ABSTRACTS BOOK

1st Session: Donor Management

Keynote lecture:
New evidences in Brain Dead donor management
Patrick Ferdinande (Leuven, Belgium)

O1 REDUCING NON-ANASTOMOTIC BILIARY STRICTURES AFTER DONATION AFTER CIRCULATORY DEATH LIVER TRANSPLANTATION, A MATTER OF TIME?

N. Gilbo, I. Jochmans, M. Sainz, J. Pirenne, D. Monbaliu
University Hospitals of Leuven – KU Leuven, Abdominal Transplantation Surgery and Coordination, Leuven, Belgium

Liver Transplantation (LT) from donation after circulatory death (DCD) is associated with increased non-anastomotic biliary strictures (NAS) and graft loss. Donor and recipient demographics, transplant and outcome data were compared between recipients with NAS (NAS+) and those without (NAS-) in a cohort of 61 consecutive DCD-LT (01/2003–12/2013). Risk factors for NAS occurring <1 year after DCD-LT were identified in multivariate regression. Median (IQR) is given.

13/61 developed NAS. Incidence of NAS decreased over time (30% in 2003–2010, 15% in 2011–2013) in parallel with a decrease in cold ischemia time (CIT) [6.8 h (5.5–8) in 2003–2010, 5.4 h (4.75–6.4) in 2011–2013, p = 0.002]. Donor and recipient age and gender, warm ischemia time [NAS+ 20 min (15–29) vs. NAS- 22 min (16–28), p = ns], donor peak AST/ALT did not differ. NAS+ had a higher DRI [3.01 (2.86–3.49) vs. 2.67 (2.37–3.05), p = 0.031], longer CIT [7.3 h (5.95–8.52) vs. 5.6 h (4.97–6.75), p = 0.004] and anastomotic time [55 min (46.5–60.5) vs. 46 min (42–52.5), p = 0.038]. Peak ALT post-LT was higher in NAS+ vs. NAS- [1114 IU/l (745–1566) vs. 645 IU/l (318–1087), p = 0.019]. No difference in early allograft dysfunction (NAS+ 23.1% vs. NAS- 20.8%, p = ns) or acute kidney injury was observed. The need for re-LT and endoscopic biliary intervention was higher in NAS+ vs. NAS- (7.7% vs. 0% and 84.6% vs. 10.4% respectively, p < 0.0001). One year censored graft and patient survival were similar between NAS+ and NAS- (84.6% vs. 89.6%, 92.3% vs. 91.7% respectively, p = ns). NAS did not influence the risk of death (HR:1, 95% CI: 0.28–3.6) or graft loss (HR:1.62, 95% CI: 0.57–4.6). CIT was the only independent risk factor of NAS (HR:1.42, 95% CI: 1.06–1.92). Even with overall short CIT (5.78 h, 5.13–7.13), the risk for NAS development in the 1st year post-transplant increases 1,42 times by every additional hour of CIT. In the absence of interventions that might directly prevent NAS, active efforts to maximally reduce CIT in DCD-LT are essential.

O2 ACTIVATION OF CYTOPROTECTION MECHANISMS IN DBD DONOR KIDNEYS DEFINE KIDNEY FUNCTION IN TRANSPLANT RECIPIENTS- A STUDY USING CLINICAL SAMPLES OBTAINED FROM THE QUOD BIOBANK

L. Van Dullemen[1,2], M. Kaisar[2,3], Z. Akhtar[2], L. Lo Faro[2], H. Huang[2,3], H. Leuvenink[1], B. Kessler[3], R. Ploeg[2]
[1]Surgery Research Laboratory, Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [2]Nuffield Department of Surgical Sciences and Biomedical Research Centre; [3]Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK

Introduction: Deceased brain dead (DBD) donors are the main source of deceased donor kidneys for transplantation. The onset of brain death adversely impacts the short and long term outcome after transplantation. Understanding the biological mechanisms that impact kidney quality will allow better allocation of donated kidneys, minimise discard, and allow the development of novel interventions during donor management to improve transplantation outcomes.
Methods: Kidney biopsies from 40 DBD donors were obtained at explantation and grouped according to kidney function following transplantation. Suboptimal kidney function was defined as the incidence of delayed graft function (DGF) and eGFR <39 ml/min 3 months post transplantation, while good function was defined as no incidence of DGF and eGFR >50 ml/min at 3 months. Using label free quantitative proteomics we compared 5 individual samples per group using tandem mass spectrometry (LC-MS/MS, Q Exactive). Proteins of interest were validated by immunoblotting on a separate cohort of 15 samples per group. Kidney biopsies from 10 living donors were analysed in parallel as the control group.
Results: With the proteomic signature we could, without "a priori" assumptions, differentiate the donor kidneys with suboptimal function after transplantation. An increased abundance of the apoptosis mediator STAT-1 and enhanced degradation of cytoskeletal- and integrin proteins of the donor kidneys associated to suboptimal function post transplantation. In addition, antioxidant proteins Theoredoxin, GST, and Peroxiredoxins were more abundant in kidneys with good outcome.
Discussion: The fingerprint of donor kidneys encompasses biological information that can discriminate grafts with suboptimal function. These results indicate that donor kidney quality depends on the balance of the degree of ischemia or repair, and interventions in the donor should aim to ameliorate injury and enhance repair mechanisms.

O3 C5L2 PROMOTES LOCAL RENAL INFLAMMATION AFTER DONOR BRAIN DEATH

N.M. Jager[1], F. Poppelaars[1], M.B. Van Werkhoven[1], Z.J. Veldhuis[3], P.J. Ottens[3], W.J. Van Son[1], H.G.D. Leuvenink[3], J.L. Hillebrands[2], M.A.J. Seelen[1]
[1]Department of Internal Medicine; [2]Department of Pathology and Medical Biology; [3]Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands

Kidneys retrieved from brain-dead donors show inferior post-transplant results compared to living donor kidneys. Donor brain death (BD) itself triggers activation of the immune system. Possibly, the systemic inflammatory response induced upon BD affects organ viability prior to organ retrieval, resulting in reduced post-transplant function and survival. As part of the inflammatory response, the complement system is activated, reflected by elevated levels of anaphylatoxin C5a circulating in the organ donor. The two known C5a receptors, C5aR and C5L2, are expressed in the kidney. Previously we reported that expression of C5aR is increased in kidney biopsies from brain-dead donors compared to living donors. The present study investigated the contribution of C5aR and C5L2 to donor BD-induced renal inflammation. To achieve this, we developed a mouse BD model, with a 3 h BD period. Subsequently, WT, C5aR$^{-/-}$ and C5L2$^{-/-}$ mice were subjected to this BD procedure. We observed that, compared to WT, C5L2$^{-/-}$, but not C5aR$^{-/-}$ mice, are protected for BD-induced decline in renal function and show reduced renal gene expression of KC, TNFα, MCP-1 and P-selectin upon donor BD. These results suggest that C5L2 is involved in BD-induced renal inflammation. Therefore C5L2 would be a target for intervention to prevent renal allograft priming in brain-dead donors.

O4 UNEXPECTED DONATION AFTER CIRCULATORY DEATH (UDCD) – A GREAT POTENTIAL FOR NEW ORGANS?

L. Venema[2], A. Brat[2], B. Bens[1], D. Van Der Vliet[5], T. Tromp[5], W. De Jongh[4], M. Van De Haak-Willems[4], M. Erasmus[3], C. Krikke[2]
[1]Emergency Medicine; [2]Surgery; [3]Thoracic Surgery, UMCG, Groningen; [4]Surgery, MUMC, Maastrich; [5]Surgery, Radboud UMC, Nijmegen, The Netherlands

The waitinglist for a transplant exceeds the number of organs suitable for transplantation. Therefore, the search for new organ sources continues. uDCD donors are scarcely used in the Netherlands as a source of organ grafts. A possible reason for this is the complexity of the protocol in terms of logistics.

Three centres started a pilot with the aim to assess the uDCD potential. The innovative part was the use of normothermic regional perfusion before procurement and ex vivo lung perfusion after retrieval to assess lung functioning.

All patients deceased on the emergency department between October 2014 and October 2015 within the age range of 18–50 were potential donors for kidneys and lungs, and between 51–65 potential lung donors. Donors could only be included when fulfilling all of the following inclusion criteria: witnessed arrest, basic life support started within 10 min, advanced life support within 20 min and resuscitation within an organ-specific time span. Furthermore, donors were excluded if there were any medical contraindications and permission for donation was not given.

208 patients died from whom 32 were potential kidney and 68 lung donor. From 32 potential kidney donors, 15 failed the inclusion criteria (47%), 7 did not consent (22%), 9 showed medical contra-indications (28%), and once logistical problems occurred (3%). From 68 potential lung donors, 22 failed the inclusion criteria (33%), 18 did not consent (27%), 24 had medical contra-indications (35%) and 3 had logistical problems (5%). One donor satisfied all criteria. However, during procurement the lungs were not suitable for transplantation.

This pilot shows there is a group of potential uDCD donors. Nevertheless, no donor resulted in transplantation. Although there were many reasons why donation in this pilot was not successful, combining new techniques with new organ sources remains a part of the solution for the shortage. We are obliged to explore these sources furthermore.

2nd Session: New aspects of cell metabolism during IRI

Keynote lecture: Metabolic changes in ischemic kidneys and metabolomics
Benedikt Kessler (Oxford, UK)

O5 DETERMINATION OF OXIDATIVE AND NITROSATIVE STRESS DURING LUNG ISCHEMIA-REPERFUSION INJURY

J. Gielis[1], J. Briedé[2], P. Cos[1], P. Van Schil[1]
[1]Antwerp University, Wilrijk, Belgium; [2]Laboratory for Toxicogenomics, Maastricht University, Maastricht, The Netherlands

Objectives: Pulmonary ischaemia-reperfusion injury (IRI) is associated with several life-threatening pulmonary disorders, and may severely compromise the outcome of lung transplantation. Highly reactive molecules such as superoxide, nitric oxide (NO.) and peroxynitrite ($ONOO^-$) are presumed to contribute to IRI pathogenesis, but this assumption is based on indirect measurements. We use electron spin resonance (ESR) to directly quantify free radical formation after pulmonary ischaemia and reperfusion.
Methods: Five groups of 10 Swiss mice were subjected to left pulmonary hilum clamping for 1 h of ischaemia followed by 0, 1, 4 and 24 h of reperfusion or to sham thoracotomy alone as control procedure. In five mice per group, ESR was used to measure iron-diethyldithio-carbamate trihydrate-trapped $NO^·$ in the lung. In the other group of 5, reactive oxygen species generation in the lung and in blood was quantified with ESR by detection of ascorbyl radical and CMH spin probe, respectively. Pulmonary $ONOO^-$ was monitored with nitrotyrosine Western blotting.
Results: After 1 h of reperfusion, a pulmonary $NO^·$ peak ($14.69 \pm 0.91 \times 10$ (4) Arbitrary Units (A.U.). vs. $1.84 \pm 0.75 \times 10(4)$ A.U. in sham; $p < 0.001$) coincided with a significant increase in nitrosated proteins (0.105 ± 0.015 A.U.) compared with sham (0.047 ± 0.006 A.U.); $p < 0.005$). Peripheral blood showed a significant free radical burst after 1 h of ischaemia ($11\,774 \pm 728$ A.U. vs. 6660 ± 833 A.U. in sham; $p < 0.001$).
Conclusions: Longitudinal quantification of free radicals during IRI reveals the occurrence of two major radical bursts. The radical peak in peripheral blood after ischaemia may be related to systemic hypoxia. After 1 h of reperfusion, the lung tissue shows a significant increase of superoxide, $NO^·$ and their reaction products, which are probably involved in IRI pathogenesis.

O6 INCREASED COLD ISCHEMIA TIME LEADS TO A HIGHER VASCULAR REMODELING IN A PORCINE KIDNEY AUTOTRANSPLANTATION MODEL: A PRELIMINARY MICRO-COMPUTED TOMOGRAPHY STUDY

S. Maiga[6,7], F. Favreau[6,7,3], F. Guy[4], J.M. Goujon[6,2,7], M. Dierick[1], T. Hauet[6,7,3,8,5]
[1]UGCT-Department of Physics and Astronomy, Ghent University, Ghent, Belgium; [2]CHU de Poitiers, Service d'Anatomopathologie; [3]CHU de Poitiers, Service de Biochimie; [4]CNRS INEE UMR 7262; [5]Fédération Hospitalo-Universitaire SUPPORT (Tours Poitiers Limoges) ; [6]INSERM U1082; [7]Faculté de Medecine et Pharamcie, Université de Poitiers, Poitiers; [8]Plateforme MOPICT, INRA/IBiSA, Domaine du Magneraud, Surgères, France

Introduction: Ischemia-reperfusion injury is responsible for a pathophysiological process targeting graft microvasculature in organ transplantation. The goal of this preliminary work is to characterize renal cortical microvasculature remodeling related to the increased duration of the hypothermic preservation in a porcine autotransplanted kidney model.
Materials and Methods: Three-month-old male pigs were used. Left kidneys were harvested and preserved in UW solution at 4°C for 24 h ($n = 5$) or for 48 h ($n = 4$). A contralateral nephrectomy was performed to mimic the nephron mass in the transplanted human recipient. The auto-transplanted animals were followed for 3 months (M3). At M3, kidney grafts were perfused with a radio-opaque silicone polymer and the cortical part was studied by X-ray micro-computed tomography. Vascular morphology is studied by a three-dimensional analysis of images from the cortex.
Results: At M3, the hypothermic preservation for 48 h of grafts compared to 24 h induced a significant increase of the cortical area thickness ($13{,}98 \pm 0.79$ mm vs. 11.41 ± 0.39 mm, $p = 0.03$), associated to a drastic decrease of microvessels contrasting with an increase in the percentage of vessels with a diameter >120 μm. These results are accompanied by an impairment of renal function and histological lesions characterized by tubulointerstitial fibrosis and tubular atrophy in the hypothermic preservation for 48 h group.
Conclusion: These preliminary results suggest that long preservation duration impacting the renal function in grafts could be associated with chronic hypoxia and fibrosis related to microvascular rarefaction.

O7 LEUKOCYTE-DERIVED MICROPARTICLES FAVOR ENDOTHELIAL SENESCENCE: IMPACT FOR PANCREATIC ISLET ENGRAFTMENT

A. El Habhab[1], M. Abbas[2], M. Kassem[1], G. Kreutter[1], S. Khemais-Benkhiat[2], F. Zobairi[1], V. Schini-Kerth[2], F. Toti[1,2], L. Kessler[1]
[1]EA 7293 Stress vasculaire et tissulaire en Transplantation; [2]UMR 7213, Illkirch, France

Introduction: Microparticles (MPs) are plasma membrane fragments shed from almost all cell types that act as cellular and vascular effectors. During Ischemia-Reperfusion (IR), cells and MPs expose phosphatidylserine that enhances the activity of tissue factor (TF), the cellular initiator of coagulation. During islets transplantation, IR and instant blood mediated inflammatory reaction (IBMIR), favor endothelial cell and leukocytes stimulation and the generation of noxious MPs. We aimed the impact of MPs-mediated leukocyte inflammatory response on primary endothelial cells (ECs).
Methods: Porcine coronary artery young P1 ECs were incubated with leukocytes-derived MPs (1–30 nM) isolated from rat splenocytes treated by LPS (5 μg/ml) or PMA (25 ng/ml)-A23187 ionophore (1 μM). Senescence-Associated β-galactosidase activity (SA-b-GALact) was assessed by C12FDG probe, Senescence markers, oxidative stress, local angiotensin system proteins and TF by Western blot, apoptosis by double propidium iodide/annexin-V (IP/AV) staining and caspase-3 protein expression.
Results: MPs induced a significant raise in SA-b-GALact in P1 ECs (18 ± 5 vs. 58 ± 6 MFI, $p < 0.05$) after 48 h associated with p53, p21, p16 (up to 3-fold) expression level. The 2-fold up-expression of NADPH oxidase subunits (GP91, P47 and P22phox) and 3-fold down-expression of eNOS indicated MP-mediated oxidative stress. MPs prompted procoagulant TF up-expression (up to 3-fold) and a secondary generation of MPs. AT1 and ACE expression (up to 1.5-fold) was increased. No significant variation in IP/AV labelling nor caspase-3 activation was detected (8% vs. 13%).
Conclusion: Leukocyte-MPs induce premature senescence and thrombogenicity in young primary ECs. Our data suggest that MPs prompt premature endothelial aging during IBMIR and possibly contributing to limited islets perfusion and thereby to accelerated islets loss.

O8 DRUG DEVELOPMENT IN ISCHEMIA REPERFUSION INJURY IN MOUSE MODELS – ADVANTAGE OF NON-INVASIVE LONGITUDINAL MAGNETIC RESONANCE IMAGING

F. Gueler[1], S. Rong[1], R. Chen[1], A. Thorenz[1], H. Abou-Rebyeh[2], J. Braesen[1], H. Lang[1], M. Gutberlet[1], D. Hartung[1], H. Haller[1], B. Hartleben[1], K. Hueper[1]
[1]Medical School Hannover, Hannover, Germany; [2]NMC Royal Hospital, Abu Dhabi, United Arab Emirates

Background: Ischemia reperfusion injury (IRI) is a major complication after liver and kidney transplantation. Development of new therapies is urgently needed to avoid IRI. Non invasive magnetic resonance imaging (MRI) methods to quantify IRI induced organ dysfunction allow to study organ perfusion, edema and inflammation.
Methods: 35 and 45 min unilateral renal IRI was done and mice were followed by functional MRI. Similarly, IRI of the liver was induced by clamping the ventral liver lobes for 60 and 90 min. MRI results were correlated with inflammation, fibrosis and pro-inflammatory cytokine mRNA expression. Blood liver function parameters (ALT, AST and LDH) were assessed in both models.
Results: In renal IRI short and long clamping time caused similar impairment of renal perfusion in the early phase (d1). However, after 7 days differences were observed. In the 35 min IRI model regeneration occurred and renal perfusion almost reached baseline levels after 4 weeks follow up histology showed mild renal scaring. Whereas, 45 min IRI caused progressive renal fibrosis and ongoing inflammation with kidney volume loss of almost 50%.

For liver IRI longer clamping times of 60 and 90 min were used. Hepatocyte dysfunction was monitored by impaired uptake of the liver-specific contrast-agent primovist® which correlated with glycogen storage dysfunction in PAS stained tissue. In addition, tissue edema was monitored by T1 and T2 mapping. Pro-inflammatory cytokine mRNA up-regulation correlated with the duration of IRI in both models. Pro-inflammatory cytokine up-regulation was predictive for later organ fibrosis.
Conclusion: MRI offers a valuable tool for drug development in pre-clinical studies.

3rd Session: What can we learn from our neighbors?

Keynote lectures:

1) Cardioprotective strategies outside Transplantation

-Heart protection at the crossroad: what perspectives?
Michel Ovize (Lyon, France)

-Vasculoprotection at the heart of cardioprotection
Stéphane Germain (Paris, France)

2) What can we learn from hibernators?
Yann Voituron (Lyon, France)

4th Session: Immunology

Keynote lecture:
Interactions between endothelial cells and polyclonal anti-thymocyte Globulins
Andres Beiras Fernandez (Frankfurt, Germany)

O9 DEFICIENCY OF IL-33 RECEPTOR (ST2) ATTENUATES KIDNEY ISCHEMIA-REPERFUSION INJURY IN EUGLYCEMIC BUT NOT IN STREPTOZOTOCIN-INDUCED DIABETIC MICE

M. Sehnine, M. Fehrat, J.M. Gombert, J.M. Goujon, T. Hauet, A. Herbelin, S. Hadjadj
INSERM U1082 IRTOMIT, Poitiers, France

Background/Objective: Diabetes is a risk factor for renal hypoxia, associated with acute and chronic renal failure. Ischemia-reperfusion injury (IRI) induces necrosis of renal cells and the release of "alarmins" such as IL-33 that can activate the innate immune system, thereby triggering an inflammatory response. As diabetes has been found to exacerbate the inflammatory response in kidney, we examined the impact of IL-33 receptor deficiency ($ST2^{-/-}$) on renal IRI in mice and its interaction with diabetes.
Research Design and Methods: Diabetes mellitus was induced with low-dose streptozotocin (STZ) (50 mg/kg/day) for 5 consecutive days in 8–12-week-old male C57BL/6 wild-type (WT) and $ST2^{-/-}$ mice. Control mice received citrate buffer (vehicle). Renal IRI was performed 3 months after STZ/Vehicle treatment by nephrectomy of left kidney (non-ischemic control kidney) and clamping the right renal artery for 32 min. Renal function and injury were determined at baseline (D0) and after 24 h of reperfusion (D1) by plasma creatinine, blood urea nitrogen (BUN) and acute tubular necrosis scoring.
Results: At baseline, diabetic $ST2^{-/-}$ mice had an increase ($p < 0.05$) in albuminuria compared with diabetic WT mice. When considering IRI procedure, plasma creatinine and BUN levels significantly increased at D1 compared to D0 in both diabetic and WT control mice, while no difference was found in $ST2^{-/-}$ mice. At D1, kidney IRI induced a rise in tubular injury in both diabetic and WT control mice, but also in their $ST2^{-/-}$ counterparts. Comparing diabetic and non-diabetic animals, no difference in tubular injury was found in WT mice, while diabetic $ST2^{-/-}$ mice had an increase ($p < 0.05$) in tubular injury compared to their age-matched $ST2^{-/-}$ controls.
Conclusion: Our data suggest that knockout of ST2 receptor protects against renal IRI in euglycemia but not after STZ-induced diabetes. This should be taken into account for developing future therapies targeting the alarmin pathway.

O10 IL-33 IS REQUIRED FOR KIDNEY ISCHEMIA-REPERFUSION-INDUCED INJURY IN MICE

M. Ferhat[6,7], S. Giraud[6,7,3], A. Robin[6,7,3], J.M. Goujon[6,7,2], T. Hauet[6,7,3,5], J.M. Gombert[6,7,1], A. Thierry[6,7,4], A. Herbelin[6,7]
[1]CHU de Poitiers, Laboratoire d'Immunologie; [2]CHU de Poitiers, Service d'anatomo-pathologie; [3]CHU de Poitiers, Service de Biochimie; [4]CHU de Poitiers, Service de Néphrologie-Hémodialyse-Transplantation Rénale; [5]Fédération Hospitalo-Universitaire SUPPORT (Tours Poitiers Limoges) ; [6]INSERM U1082; [7]Université de Poitiers, Faculté de Medecine et Pharmacie, Poitiers, France

Introduction: Ischemia-reperfusion (IR) injury during kidney transplantation induces necrosis of renal cells and the release of "alarmins" such as IL-33 that can activate the innate immune system, thereby triggering an inflammatory response and tissue damage leading to renal failure, dysfunction or rejection. The aim of this study was to investigate the contribution of IL-33 in kidney IR injury.
Methods: 10–12-week-old wild-type (WT) and IL-33-deficient ($IL\text{-}33^{Gt/Gt}$) male C57/Bl6 mice were subjected to 32 min of unilateral kidney ischemia or a Sham operation. After 24 h, kidneys were harvested and leucocyte infiltration (macrophages, neutrophils, NK and NKT cells) was analyzed by flow cytometry. Renal injury was assessed by measurement of plasma creatinine and histological grading.
Results: Plasma creatinine level and tissue damage significantly increased after renal IR in WT mice, as compared with Sham-operated animals, a difference that disappeared when WT mice were replaced by $IL\text{-}33^{Gt/Gt}$ mice. Even though intra-renal neutrophil (CD11b(+)GR-1(+)) were significantly increased 24 h-post IR in both WT and $IL\text{-}33^{Gt/Gt}$ mice, this phenomenon was found to be significantly attenuated in $IL\text{-}33^{Gt/Gt}$ mice. Moreover, monocyte/macrophages (CD11b(+)F4/80(+)) and NKT cells, also known for their deleterious effect during renal IR injury, seemed to be less recruited 24 h-post IR in $IL\text{-}33^{Gt/Gt}$ mice compared with WT mice.
Conclusion: $IL\text{-}33^{Gt/Gt}$ mice are less sensitive to kidney damage 24 h-post IR, consistent with a deleterious effect of IL-33 during renal IR injury. This study underlines a new possible role of IL-33 as an innate-immune mediator during kidney IR injury.

O11 CIRCULATING CYTOKINE LEVELS AND OUTCOME FOLLOWING HUMAN ORTHOTOPIC LIVER TRANSPLANTATION (OLT)

F. Robertson[3], V. Male[2], G. Wright[1], B. Fuller[3], B. Davidson[3]
[1]Department of Immunology, Edinburgh Napier University, Edinburgh; [2]Department of Infection and Transplantation; [3]Department of Surgery and Interventional Science, University College London, London, UK

Ischaemia reperfusion (IR) injury is a major factor in patient and graft survival following OLT. Circulating cytokines are felt to be key effectors of the injury in the early phase. However the association between cytokine levels and outcome is unknown.

The aim of this study was to correlate levels of serum cytokines following OLT with outcome.
Methods: Cytokines were measured as part of a prospective trial on remote ischaemic pre-conditioning in human orthotopic liver transplantation (RIPCOLT trial). Following ethical approval and informed consent, peripheral samples were collected from 28 patients (24M, 4F) undergoing OLT on induction of anaesthesia, at the end of the transplant surgery and at 24 h post op.

Samples were centrifuged and serum was stored at −80 °C. Circulating levels of IL-2, IL-6, IFNγ and TNFα were measured by Legendplex (Biolegend) and levels of IL-8 and IL-17A were measured by ELISA (Biolegend).
Results: Of the 28 patients, 1 died peri-operatively from severe IR injury, primary graft non function, coagulopathy and blood loss.. There was no other graft loss. Baseline values for all cytokines measured were very low. Circulating levels of IL-6 ($p < 0.001$), IL8 ($p = 0.001$), IL-10 ($p < 0.001$) and IL-17A ($p = 0.028$) were significantly raised post reperfusion but returned to undetectable levels 24 h post transplantation. Circulating levels of IL-2 ($p = 0.4$), IFNγ ($p = 0.3$) and TNFα ($p = 0.7$) showed no significant change during the perioperative period. Patients who developed AKI, post-operative complications or infective complication did not have higher cytokine levels than those that did not.
Conclusions: This study shows that circulating levels of pro-inflammatory cytokines Il-6, IL-8 and Il-17A are raised following reperfusion however the levels did not correlate with post-operative outcome in this patient population.

O12 C5L2 IN RENAL ISCHEMIA-REPERFUSION INJURY

F. Poppelaars[2], M. Molenaars-Van Werkhoven[2], J. Kotimaa[6], Z. Veldhuis[4], A. Ausema[5], J. Damman[1], H. Leuvenink[4], R. Daha[6,2], W. Van Son[2], C. Van Kooten[6], R. Van Os[5], J. Hillebrands[3], M. Seelen[2]
[1]Department of Pathology, AMC, Amsterdam; [2]Department of Nephrology; [3]Department of Pathology and Medical Biology; [4]Department of Surgery; [5]European Research Institute for the Biology of Ageing, UMCG, Groningen; [6]Department of Nephrology, LUMC, Leiden, The Netherlands

The complement system, and specifically C5a, is involved in renal ischemia-reperfusion injury (IRI). The two receptors for C5a, C5aR and C5L2, are expressed on leukocytes as well as in the kidney. Extensive evidence shows that C5aR inhibition protects kidneys from IRI, but the role of C5L2 in IRI has not been studied so far. Therefore, WT, C5aR-/- and C5L2-/- mice were subjected to 40 min of bilateral renal ischemia, followed by reperfusion for 1, 3 or 7 days. It was found that C5L2-/- mice were protected against IRI, resulting in significant lower plasma creatinine and BUN levels, and reduced acute tubular necrosis. Next, an *in vivo* migration study, where WT, C5aR-/- and C5L2-/- mice were injected intraperitoneally with complement ligands, revealed that C5L2 is not involved in leukocyte migration. To investigate the contribution of renal-expressed C5L2 versus leukocyte-expressed C5L2 in renal IRI, bone marrow chimeras were created. Our data show that renal-expressed C5L2 and leukocyte-expressed C5L2 mediate IRI-induced renal dysfunction. Therefore, C5L2 is a functional receptor in renal IRI rather than a simple decoy receptor. For that reason, next to C5aR, C5L2 is a potential target for intervention during renal IRI.

5th Session: IRI and cells

Keynote lecture: Mesenchymal stromal cell therapy in ischemia/reperfusion injury : review of the experimental and clinical evidence
François Jouret (Liège, Belgium)

O13 ADMINISTRATION OF THIRD-PARTY MESENCHYMAL STROMAL CELLS AT THE TIME OF KIDNEY TRANSPLANTATION: INTERIM SAFETY ANALYSIS AT ONE-YEAR FOLLOW-UP

L. Weekers[3], P. Erpicum[3], O. Detry[1], C. Bonvoisin[3], A. Briquet[2], C. Lechanteur[2], E. Baudoux[2], F. Jouret[3], Y. Beguin[2]
[1]Abdominal Surgery; [2]Hematology; [3]Nephrology, ULg CHU, Liège, Belgium

Introduction: Mesenchymal stromal cells (MSC) therapy has been suggested in kidney transplantation (KTx). We report on the 1-year follow-up of an open-label phase I trial using MSC at the time of KTx.
Patients and Methods: On postoperative day 3 (D3), third-party MSC (~2.0 $\times$ 10^6/kg) were administered to 7 non-immunized first-transplant recipients from deceased donors, under standard immunosuppression (Basiliximab, Tacrolimus, MMF and steroids). No HLA matching was required for MSC donors. In parallel, 7 comparable KTx recipients were included as controls. Informed consent was obtained from all participants.
Results: No hemodynamic or immune-allergic side-effect was noted at the time of MSC injection. Still, 1 patient with a history of ischemic heart disease had a NSTEMI ~3 h after MSC infusion. Ten months after KTx, 1 MSC patient had type B aortic dissection and STEMI. Four MSC patients had at least 1 opportunistic infection, whereas 3 controls had polyoma-BK viremia. Three MSC patients were affected by at least 1 (pulmonary) infection, whereas 3 controls had urinary infection. No MSC engraftment syndrome was observed. At D14, eGFR in MSC and control groups was 47.1 $\pm$ 6.8 and 39.7 $\pm$ 5.9 ml/min, respectively (p, 0.05). Nevertheless, eGFR in MSC and control groups at 1 year was 43.1 $\pm$ 17.8 and 53.9 $\pm$ 13.4 ml/min, respectively (p, 0.25). At 3-month protocol biopsy, borderline rejection (BR) was evidenced in 1 MSC patient. Later on, 1 BR and 1 AR were diagnosed at D240 and D330, respectively. No biopsy-proven AR was noted in controls. Three patients developed anti-HLA antibodies against MSC (n = 1) or shared kidney/MSC (n = 2) mismatches.
Conclusions: MSC infusion was safe in all patients except one. Incidence of opportunist and non-opportunist infections was similar in both MSC and control groups. No MSC engraftment syndrome was documented. No difference in eGFR was found at 1 year *post* KTx. Putative immunization against MSC was observed in 3 patients.

O14 CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM PORCINE ADIPOSE TISSUE AND THEIR EFFECTS ON KIDNEY GRAFT RECOVERY IN A PRECLINICAL PORCINE MODEL OF RENAL AUTO-TRANSPLANTATION MIMICKING THE NON-HEART-BEATING-DONOR CONDITIONS

A. Kasil[2], X. Matillon[2], C. Auxenfans[1], T. Hauet[4,3,5], F. Favreau[3,4], W. Hebrard[6], P. Couturier[6], L. Badet[2,3,4]
[1]Banque de Tissus et Cellules, Lyon; [2]Service d'urologie, Hospices Civils de LYON, Lyon; [3]Service de Biochimie, CHU de Poitiers; [4]INSERM U1082, Université de Poitiers, Faculté de Medecine et Pharmacie, CHU de Poitiers, Service de Biochimie, Poitiers; [5]Plateforme MOPICT Labelisée IBiSA, Unité GENeSI; [6]Plateforme MOPICT Labelisée IBiSA, Unité GENeSI, INRA, Surgères, France

Introduction: Ischemia reperfusion (IR) is a key process involved in acute and chronic renal graft dysfunction. The objective of this study was to characterize mesenchymal stem cells from porcine adipose tissue (pASC) and their role in the graft function recovery in conditions mimicking Non-Heart-Beating-Donors (NHBD).
Materials and Methods: Morphology, proliferative capacities, phenotype by flow cytometry and the metabolic profile in Nuclear Magnetic Resonance (NMR) of porcine ASC (pASC) were determined. Their resistance to a sequence of hypoxia-reoxygenation (HR) was tested by analyzing their viability and metabolic profile in NMR. Feasibility, functional and histological outcomes of an autologous injection of 10^6 pASC/kg in the renal artery of 3 auto-transplants kidneys after 1 h of warm ischemia and 24 h of storage at 4°C in UW solution and contralateral nephrectomy were compared to a group of autotransplanted pigs without injection of pASC.
Results: The cell extraction technique was reproductible and allowed having sufficient pASC with the characteristics of mesenchymal stem cells. The metabolic profile in NMR of pASC was not changed with the passages, characterizing the stability of the cell lines. The cell viability after a sequence of HR exceeded 70%. The injection of 10^6 pASC/kg was practicable 15 days after removal of adipose tissue. The function recovery was significantly improved and the histological lesions were significantly reduced in the group treated by pASC.
Conclusion: Injection of pASC in renal graft artery at reperfusion of the grafts in a porcine model mimicking Non-Heart-Beating-Donors conditions could improve graft function recovery and limits tubular damages at day 7. These therapeutic potentials will be confirmed by further studies at the end of the follow-up at 3 months of 6 animals.

O15 CONTRIBUTION OF GD T-CELL SUBSETS AND IL-17A ACTIVATION TO RENAL ISCHEMIA REPERFUSION INJURY IN MICE

A. Thorenz, S. Rong, N. Voelker, R. Chen, J. Braesen, H. Haller, C. Klemann, F. Gueler
Medical School Hannover, Hannover, Germany

Background: Ischemia reperfusion injury (IRI) contributes to acute kidney injury (AKI) and to delayed graft function (DGF) after kidney transplantation. After initial activation of myeloid cells in the first 48 h after IRI, T-cells invading the renal tissue are relevant producers of the pro-inflammatory mediator IL-17A. In this project, we evaluated the role of T-cell subsets (ab versus gd T-cells) and IL-17A on inflammation and fibrosis induced by ischemia reperfusion injury in mice.
Methods: IRI was induced by unilateral clamping of the renal pedicle for 45 min and mice were sacrificed after 7 days when infiltrating T-cells were dominant. In a second model IRI induced delayed graft function (DGF) was studied after allogenic transplantation (ktx) and again leukocyte composition was studied at day 7 and compared to IRI alone. T-cell receptor (TCR-gd) and IL-17A deficient and wildtype (WT) control mice were tested in the IRI model as well. FACS analysis, histology and immunohistochemistry for inflammation and fibrosis as well as qPCR was done.
Results: IRI and ktx resulted in substantial T-cell infiltration but the distribution of T-cell subsets were different. In IRI ab T-cell infiltrates were 2.5 fold higher compared to gd T-cells whereas in the combination of IRI with ktx ab T-cell infiltrates were about 8 fold higher compared to gd T-cell infiltrates. The gd T-cells contributed substantially to elevated IL-17A production. Surprisingly, gd T-cells and IL-17A deficient mice were not protected from IRI and showed progressive renal fibrosis similar to WT mice. In both mouse strains (TCR-gd and IL-17A deficient mice) IL-17A production of gd T-cells was totally abrogated in ex vivo T-cell stimulation with PMA/ionomycin.
Conclusion: Surprisingly, neither gd T-cell nor IL-17A deficiency attenuated IRI, inflammation or tissue fibrosis.

O16 ENDOTHELIAL MICROPARTICLES RELEASED BY ACTIVATED PROTEIN C EXERT A CYTOPROTECTIVE EFFECT ON BETA CELLS: INTEREST IN ISLET PANCREATIC TRANSPLANTATION

G. Kreutter[2,3], M. Abbas[1], M. Kassem[2], A. El Habhab[2], F. Zobairi[2], L. Kessler[2,3], F. Totl[2], G. Ubeaud-Sequier[2,3,4]
[1]UMR 7213, Laboratoire de Biophotonique et Pharmacologie, CNRS; [2]EA 7293, Stress vasculaire et tissulaire en transplantation, Université de Strasbourg, Illkirc; [3]Endocrinologie-Diabète-Nutrition et Addictologie, Hôpital Civil; [4]Pharmacie-Stérilisation, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

During islet transplantation, Ischemia/reperfusion leads to inflammation and graft loss. Early events combine endothelial damage, the local recruitment of leukocytes and activation of coagulation. The Activated Protein C (APC) limits thrombin generation and exerts endothelial cytoprotection by targeting the Protease Activated Receptors (PARs). In blood flow, procoagulant microparticles (MP) shed from the plasma membrane of activated cells are cellular effectors. APC-treated endothelial cells (EC) release MP bearing protein C receptor. This study characterized the MP shed by APC-treated EC or β-cells and compared their effects on β target cells submitted to oxidative stress.

Rat β cells (Rinm5f) and porcine coronary artery EC were treated with 2–70 nM APC (Xigris®) for 24 h. Washed MP isolated from EC supernatant were applied for 6 h to Rinm5f, before addition of 100 μM H202. After 24 h, apoptosis was assessed by hypodiploïde DNA staining, secreted insulin by ELISA; expression of glycosylated PAR, endothelial NO synthase (eNOS) and annexin1 (A-1) by Western blot. APC activity was measured using a chromogenic substrate, MP concentration by prothrombinase assay.

APC enhanced APC activity at both cell surfaces (rinm5f: 2.4, EC: 1.4-fold $p < 0.001$) whilst apoptosis remained low (4 $\pm$ 0.9%, 3.2 $\pm$ 0.5%). APC activated PAR1 in both cells, and up-regulated the expression of A1 and eNOS, mainly in EC. APC (>20 μM) enhanced MP release from EC and Rinm5F (by 32% and 28%) with a 6-fold rise in MP-borne APC activity ($p < 0.001$). MP from APC-treated β-cells had no cytoprotective effect. Conversely, MP from EC (>10 nM eq Phosphatidylserine) reduced the apoptosis of H_2O_2 treated β-cells (5 $\pm$ 1% vs. 21 $\pm$ 1%, $p < 0.001$), and restored insulin secretion (10 ng/ml vs. 0.8 ng/ml, n = 3) whereas APC alone remained inactive (up-to 70 nM).

MP from APC-treated EC protect β cells against oxidative stress. They may prove a promising therapeutic tool in the protection of transplanted pancreatic islets.

6th Session: Temperature and Preservation

Keynote lectures:

1) -Should organs be preserved in normo, subnormo or hypothermia?
Gabriel Oniscu (Edinburgh, UK)

2) -Thermosensors and cold-mediated pathways : perspectives for organ preservation
Gabriel Bidaux (Lyon, France)

O17 EX-VIVO NORMOTHERMIC PERFUSION WITH THE NOBLE GAS ARGON IN AN EXPERIMENTAL MODEL OF KIDNEY TRANSPLANTATION

S. Hosgood, M. Hamed, T. Adams, M. Nicholson
Department of Surgery, University of Cambridge, Cambridge, UK

Introduction: Noble gases can exert biological actions that may help to reduce transplant related ischaemic injury. The aim of this study was assess the effects of argon administered directly to the kidney during ex-vivo normothermic perfusion (EVNP).
Methods: Under Home Office Animals (Scientific Procedures) Act 1986 porcine kidneys were retrieved after 10 min of warm ischaemia. After 17 h of static cold storage, kidneys underwent 1 h of EVNP with a leukocyte depleted blood based solution with either argon (n = 6) [70% argon/25% O2/5% CO2], oxygen (n = 6) [95% O2/5% CO2] or nitrogen (n = 6) [70% nitrogen/ O2/5% CO2]. After EVNP kidneys were reperfused ex-vivo for 3 h with oxygenated whole blood to assess renal function and injury.
Results:
EVNP: The argon treated kidneys produced significantly more urine during EVNP compared to the oxygen treated kidneys (argon 278 ± 88 vs. oxygen 180 ± 42 ml vs. nitrogen 199 ± 88 ml; $p = 0.049$). Levels of HIF-1α detected in the urine were significantly higher in the nitrogen kidneys (0.33 ± 0.37 ng/ml) compared to the control (0.04 ± 0.09 ng/ml) and argon (0.0 ng/ml).
Reperfusion: During reperfusion levels of oxygen consumption at 1 h were significantly higher in the argon kidneys (argon 31.4 ± 8.8 vs. oxygen 16.3 ± 11.0 vs. nitrogen 32.0 ± 14.8 ml/min/g; $p = 0.027$). Creatinine clearance (CrCl) was also significantly higher [(Area under the curve (AUC) CrCL, argon 4.5 ± 3.5 vs. oxygen 1.8 ± 1.0 vs. nitrogen 3.4 ± 1.9 ml/min/100 g; $p = 0.030$). There was no significant difference in levels of HIF-1α ($p = 0.623$), IL-6 ($p = 0.657$) or TNFα ($p = 0.328$) after reperfusion. Tissue levels of protein carbonyl were similar in the 3 groups after reperfusion ($p = 0.228$).
Conclusion: EVNP can be used to deliver therapies directly to the kidney. Kidneys treated with argon had improved renal function and oxygen consumption during reperfusion compared to kidneys treated with oxygen. Nonetheless, argon did not reduce the level of inflammation or oxidative damage.

O18 THE FIRST REPORTED USE OF EX-VIVO NORMOTHERMIC PERFUSION FOR THE ASSESSMENT AND TRANSPLANTATION OF KIDNEYS DEEMED UNTRANSPLANTABLE DUE TO INADEQUATE IN-SITU PERFUSION.

S. Hosgood, M. Hamed, K. Saeb-Parsy, M. Nicholson
Department of Surgery, University of Cambridge, Cambridge, UK

Introduction: Herein, we report the first case of a pair of human kidneys that were declined for transplantation due to inadequate *in-situ* perfusion but subsequently transplanted after perfusion and assessment using *ex-vivo* normothermic perfusion (EVNP).
Methods: The pair of kidneys were from a 35 year male, donation after circulatory death (DCD) donor. The warm ischaemic time was 13 min. The recipient details and ischaemic times are detailed in Table 1. Both kidneys were declined by all UK transplant centres. On arrival, both kidneys had significant areas of incomplete clearance of blood from the microcirculation. This didn't clear after a further attempt to flush the kidneys. Kidneys underwent 60 min of EVNP with an oxygenated packed red blood cell based solution warmed to 35.2°C.
Results: During EVNP the patchy areas cleared immediately in the right kidney and within 30 min in the left. The mean renal blood flow (RBF) and total urine output (U/O) was higher in the right kidney compared to the left (RBF 68.0 vs. 59.9 ml/min/100 g), (U/O 560 vs. 430 ml). The EVNP assessment score was 1 for the right kidney and 2 for the left.

Based on the EVNP perfusion parameters both kidneys were deemed suitable for transplantation.

The right kidney was transplanted first and therefore had a shorter total ischaemic time (Table 1).

Both kidneys were transplanted without any complications and had initial graft function. The serum creatinine levels at 1 month were 100 μmoL/L in the recipient of the right kidney and 182 μmol/L in the left.
Conclusion: EVNP technology can be used to assess and rescue kidneys previously deemed unsuitable for transplantation.

Table 1

Recipient	1 (Right kidney)	2 (Left kidney)
Age (y)	30	68
Gender	Male	Female
Cold ischaemic time (h)1st	17.24	19.44
Cold ischaemic time (min)2nd	132	247
Total ischaemic time (h)	21.09	26.24

7th session: Ischemia Reperfusion Injuries we never talk about

Keynote lecture:
Intestinal Ischemia Reperfusion Injury
Kaatje Lenaerts (Maastricht University, NL)

O19 EVALUATION OF PANETH CELL ALTERATIONS AFTER INTESTINAL TRANSPLANTATION AND DURING GRAFT REJECTION

M. Kip[3], I. Hundscheid[3], L. Ceulemans[1], H. Hartog[4], R. Brown[4], E. Canovai[1], O. Corcos[2], F. Joly[2], G. Hertogh[1], G. Gupte[4], C. Dejong[3], S. Olde Damink[3], D. Mirza[4], J. Pirenne[1], K. Lenaerts[3]
[1]University Hospitals Leuven, Leuven, Belgium; [2]Beaujon Hospitals, Paris, France; [3]Department of General Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands; [4]Birmingham Children's Hospital and University Hospitals Birmingham, Birmingham, UK

Intestinal transplantation (ITx) has become an accepted treatment for patients with irreversible intestinal failure, but remains a challenging procedure. Acute rejection is the most common complication. Ischemia reperfusion injury (IRI) plays a pivotal role in the cascade leading to rejection. We have shown that Paneth cells, important gatekeepers of intestinal crypts, are highly susceptible to IRI in humans. Therefore, our aim was to study Paneth cell homeostasis in IRI and rejection in ITx patients.

Archived endoscopic mucosal biopsies of 33 ITx patients were used, and clinical information was collected. Consecutive biopsies were double-stained for Paneth cell marker lysozyme and apoptotic marker M30 to visualize antimicrobial expression and Paneth cell loss at reperfusion (T0), and during the first week (W1), month (M1), and year (Y1) after ITx, as well as prior to, during, and after a rejection episode. The number of lysozyme-positive and lysozyme-positive/M30-positive cells per crypt, and lysozyme intensity were quantified by two independent observers.

Within W1 after ITx, there was a significant decrease in lysozyme intensity ($p < 0.05$), and a tendency towards lower Paneth cell number per crypt ($p = 0.09$) compared to T0. Within Y1, the Paneth cell number was comparable to T0, and increased compared with W1 and M1 ($p < 0.01$). Prior to rejection, lysozyme intensity was significantly reduced compared with T0 ($p < 0.01$). Higher lysozyme expression was observed after rejection compared with levels prior to ($p < 0.05$), and during rejection ($p = 0.08$). Paneth cell numbers were increased after a rejection episode compared with levels prior to, and during rejection ($p < 0.05$).

In conclusion, this study shows reduced Paneth cell numbers and antimicrobial expression during the first week after ITx, as well as prior to, and during a rejection episode. These data suggest an interplay between innate immunity, reduced antimicrobial defense mechanisms, and alloimmune response.

O20 UTERUS TOLERANCE TO LONG-TIME COLD ISCHEMIC STORAGE AFTER AUTO-TRANSPLANTATION IN EWE

J. Tricard[1,2,3], S. Ponsonnard[1], Y. Tholance[1], L. Mesturoux[1], D. Lachatre[1], C. Couquet[4], C. Yardin[2,1], F. Terro[2], P. Marquet[3,1], A. Piccardo[1], T. Gauthier[1,2,3]
[1]CHU Dupuytren; [2]Equipe de Recherche Médicale Appliquée (ERMA) ; [3]INSERM UMR-850; [4]Laboratoire Départemental d'Analyses et de Recherches, Limoges, France

Objective: To study how does the uterus tolerate long-time cold ischemic storage before auto-transplantation in ewe.
Methods: Fourteen uterus auto-transplantations (UAT) were performed in ewe: 7 after 3 h of cold ischemia time (CIT), 7 after 24 h of CIT. The uterus body and one uterine horn with the ipsilateral arterio-venous pedicle were retrieved and then individually flushed "in situ" and stored with Celsior® at 4°. They were branched on the recipient's external iliac vessels. The transplant was assessed 8 days after the procedure and arterial Doppler performed. Histology and apoptosis analysis (TUNEL and cleaved caspase-3) were performed before uterus retrieval, after 90 min of reperfusion (T+90) and ≥8 days

after transplantation (≥D8). Pelvic magnetic resonance imaging (MRI) was performed in one ewe of each group after surgery.
Results: Twelve UAT were successfully performed. Seven ewes were alive at ≥D8. The histological analysis at ≥D8 revealed two viable uteruses in the 3-h CIT group and three in the 24-h CIT group, with no significant apoptotic signal in any case. One uterus was necrotic in both groups. The histological analysis revealed at T+90 a moderate inflammation of the endometrium and serosa in the 3-h CIT group and a severe inflammation in the 24-h CIT group, but no significant apoptotic signal in either group. The MRI results correlated with the macroscopic observations of the 'second look' laparotomy.
Conclusion: A cold ischemia of 24 h doesn't cause significant histological modification and apoptosis in the uterus of ewe. Uterus transplantation with a brain dead donor could be performed even if uterus retrieval (multiorgan procurement site) is remote from the transplantation site.

O21 PRETREATMENT OF HEART ALLOGRAFTS WITH THE CYTOTOPIC ANTI-THROMBIN INHIBITOR THROMBALEXIN PROLONGS TRANSPLANT SURVIVAL

Q. Peng, A. Nowocin, L. Smyth, R.A. Smith, A. Dorling, R. Lechler, G. Lombardi

MRC Centre for Transplantation, King's College London, London, UK

Cardiac transplantation is the treatment of choice for end-stage heart failure. Its efficacy is limited by the occurrence of ischemia-reperfusion (IR) injury as well as the development of cardiac allograft vasculopathy. IR triggers the activation of innate components of the immune system including complement and coagulation, which are major contributors to early graft injury, local inflammation and graft dysfunction. Limiting the effects of IR and preserving heart function by inhibiting the aforementioned is therefore of primary importance.

In this study, we tested the efficacy of a novel strategy whereby murine heart allografts were perfused with a cytotopic anti-thrombin inhibitor (Thrombalexin, which contains a Hirulog-like sequence and a membrane-interacting component) prior to transplantation. Thrombin is a major molecule involved in the coagulation pathway and also interacts with the complement system. Perfusion of the BALB/c heart grafts with Thrombalexin for 15 min before transplantation significantly reduces graft injury and prolongs graft survival in C57BL/6 recipient mice (n = 8, MST = 15 days) as compared to untreated hearts (n = 8, MST = 11.5 days). This protection was associated with a significantly reduced macrophage and T cell infiltration. To shed some light on the effects observed *in vivo*, murine heart endothelial cells (EC) were incubated *in vitro* with Thrombalexin. The pre-treatment of EC with Thrombalexin resulted in down-regulation of TNFα-induced expression of adhesion molecules such as ICAM-1.

In conclusion, coagulation mediated allograft heart IR injury and graft rejection may be prevented/delayed by pretreatment of the grafts prior to transplantation with the anti-thrombin drug Thrombalexin. The results from our study suggest that localized anti-thrombin inhibition is effective in reducing early local inflammation and provides a window of opportunity to combine Thrombalexin-treatment with additional therapies to induce transplantation tolerance.

O22 TOTAL LIQUID VENTILATION MITIGATES MULTI-ORGAN FAILURE AND PRESERVES KIDNEY FUNCTION THROUGH ULTRA-FAST COOLING AFTER AORTIC CROSS CLAMPING

N. Mongardon[3,2], M. Kohlhauer[3], F. Lidouren[3], B. Costes[3], C. Barau[3], S. Giraud[5], T. Hauet[5], P. Micheau[1], A. Cariou[4], G. Dhonneur[2], A. Berdeaux[3], B. Ghaleb[3], R. Tissier[3]

[1]Université de Sherbrooke, Sherbrooke, Canada; [2]Service d'Anesthésie et des Réanimation Chirurgicales, Hôpital Henri Mondor, Créteil; [3]INSERM U955, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est Créteil, France, Maisons-Alfort; [4]Service de Réanimation Médicale, Hôpital Cochin, Paris; [5]INSERM U1082, Poitiers, France

Introduction: Total liquid ventilation (TLV) provides ultrafast cooling and potent neuro- and cardioprotection after experimental cardiac arrest.
Hypothesis: We determined whether it could also mitigate multi-organ failure and sepsis-like response after abdominal ischemia/reperfusion.
Methods: Anesthetized rabbits were submitted to 30 min of supracoeliac aortic cross-clamping and 300 min of reperfusion. They underwent a normothermic procedure (Control group) or rapid cooling toward 32–33°C with TLV started either before, during or after aortic clamping (PRE, PER and POST groups, respectively). TLV was maintained during 75 min after which rabbits were rapidly rewarmed and switched to conventional mechanical ventilation.
Results: Control animals elicited a dramatic shock state with a low cardiac output, high demand in norepinephrine, liver failure and acute kidney injury. Cardiac output was gradually improved in POST, PER and PRE groups as compared to Control. A significant protection was also observed in the TLV groups regarding liver enzymes (ASAT, ALAT, L-FABP), acute kidney injury markers (nNAG, KIM-1, NGAL, β2-microglobulin) and renal function parameters (creatinine clearance and fractional Na+ excretion). The protection was maximal in the PRE group and achieved a lower extent in PER and POST groups when compared to Control. As example, urinary clearance of creatinine achieved 4.8 ± 1.2, 1.3 ± 0.9, 0.9 ± 1.4 in PRE, PER and POST groups vs. 0.5 ± 0.4 ml/kg/min in Control (all $p < 0.05$), respectively. Importantly, multi-organ failure was associated with elevated blood transcripts of inflammatory markers (e.g., IL-1β, IL-8, IL-10). This was attenuated in all groups submitted to TLV with no difference between PRE, PER and POST groups.
Conclusions: Ultra-fast cooling with TLV attenuates multi-organ failure in a model of abdominal ischemia/reperfusion. The cardiac, liver and renal protections but not anti-inflammatory effects depend upon the window of application.

O23 ANTIOXIDATIVE NANOPARTICLE FOR ORGAN TRANSPLANTATION

Y. Nagasaki[2], A. Takashima[2], B. Shashi[2], R. Thuillier[1], T. Hauet[1]

[1]Insert U927, Université Poitiers; [2]Materials and Medical Sciences, WPI-MANA, University of Tsukuba, Tsukuba, Japan

During organ transplantation, it is important to control its oxidative damages before, during and after the organ preservation. After an organ transplantation, an oxygen concentration significantly increases, which also causes the oxidative damage to the organ. We have developed antioxidative nanoparticle (RNP), which strongly scavenge reactive oxygen species (ROS). Since the nanoparticles with several tens nanometer do not internalize healthy cells, they are not disturb normal redox reactions in normal cells such as electron transport chain. We have so far applied our RNP for renal, cerebral, myocardial and intestinal ischemia reperfusion injuries and confirmed to suppress oxidative damage, without severe adverse effects. We try to use our RNP for cold preservation of organs. Now we obtained several data on cold preservation of cells in the presence of RNP. We would like to present these data on the meeting and will discuss in detail.

8th Session: Organ Ex vivo repair and Machine perfusion

Keynote lecture:
Underlying mechanisms and protective effects of HOPE in liver machine perfusion preservation
Philipp Dutkowski (Zurich)

O24 THE TRANSPORTABLE OXYGENATED MACHINE PERFUSION AIRDRIVE®, AN INNOVATIVE APPROACH TO SAFELY EXPAND THE DONOR POOL FOR LIVER TRANSPLANTATION

P. Compagnon[3], H. Hentati[3], E. Lesveque[4], M. Disabato[3], J. Calderaro[2], J. Cohen[1], I. Ben Mosbah[1], D. Azoulay[3]

[1]Institut Mondor Recherche Biomédicale (IMRB), INSERM U955; [2]Service d'Anatomopathologie; [3]Service de Chirurgie Hépato-Biliaire et Transplantation Hépatique; [4]Service de Réanimation Digestive, Hôpital Henri Mondor, Assistance Publique–Hôpitaux de Paris, Université Paris-Est, Créteil, France

The Airdrive® is the first transportable oxygenated machine perfusion (MP) unit suitable for liver preservation allowing immediate liver oxygenated perfusion upon graft harvesting. We hypothesized that the Airdrive® MP would improve the quality of livers derived from donation after circulatory death (DCD), using a large animal model. Female large white pigs were used. Cardiac arrest was induced by IV injection of KCL. After 60 min of WI, livers were flushed in situ with HTK and subsequently preserved either by SCS (WI-SCS group) or hypothermic MP (WI-MP group) using MPS-Belzer solution. Liver allografts procured from heart beating donors and preserved by simple cold storage (SCS) served as controls. After 4 h of preservation, livers were transplanted. The main judgment criterion was animal survival at day 5. PNF and death occurred within 6 h in all animals of WI-SCS group. In contrast, 5-day survival was 100% in WI-MP group and controls. A post-reperfusion syndrome was observed in all animals of the WI-SCS group but none of the control or WI-MP groups. At the end of cold preservation, ATP content was higher in WI-MP group vs. WI-SCS group. After reperfusion, MP livers functioned better (INR, total bilirubin) and showed less hepatocellular and endothelial cell injury, in agreement with better preserved liver integrity (histology) relative to WI-SCS group. MP livers also exhibited improved ATP recovery after transplantation compared with SCS livers. The protective effect of the Airdrive® was associated with an attenuation of inflammatory response (TNF-a) and tissue oxidative stress (lipid peroxidation) and a better endoplasmic reticulum adaptation (caspase12, CHOP, GRP78), leading to reduced mitochondrial damage (cytochrome C, caspase9, GLDH), and apoptosis (caspase3 and TUNEL). This study demonstrates for the first time the efficacy of the transportable MP Airdrive® device to enhance donor liver viability for transplantation in a clinically relevant DCD model.

O25 TRIMETAZIDINE IS PROTECTIVE DURING KIDNEY PRESERVATION WITH MACHINE PERFUSION

R. Codas[6,1,2], A. Kasil[1,2], J. Cau[6], A. Thierry[6,4,5,7,8], T. Hauet[6,8,3,7,5], L. Badet[6,1,2]

[1]Faculté de Médecine, Université de Lyon 1; [2]Groupement Hospitalier Edouard Herriot, Service d'Urologie, Lyon; [3]CHU de Poitiers, Service de Biochimie; [4]CHU de Poitiers, Service de Nephrologie; [5]Fédération Hospitalo-Universitaire SUPPORT (Tours Poitiers Limoges); [6]INSERM U1082; [7]Plateforme MOPICT, INRA/IBiSA, Domaine du Magneraud; [8]Université de Poitiers, Faculté de Medecine et Pharamcie, Poitiers, France

Introduction: The use of machine perfusion for kideny preservation is recommended for the conservation of organs obtained from donors after cardiac arrest (warm ischemia) or from marginal donors. Preservation with machine perfusion allows organs evaluation and potential additon of drugs modifying the protocols with a predictive and therapeutic approach

Materials and Methods: Two experimental groups of 6 animals were studied using Large White pigs. The left kidney was removed after 1 h of warm ischemia, washed by KPS-1® solution (group KPS) KPS-1® or with trimetazidine (KPS + T group) and then placed in perfusion machine. Kidneys were then transplanted in the same animal and contralateral kidney removed. Perfusion milieu was sampled for tissue infury markers. The recovery of glomerular and tubular function was studied every day for one week. A kidney biopsy was peformed at D7 to assess tissue injury. Markers of lesions (KIM-1, NGAL, AST and H-FABP), oxidative stress and innate immunity was evaluated. Three months after auto transplantation, animals were sacrificed and a histomorphological analysis and immunoblotting were performed

Results: The contribution of trimetizidine allows a significant improvement in recovery of function and limiting the oxidative stress in association with a reduction in tubular necrosis. This is also related to a significant limitation of tissue damage markers release during machine preservation. The expression of markers of innate immunity is also significantly limited. At 3 months, the glomerular filtration level is improved in the KPS + T group with a reduction of interstitial fibrosis and tubular atrophy

Conclusion: Kidney preservation with machine perfusion is potentially interesting for organ evaluation and potential addition of protective drug which could change protocole for patients

O26 TECHNIQUE FOR EX VIVO LUNG PERFUSION (EVLP) OF LUNGS FROM BRAIN-DEAD DONOR RATS AND THE EFFECT OF PREDNISOLONE

J. Van Zanden[1], H. Leuvenink[3], E. Verschuuren[2], D. De Jong[3], S. Veldhuis[3], P. Ottens[3], M. Erasmus[1], M. Hottenrott[1]

[1]Department of Cardiothoracic Surgery; [2]Department of Pulmonary Diseases; [3]Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands

Ex vivo lung perfusion (EVLP) systems have become an important tool to treat marginal brain dead donor lungs with edema. Prednisolone is added routinely, and enables prolonged perfusion of preinjured lungs in experimental setting. This is probably the result of reduced edema and modulation of immune response. Here, the newly established ex vivo lung perfusion model and effect of prednisolone in the EVLP were investigated.

Heart-lung blocks were procured from Lewis rats 3 h after acute brain death induction and were cold preserved for 1 h. Thereafter, lungs were placed for 6 h in the normothermic EVLP model. Lungs were ventilated with a tidal volume of 7 ml/kg of body weight, a PEEP of 5 cmH_2O, a frequency of 60 and a FiO_2 of 21%. Perfusion was performed with a modified Steen solution, cefuroxime with and without 40 mg prednisolone at a maximal pulmonary arterial pressure of 12 mmHg. Ventilation parameters, lung oxygenation capacity, glucose levels, lactate and flow were recorded and perfusate samples collected, over time. Lungs were macroscopically scored and analyzed for wet/dry ratio, qPCR and patho-histological changes.

Ventilation parameters, lung oxygenation capacity and flow indicate that we have established a stable EVLP system. Macroscopic scoring, ventilation parameters and lung oxygenation capacity suggest a beneficial effect of prednisolone in the EVLP. While the usage of prednisolone is advisable clinically, it might interfere with other treatment opportunities in experimental studies.