and release, liver neutrophil infiltration, MAPK, GSK3b, Caspase 3 and LC3 expression levels.
Results: Treatment with lithium protected against I/R injury in steatotic liver and aged liver, as indicated by lower serum aminotransferase levels, lower inflammatory response (less neutrophil infiltration), lower intracellular stress (less HMGB1 translocation), less apoptosis (lower Caspase 3 expression) and higher levels of autophagy (higher LC3b expression). Treatment with lithium prevented the dephosphorylation of GSK3b and modulated the activation of MAPK pathways after reperfusion.
Conclusion: On the basis of these data, we conclude that treatment with lithium may be a simple way for protecting against I/R injury in steatotic and aged liver. Lithium treatment reduced inflammation and apoptosis via a modulation of MAPK pathway, as well as induced autophagy and reduced necrosis via a modulation of GSK3b pathway.

P28 POTASSIUM AS A POTENTIAL BIOMARKER IN RENAL EX VIVO NORMOTHERMIC PERFUSION

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Background: Ex vivo normothermic perfusion (EVNP) can be used to assess organ viability prior to transplantation. In this study, we investigated the role of potassium as a viability marker in human kidneys undergoing EVNP and determined the association with renal function after transplantation.
Methods: Ninety-three human kidneys allocated for research were assessed using EVNP after being deemed unsuitable for transplantation. EVNP was performed using a modified paediatric cardiopulmonary bypass circuit for 60 min at 36°C after a period of static cold storage. Levels of potassium were measured in the perfusate pre and post EVNP and the percentage change calculated. Levels were correlated with functional parameters.

In a separate series, thirty-four kidneys were transplanted after EVNP. The percentage change in potassium was correlated with graft outcome.
Results: In the discarded series during EVNP, an increasing hyperkalaemia was associated with poor renal function (% creatinine fall $r^2 = 0.36$, $p < 0.0001$; creatinine-clearance $r^2 = 0.36$, $p < 0.0001$; urine output $r^2 = 0.36$, $p < 0.0001$), oxygen consumption ($r^2 = 0.16$, $p = 0.0007$) and renal blood flow ($r^2 = 0.16$, $p = 0.0009$).

In the transplant series, rising levels of potassium during EVNP were associated with a lower level of eGFR ($r^2 = 0.16$, $p = 0.050$) three months after transplantation. There was no correlation with graft function at 12 months post-transplant.
Conclusion: An increase in perfusate levels of potassium during EVNP was associated with a higher level of injury. Measurement of potassium during EVNP provides additional information on kidney viability and could be included in an assessment score.

P29 INCREASED INFLAMMATION TRIGGERED BY HYPEROXALURIA EXACERBATES RENAL ISCHEMIA AND REPERCUSSION INJURY

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Background/Aims: Acute kidney injury (AKI) caused by ischemia and reperfusion injury (I/R) induces renal dysfunction associated with an inflammatory response. On the other hand, renal I/R may contribute to crystal deposition of calcium oxalate (CaOx) in renal tubules causing additional damage to epithelial cells. The objective of this work was to assess whether the interplay between I/R and deposition of CaOx crystals would impact the inflammatory response and the development of renal fibrosis.
Methods: Male rats received or not a solution with 0.8% ethylene glycol (EG) in drinking water for a period of 4 weeks. After that, they were subjected to 60 min of renal I/R. Animals were sacrificed 24 h after. Biochemical and histological parameters were evaluated. Gene and protein expression of pro and anti-inflammatory and -fibrotic molecules were measured. Results

EG increased urine volume and reduced urinary pH. Serum creatinine and urea levels increased in animals subjected to renal I/R as compared to control group. EG further increase these levels, with a significant increment as compared to group I/R. EG treatment also induced higher gene and protein expression of CINC2, CINC3, TNF-alfa, IL-6 and IFN-gamma, with subsequent higher type I collagen, alfa-SMA expression and CD11b infiltration. Increased crystals presence was observed in tubules after I/R, a characteristic of CaOx deposition.
Conclusions: Renal injury is increased after CaOx crystals deposition in renal tubule, leading to increased inflammation and thus, facilitating renal fibrosis.

P30 COST-EFFECTIVENESS OF MACHINE PERFUSION USE AFTER LONG COLD ISCHEMIC TIME IN A KIDNEY TRANSPLANTATION PROGRAM

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In Brazil the incidence of DGF (delayed graft function) is high (60–70%) mainly due to an inadequate care of the donors and long cold ischemia time (CIT). This high incidence of DGF is associated to a longer hospitalization and poorer long-term graft survival. The use of perfusion machine (MP) as a preservation method is associated with a reduction of DGF.
Objectives: The objective of this study is to evaluate the cost-effectiveness of MP use after long CIT in comparison to a cold storage (CS) in a kidney transplantation (KT) program.
Methods: A probabilistic decision tree was developed to compare MP versus CS. The structure of the model was populated by review of the literature and outcomes of KT in our center. The model estimated the incremental cost-effectiveness ratio (ICR) in terms of DGF. The costs analyzed were: transplant surgery; hospitalization stay; dialysis; hemotherapy; laboratory tests; preservation solution and kits. The values were from 09/2014.
Results: 54 KT preserved in the MP from 2/13 to 07/14 was compared to 101 KT preserved by CS from 11/08 to 5/12, showed a DGF rate of 61% for MP and 79% in the CS group ($p = 0.02$), the DGF duration was 5 days in the MP and 11 days in the CS group ($p < 0.001$) and the hospital discharge was 13 days for the MP and 18 days for the CS ($p < 0.01$). Resource consumption for CS was $17,668.39 for immediate graft function (IGF) and $28,902.05 for DGF recipients. In MP group was $16,939.86 for IGF and $22,046.07 for DGF recipients. The incremental cost and effectiveness were: $6,509.60 and 18% respectively. The ICR was $360.42 for each 1% of DGF saved.
Conclusions: The use of the MP decreases DGF and hospital stay, and is cost-effective in terms of savings for DGF.

P31 ANALYSIS OF DOSE-EFFECT OF HEMARINA-M101 IN A PORCINE AUTOTRANSPLANTED AND PERFUSED KIDNEY MODEL

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Introduction: To reduce the risk of Delayed Graft Function, the quality of conservation has to be improved. We analysed the role of HEMARINA-M101 in a preclinical model using a perfusion machine (WAVES®) which can oxygenate the perfusion medium.
Methods: The perfused kidneys without or with addition of 1 g/l or 2 g/l of M101 (WAVES, WAVES 1 g and WAVES 2 g groups respectively) were compared to a sham operated group (SHAM) and a unilateral nephrectomy (NEP) group. We evaluated acute and chronic lesions: graft function evolution, necrosis, histological lesions, inflammation and fibrosis. The pigs were studied during 3 months after a kidney autotransplantation mimicking a NHBD model (warm ischemia: 1H, Preservation by the WAVES perfusion machine: 24 h, contralateral nephrectomy).
Results: Addition of 2 g/l of M101 decreased acute lesions and restored graft function at the end of the first week. For WAVES and WAVES 1 g/l groups, the function recovery was longer and less important. WAVES 2 g group had the lowest level of circulating AST. Cellular necrosis was dose dependant. At 3 months: graft function and Proteinuria/Creatininuria Ratio were the highest, the intermediate and the lowest level in WAVES, WAVES 1 g/l and WAVES 2 g/l groups respectively. There were no differences of chronic histological lesions between Sham, NEP and WAVES 2 g/l. In the other WAVES groups, kidney integrity was decreased. The inflammatory response was absent (Sham), moderate (NEP) and increased (WAVES groups) but with a partial protection at the dose of 1 g/l and a better protection at 2 g/l. Interstitial fibrosis was <10% (Sham) and moderately increased for NEP group. There was a dose-dependant decrease of fibrosis generation in the perfused groups (10–12% in WAVES 1 g and <10% in WAVES 2 g), confirmed by analysis of TGF-ß pathway.
Conclusion: The use of HEMARINA-M101 at a dose of 2 g/l provide an increased level of protection for kidneys during perfusion in a preclinical model of NHBD.