TRANSPLANT INTERNATIONAL

ORAL PRESENTATIONS

1ST SESSION: NEW ASPECTS OF CELL METABOLISM DURING IRI

O1-0002 AN ANGIOGENIC RESPONSE ACTIVATED BY THE UNFOLDED PROTEIN RESPONSE DURING RENAL ISCHEMIA INDEPENDENTLY OF HIF-1ALPHA

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Introduction: Acute and chronic ischemic injuries permanently stress kidney tissue that challenge cell viability, promote inflammation and fibrogenesis. Adaptive responses to ischemia are mostly mediated by the activation of the transcriptional factor HIF-1α in response to hypoxia. Ischemia also promotes nutrients deprivation that triggers Endoplasmic Reticulum (ER) Stress followed by the Unfolded Protein Response (UPR). The aim of this study was to test whether the UPR would promote an angiogenic response independently of HIF-1α pathway during ischemic stress in the human epithelium.

Materials and methods: qPCR, immunoblots and ELISA were performed on human kidney tubular cell lines (HK2) exposed to glucose deprivation and/or hypoxia to evaluate the expression of the UPR markers BiP, CHOP, ATF4, EDEM and spliced XBP1 and the expression of angiogenic inducers Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), Angiogenin (ANG) and PDGF-BB. ARN interference directed against the three transducers of the UPR, *ATF6, PERK* and *IRE1* was performed to test which UPR axis is involved in mediating angiogenic markers. To characterize acute kidney ischemia, nephrectomized rat kidneys were preserved in conservation solution, IGL1, to mimic cold ischemia.

Results: Glucose deprivation induced the secretion of VEGF, bFGF and ANG, but not PDGF-BB. Glucose deprivation, unlike hypoxia, did not induce HIF-1α expression, whereas glucose deprivation, and not hypoxia, triggered ER stress and activated the expression of the UPR genes BiP, CHOP, ATF4, EDEM and the spliced form of XBP1 mRNA. Using RNA interference against the three transducers of the UPR, we demonstrate that VEGF and bFGF secretion depends on the *Perk* pathway whereas ANG expression depends on *Ire1*α pathway. ATF6 is not involved. *In vivo*, UPR and angiogenic markers are simultaneously increased in rat kidneys at 16 hours of cold ischemia.

Discussion: This work demonstrates that nutrient deprivation promotes an angiogenic response characterized by the secretion of VEGF-A, bFGF and ANG independently of the HIF-1α pathway in human kidney tubular cells. *Perk* and *Ire1*α pathways are involved but regulate different angiogenic markers during kidney ischemia.

O2-0014 UPREGULATION OF HIF-1 ALPHA ATTENUATES HYPOXIA/REOXYGENATION INJURY THROUGH HIF-1 ALPHA -INOS-PGC-1ALPHA PATHWAY

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Background: Studies have shown that activation of HIF-1 alpha attenuated ischemia/reperfusion (I/R) injury, which was also confirmed by our previous study. But the functional relationships between HIF-1 alpha protective mechanism and mitochondrial metabolism have not been fully clarified. So we investigated the effect of HIF-1 alpha upregulation on I/R injury and the mitochondrial metabolism.

Methods: HK-2 cells were exposed to 1% O_2 for 16 hours before reoxygenation for 3 hours. Five groups were studied: PHD2 siRNA (50nm) group (I), nontargeting siRNA control group (NTSC) (II), PHD2 siRNA+L-NIL [the selective iNOS inhibitor L-N6-(1-iminoethyl) lysine hydrochloride, 1 mM] group (III), I/R group (IV), normal group (V). The number of viable cells, the activity of ATP, LDH production, mitochondrial integrity and cytochrome-c oxidase (COX) activity were measured. Protein and mRNA levels of HIF-1 alpha, iNOS, PPAR-γ cofactor-1alpha, mtDNA-encoded genes COX I, II and III were also analyzed separately by real-time PCR and western blot.

Results: The number of viable cells, contents of LDH were markedly enhanced in group I compared with group IV ($P < 0.05$), while no statistically significant difference of ATP levels in these two groups. It suggested that equal ATP levels cells could withstand hypoxia better if the source of ATP production is glycolytic rather than mitochondrial in group I, which was consistent with other previous study. Moreover, PHD2 siRNA increased HIF-1 alpha expression, and also mRNA and protein expressions of its downstream gene iNOS, and PPAR-γ cofactor-1alpha (PGC-1 alpha, a mitochondrial biogenesis which was a key regulator of mitochondrial ATP production). Furthermore, the changes of mitochondrial membrane potential (Fig. 1) and COX activity induced by I/R injury were minimized in group I. These protective effects were abolished by L-NIL.

Conclusions: We found that upregulation of HIF-1 alpha by PHD2 siRNA significantly attenuated I/R injury by rescuing mitochondrial dysfunction. Through increasing iNOS and PGC-1 alpha expressions, upregulation of HIF-1 alpha reduced the mitochondrial membrane potential changes and restored the activity of COX. And the protection was abrogated in the presence of the iNOS inhibitor L-NIL. These findings suggested that the intervention through HIF-1 alpha-iNOS-PGC-1alpha pathway may have the therapeutic potential for the better management of I/R injury.

O3-0076 PKC ALPHA/BETA INHIBITION ATTENUATES HYPOXIA INDUCED INTERSTITIAL RENAL FIBROSIS AND INFLAMMATION VIA REDUCED ACTIVATION OF TGF-BETA SIGNALING

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Background: Ischemia reperfusion injury (IRI) leads to progressive renal fibrosis and loss of renal function. In this study we tested the efficacy of a PKC alpha/beta inhibitor (PKC-I) to attenuate post-ischemic renal fibrosis and inflammation.

Methods: IRI was induced in mice by transient unilateral clamping of the left renal pedicle for 45 min. Treatment with the PKC alpha/beta inhibitor (0.6 mg/day) was initiated either prior to or 24 hours after ischemia and continued over 28 days. Renal morphology, glomerular filtration rate (GFR), renal blood flow (RBF), expression of alpha-SMA, collagen 4, and fibronectin were examined. Furthermore, expression of CTGF and PAI-1 down-stream targets of TGF-beta as well as inflammatory cell infiltration (macrophages, CD4+ T-cells) was investigated.

Results: In untreated mice GFR and RBF were significantly reduced 28 days after unilateral ischemia. In contrast, treatment with the PKC inhibitor improved renal function markedly. IRI caused severe renal fibrosis and up-regulation of pro-fibrotic proteins (a-SMA, fibronectin, collagen4). PKC-I pre-treatment partially blocked up-regulation of pro-fibrotic proteins. Furthermore, up-regulation of CTGF and PAI-1 expression was significantly ameliorated by PKC-I pre-treatment ($P < 0.005$) and inflammatory cell infiltration was markedly attenuated.

Conclusion: PKC alpha/beta inhibition reduces experimental renal fibrosis via inhibition of TGF-beta signaling and attenuates renal inflammation.

O4-0050 CYCLOSPORIN A AND METFORMIN INHIBIT ISCHEMIA-REPERFUSION INJURY ON INS-1 CELLS THROUGH PERMEABILITY TRANSITION PORE INHIBITION

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Objective: The loss of islet graft during post-transplant period is one of major limitations to islet transplantation. Ischemia-reperfusion (I/R) injury is a factor of importance in the β cell loss during post-transplant period. The permeability transition pore (PTP) is a mitochondrial channel involved in cell death. Here, we studied the impact of simulated I/R on cell viability and the involvement of oxidative stress and PTP opening during I/R in INS-1 cells.

Research design and methods: Cell death was analyzed by flow cytometry. The open/closed PTP state and the superoxide production were studied by confocal microscopy.

Results: The incubation of INS-1 cells in the absence of energy substrates in hypoxic condition for 1 hour followed by an incubation in normal condition led to PTP opening and to a dramatic increase in cell death. Both event were totally prevented when the cells were incubated in the presence of the antioxidant *N*-acetyl cystein (NAC), in anoxia, or when PTP opening was inhibited by either Cyclosporin A (CsA) or Metformin. Superoxide production increased during the removal of energy substrates and again increased when normal energy substrate and O_2 were restored. NAC, anoxia or Metformin prevented the two phases of oxidative stress, while CsA prevented only the second one. Hypoxia alone did not induce oxidative stress, PTP opening or cell death.

Conclusions: A 1 hour removal of energy substrates in INS-1 cells led to an oxidative stress followed by PTP opening, this latter being responsible for a secondary and robust oxidative stress.

O5-0015 TRANSITION FROM REVERSIBLE TO IRREVERSIBLE INJURY FOLLOWING PROLONGED MYOCARDIAL HYPOTHERMIC PRESERVATION. IMPLICATION OF MITOCHONDRIAL PERMEABILITY TRANSITION PORE (MPTP) OPENING

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Introduction: The aim of this study was to evaluate the ischemic functional recovery related with mitochondrial dysfunction during different time of hypothermic heart preservation.

Methods: Rat hearts ($n = 65$) were perfused according to Langendorff technique, arrested and preserved with Saint-Thomas' Hospital solution during 4, 8, 12, 16 or 24 hours at 4 °C. Cold ischemia was followed by a 60 min reperfusion for functional assessment. Necrosis was evaluated by TTC staining method and by measuring CK and LDH leakage in the coronary effluents. Hearts were also reperfused 10 min and mitochondria were isolated to assess Ca^{2+} resistance capacity of the MPTP.

Results: Data showed that a transition from reversible to irreversible ischemia occurred between 8 and 12 hours cold preservation. Hearts preserved 4 and 8 hours showed better functional recovery with RPP averaging at the end of the reperfusion 17664 ± 1941and 14278.4 ± 2176. On the contrary, hearts preserved 12, 16 and 24 hours were irreversibly damaged (with RPP averaging respectively 2683 ± 895, 2711.5 ± 378 and 1776 ± 98 mmHg/min, $P < 0.001$ vs. 4 and 8 hours preservation). Enzymes releases (CK and LDH) and TTC staining showed an absence of necrosis prior to 8 hours preservation. Identically, mean calcium load inducing MPTP opening after 8 hours of preservation was significantly less in comparison with hearts preserved 12 hours or more ($P < 0.001$).

Conclusion: This study is the first to show a transition from reversible to irreversible cold ischemia between 8 and 12 hours. Irreversible cold ischemia would involve higher sensitivity of MPTP.

O6-0033 HYPERCHOLESTEROLEMIA PROMOTES INTRARENAL REMODELING IN A PORCINE AUTO-TRANSPLANTED KIDNEY MODEL

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Introduction: Hypercholesterolemia is more and more present in kidney transplantation. This study aimed to characterize the role of hypercholesterolemia on renal graft outcome, particularly oxidized low-density lipoprotein (oxLDL) and its pivotal lectin-like oxLDL receptor-1 (LOX-1).

Materials and methods: In this study, pigs underwent renal auto-transplantation 2 months after starting either a normal (ND) or hyperlipidemic (2% cholesterol, HD) diet maintained during the follow-up. Kidney graft function and cholesterol levels were monitored for 3 months after transplantation. Renal remodeling, inflammation, LOX-1 and TGFβ signaling pathways were evaluated 3 months after surgery.

Results: HD-induced an increase in plasma oxLDL levels at the time of surgery were associated with high proteinuria 3 months later. Increased oxLDL levels at 3 months promoted concomitant activations of renal LOX-1 and TGFβ signaling pathways, results supported by in *vitro* studies using primary renal artery endothelial cell cultures incubated with oxLDL in hypoxia-reoxygenation conditions. Furthermore, hypercholesterolemia induced monocyte infiltration in graft and NFKb expression promoting the interstitial fibrosis-tubular atrophy.

Conclusion: These results reinforce the need to control cholesterol levels in both donor and recipient kidney transplants to better manage graft outcome.

2ND SESSION: IMMUNOLOGICAL CONSEQUENCES OF IRI

O7-0023 INVOLVEMENT OF IGM NATURAL ANTIBODIES IN RENAL ISCHAEMIA REPERFUSION INJURY: A POTENTIAL NEW THERAPEUTIC TARGET?

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Introduction: We have previously demonstrated that i.v. administration of apoptotic cells (AC) prior to renal ischaemia reperfusion injury (IRI) results in functional protection via an unknown mechanism. IgM natural antibodies (nAb) bind to injured tissue and conserved bacterial antigens. They are recognised to act as an innate arm of the adaptive immune system, with deposition of nAb shown to be important in initiating mesenteric, skeletal and cardiac IRI. The role of nAb in renal IRI is not known, This work examined the primary hypothesis that nAb deposition could be present and important in renal IRI. Secondly, if nAb were bound by AC this could contribute to their protective effects in IRI.

Methods: IRI was induced in Balb/c and Balb/c-SCID by 20 min clamping of the left renal pedicle with right nephrectomy ($n = 8-10$/group). Blood and kidneys were collected at 1 and 24 hours post IRI. Murine thymocytes were rendered apoptotic *ex vivo* (> 98% apoptotic) and $2x10^7$ ACs injected i.v prior to experimental IRI. Biochemical and histological injury was assessed. Frozen sections were examined by immunofluorescence (IF) for IgM and C3 deposition. In vitro studies examined binding of IgM by ACs using flow cytometry with anti-IgM-APC Ab.

Results: IgM deficient SCID mice had preserved renal function compared to control Balb/c mice (serum Creatinine (sCr) 94 ± 16 vs. 140 ± 14μmol/L; $P < 0.05$) after IRI. SCID kidneys showed structural protection, with less necrosis of outer medullary tubules (30 ± 1% vs. 56 ± 4% necrotic tubules; $P < 0.01$). $2x10^7$ ACs injected 24 hours prior to IRI resulted in a 51% reduction in sCr ($P < 0.05$) in Balb/c mice but had no protective effects in SCID mice, even when IRI was increased to 25 min to augment injury. IF studies demonstrated that IgM is not present within the normal mouse medulla but is deposited in the first hour after IRI. This binding was absent in SCID kidneys. IgM was not detectable 24 hours after IRI indicating that it had either been cleared or the bound cells had died and been phagocytosed. In vitro studies demonstrated that serum from Balb/c mice contains nAb which bind to ACs, but SCID serum does not.

Conclusion: These data demonstrate that nAb deposition is a feature of the immediate reperfusion phase of renal IRI. SCID mice lack nAb capable of recognising injured/dying cells *in vitro* and *in vivo*, whilst displaying a protected phenotype. Further studies are ongoing to characterise the effects of nAb repletion/depletion as a therapeutic strategy in IRI given the potential translational implications for both transplantation and acute kidney injury.

O8-0046 IL-17 PRODUCING UNCONVENTIONAL TCELLS CONTRIBUTE TO HEPATIC ISCHEMIA REPERFUSION INJURY

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Hepatic ischemia reperfusion injury (IRI) is a complication of organ transplantation, which leads to liver dysfunction and failure and may result in graft loss. CD3+ T cells are major effector cells in the pathogenesis of hepatic IRI. Nevertheless, the precise role that distinct T cell subsets such as IFN -γ- and IL-17-producing T cells (Th1 and T17, respectively) play in IRI remains unknown. The purpose of this study was to determine the basic mechanisms underlying T17-mediated IRI in transplantation, particularly downstream of the key T17 effector cytokine IL-17, and with respect to regulation by the hallmark T17 transcription factor ROR-gamma-t (RORγt). We tested whether RORγt+ T17 cells were truly involved in hepatic IRI by applying partial warm IRI to Rorγt-*reporter* and wild-type (wt) control animals. We found that both RORγt-*reporter* and wt mice show comparable levels of fulminant hepatocellular damage 3 and 24 hours after reperfusion, as indicated by similar levels of ALT and histological injury. Importantly, further immunological analysis by FACS and ELISA revealed that a fraction of hepatic CD3+ T cells in the RORγt-*reporter* (and wt) mice undergoing IRI express high levels of both IL-17 and GFP(RORγt), suggesting a crucial pathophysiological role for RORγt+ T17 cells in IRI. Next, we tested the hypothesis that RORγt critically determines IRI severity through the induction of hepatic T17 cells by studying Rorγt-*ko* mice under IRI conditions. We found that the frequency of hepatic T17 cells (by FACS) was dramatically lower in Rorγt-*ko* mice after 24 hours of reperfusion, compared to *reporter* and wt controls. Intriguingly, this lack of RORγt+ T17 cells in Rorγt-*ko* mice significantly reduced IR-induced hepatocellular injury in comparison to Rorγt-*reporter* and wt control mice (ALT, histology). This study shows that T17 cells may play an important pathophysiologic role in IRI and are dependent on RORγt. In liver transplantation, our studies point to a major and as yet understudied role of RORγt+ T17 cells in early IRI.

O9-0011 IL-33 EXACERBATES LIVER ISCHEMIA-REPERFUSION INJURY

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Background: Liver ischemia−reperfusion (I/R) injury is a multifactorial process that affects graft function after liver transplantation. In?ammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and IL-18, have been shown to play key roles in the pathophysiology of liver I/R injury. Interleukin-33 (IL-33), the most recently identified member of the IL-1 family, can enhances Lipopolysaccharide-Induced In?ammatory cytokine production from mouse macrophages by regulating Lipopolysaccharide Receptor Complex.

Objective: The aim of this study was to examine the role of IL-33 on liver I/R injury in mice.

Method: A partial lobar liver warm ischemia model was performed. IL-33 and ST2 gene expression were analyzed in sham mice and liver I/R injury mice, using real-time polymerase chain reaction. IL-33 protein was detected in sham and I/R injury liver sections using western blotting and immunohistochemistry staining approaches.

Results: Our results show that both mRNA and protein of IL-33 were overproduced in I/R injury mice but not sham mice. The major sources of IL-33 in I/R injury mice were the vascular endothelial cells and injury hepatocytes. Those mice pretreated with anti-IL-33 ployantibody 1 hr before ischemia showed decreased serum alanine aminotransferase levels; inhibited production of proinflammatory cytokines such as IL-1β, IL-18, TNF-a, and IL-6 as well as decreased of inflammatory cell infiltration by downregulation of TLR4 on kupffer cell, leading to the prevention of liver I/R injury, when compared with controls. Histology revealed that pretreatment with anti-IL-33 ployantibody significantly ameliorated hepatocellular damage. At the same time, those mice pretreated with rIL-33 protein revealed more serious liver injury than control mice.

In conclusion: our study confirms that IL-33 signaling is involved in liver I/R and that inhibition of IL-33 can protect the liver from I/R injury by reducing IL-1β, IL-18, TNF-a, IL-6 release and inflammatory cell infiltration through downregulation of TLR4 expression on kupffer cell.

Keywords: Liver ischemia and reperfusion injury; IL-33; TLR4

O10-0060 FEASIBILITY OF CONTINUOUS *EX VIVO* KIDNEY PERFUSION WITH MESENCHYMAL STEM CELLS TO PREVENT ISCHEMIA-REPERFUSION INJURY IN MICE

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Continuous machine perfusion of solid organs after explantation has been discussed for decades, while its clinical superiority in kidney transplantation was shown just recently. This form of organ preservation not only represents a more physiological storage modality ex vivo, but also offers new possibilities to modify the graft before transplantation. One way of modifying the graft during continuous perfusion might be the application of mesenchymal stem cells (MSC): These cells have several abilities that could be highly beneficial to the graft, as MSC have both regenerative and immunomodulatory properties. In an isolated organ approach, the application of MSC could be more effective with decreased systemic side effects. In a first set of experiments, we tested the general feasibility of murine MSC in a continuous perfusion system under different perfusion conditions. It was shown that viable MSC can be harvested over at least 2 hours of continuous pumping under hypothermic conditions using commercially available Custodiol® solution, albeit the number of living MSC decreases over time. After 2 hours, MSC moved over the roller pump survived at a rate of 38% (as compared to 104% when kept under culture conditions). Next, we perfused murine kidneys with CFSE-labeled MSC under hypothermic conditions at 90 mm HG perfusion pressure. After 30 min of perfusion, MSC can be found in or near glomerular structures. This set of experiments shows that MSC can be applied during *ex vivo* kidney perfusion in a technically successful fashion. This opens a whole new era in cellular therapy, allowing investigators to replenish grafts with cells favoring a changed microenvironment, thereby possibly leading to decreased ischemia reperfusion injury and less immunogenicity.

O11-0008 CULTURED HUMAN MSCS SECRETOME CONTAINS ANTI INFLAMMATORY CYTOKINES AND REDUCES IN VITRO ISCHEMIA REPERFUSION LESIONS AND T LYMPHOCYTE PROLIFERATION AFTER ALLOGENEIC STIMULATION

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Mesenchymal stem cells (MSCs) may reduce ischemia reperfusion injury by paracrine activation of cardioprotective pathways. MSCs may also modulate allogeneic rejection by secretion of cytokines. We hypothesised that human MSCs secretome may contain an anti inflammatory cocktail of cytokines that could reduce both ischemia reperfusion injuries and allogeneic rejection at the time of organ transplantation.

Methods and results: Serum free conditioned media (secretome) was harvested after 24, 48 and 72 hours from cultured human MSCs. The best results were obtained with secretome harvested after 48 hours of culture. Addition of human MSCs secretome at the time of reperfusion in an in vitro model of ischemia reperfusion of neonatal rat's cardiomyocytes significantly reduced (-23% ± 5%) cell death assessed by MTT staining. Addition of human MSCs secretome on mixed lymphocytes reaction significantly reduced (-22% ± 3%) T lymphocytes proliferation assessed by the amount of radioactivity measured in a scintillation counter after ^{3}H thymidine incorporation. Multiplex analysis of human MSCs secretome (IL-1, IL-6, IL-10, IL-12, IL-17, TNF-a, VEGF) showed an anti inflammatory profile of cytokine concentration.

Conclusion: Our preliminary in vitro data suggest that human MSCs secretome features an anti inflammatory cytokines profile that may favor the reduction of ischemia reperfusion injuries and modulate allogeneic rejection.

O12-0059 INVOLVEMENT OF HMGB1 ALARMIN AND IL33/ST2 PATHWAY AFTER ISCHEMIA REPERFUSION IN HUMAN RENAL TRANSPLANTATION

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The nature of the signals activating the innate immune response following a sequence of ischemia-reperfusion (IR) remains unknown. Sharing many properties with alarmin, IL33 is a cytokine produced during necrosis or apoptosis processes. Its transmembrane receptor ST2, also present in serum in soluble form (sST2), is expressed on the cell surface of immune cells (NK / iNKT cells, Dendritic cells). Activation by IL33 results in the production of inflammatory cytokines and Th2 polarization. However the role of the IL33/ST2 pathway in the IR injury is not established. The aim of this work was to demonstrate the activation of this pathway after kidney transplantation in humans.

This is a single-center prospective pilot study including 27 renal transplant patients (mean age 51.4 years), conducted between December 2009 to October 2010. The average duration of cold ischemia is 14.22 ± 4.0 hours. Leucocyte activation was evaluated by transcriptomic analyses and IL33, sST2 and HMGB1 serum level were measured by ELISA after blood collection at different points: pre-transplant, 30 min and 3 hours after declamping, Day 1, Day3, Day7 and Day14. The Wilcoxon test for paired data was used for comparing the values 2-2, the Spearman test was used for correlations ($P < 0.05$ was considered significant).

ST2 mRNA as well as sST2 protein levels increased significantly from 3 hours after declamping compared to pre-transplant values ($P < 0.01$), marked by a protein peak at Day1 (values significantly higher than 3 hours and Day3, $P < 0.05$). IL33 mRNA and protein levels had a tendency to be elevated after transplantation (not significantly). HMGB1 protein and its receptors mRNA levels (Toll Like Receptors) increased significantly from 30 min after declamping, to Day1 for TLR mRNA and to Day3 for the HMGB1 protein ($P < 0.05$). Finally, IL33 circulation levels at all times post transplantation, were correlated with the duration of cold ischemia ($P < 0.05$).

These data suggest the involvement of the IL33/ST2 pathway in human kidney transplantation. The significant correlation between the duration of cold ischemia and IL-33 circulating levels may promote this alarmin as a biomarker of the intensity of IR injury. These data provide new therapeutic target to neutralize the early activation of innate immunity.

3RD SESSION: NEW TRENDS FOR ADDITIVES TO PRESERVATION SOLUTIONS

O13-0032 KIDNEY PRESERVATION WITH CARDIOTROPHIN-1 PREVENTS ISCHEMIA/REPERFUSION INJURY AND INFLAMMATORY RESPONSE IN A SYNGENEIC RAT KIDNEY TRANSPLANT MODEL

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Injury caused by ischemia and reperfusion plays a major role in long-term survival of renal allografts. Cardiotrophin-1 (CT-1) is a member of the interleukin-6 (IL-6) family of cytokines and it has been shown to have a protective effect on myocardial and liver damage induced by ischemia/reperfusion and other insults.

The purpose of the present study has been to assess the effect of CT-1, added to the fluid for perfusion and preservation of donor kidney, on renal function after transplantation in the Fischer-Fischer rat model of syngenic kidney transplantation (KTx). Orthotopic transplantation of the left donor kidney was performed after 24 hours of cold storage in University of Wisconsin fluid with or without CT-1 (0.02 mg/l). The right kidney was removed at the time of KTx. Recipient animals were followed up for 30 days. At several times, renal function was measured (creatinine clearance in metabolic cages), and kidneys were obtained, frozen and some markers of injury and inflammation such as superoxide anion production, TNF-α release, iNOS expression, serum levels of soluble ICAM-1 and VCAM-1 and GP130, and NFκB activation (nuclear p65 translocation) were evaluated.

The addition of CT-1 to the preservation fluid improved early (5 – 7 days) and long-term (14 – 30 days) survival and reduced acute renal injury (significantly lower serum creatinine and higher creatinine clearance in the CT-1-treated group) at days 1, 3, 7 and 14 after grafting. CT-1 addition to the preservation fluid also significantly reduced superoxide anion production, TNF-α release, iNOS expression, serum levels of soluble ICAM-1 and VCAM-1 and NFκB activation. In conclusion, preservation of kidney to be grafted with CT-1 improves short- and long-term outcomes, renal function and inflammatory response after syngenic KTx.

O14-0074 SUPERIOR LUNG PRESERVATION WITH A POLYETHYLENE GLYCOL BASED SOLUTION IN A PORCINE SINGLE LUNG TRANSPLANT MODEL

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Objectives: Scot15® is a low-K+ preservation solution including polyethylene glycol (PEG) as a colloid for protection of vascular endothelium during cold ischemia. PEG was previously demonstrated to have ''immunocamouflage'' properties. The aim of this study was to assess whether these properties would be beneficial in a pig lung transplant model in comparison to Perfadex® as golden standard solution.

Methods: Domestic pig donor lungs were flushed either with Scot15®[S; $n = 6$] or Perfadex®[P; $n = 6$] and stored on ice for 22 hours. The left lung was transplanted in a recipient animal observed during 6 hours after reperfusion with intermittent clamping or the right hilum during measurements. Pulmonary vascular resistance (PVR) and partial arterial oxygen tension (PaO2) were measured hourly. Peripheral lung biopsies were taken for HRMAS detection of the respective colloid in the lungs and metabolic evolution along reperfusion. Bronchoalveolar lavage (BAL) was taken to assess neutrophilic alveolar recruitment. At the end of reperfusion, wet to dry weight ratio (W/D) was measured as a marker of lung edema.

Results: At the end of cold ischemia, HRMAS metabolic state was better preserved in [P] versus [S] but there was no difference in cellular gluthatione levels. HRMAS showed presence of PEG in the lungs in [S] before and during the reperfusion. Dextran was not detected in [P]. After 6 hours of reperfusion, PaO2 was significantly better in [S] (310 ± 51 mmHg vs. 198 ± 71 mmHg in [P];$P = 0.03$)(Fig.). PVR remained lower in [S] but the difference did not reach significance. Differential cell count of BAL showed lower neutrophilic cell count in [S] (89 ± 67 neutrophilic cells in [S] versus 139 ± 53 neutrophilic cells in [P];$P = 0.001$). There was no difference in W/D weight ratio ($P = 0.9$).

Conclusion: After 22 hours of cold ischemia, oxygenation capacity was superior with less inflammatory reaction in lung grafts preserved with a low-potassium PEG solution compared to the current standard solution. Further experiments to study the ''immunocamouflage'' properties of PEG based solutions in lungs are warranted.

O15-0013 INTRALUMINAL POLYETHYLENE GLYCOL STABILIZES THE TIGHT JUNCTIONS AND IMPROVES INTESTINAL PRESERVATION IN THE RAT

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The rapidly progressing mucosal breakdown limits the intestinal preservation time below 10 hours. Recent studies indicate that certain solutions containing polyethylene glycol (PEG) introduced intraluminally alleviate preservation injury of intestines stored in UW-Viaspan. We investigated whether a low-sodium PEG solution is beneficial for the rat intestines stored in histidine-tryptophane-ketoglutarate (HTK) preservation solution (high sodium/low potassium). Rat intestines were perfused with ice-cold HTK and a customized PEG-3350 solution was introduced intraluminally (L group) before cold storage (omitted in controls). After 8, 14 and 20 hours we analysed tissue injury, edema, brush-border maltase activity and zonula occludens-1 (ZO-1) and claudin-3 expression in the tight junctions (TJ). We measured epithelial resistance and permeability (Ussing chamber) after 8 and 14 hours. The L group had superior morphology while maltase activity was similar with the controls. TJ proteins rapidly decreased and de-colocalized in controls; the alterations were slower in the L group, where colocalization persisted about 14 hours. L intestines had higher epithelial resistance and lower permeability that the controls. These results show that a customized PEG solution intraluminally reduces the intestinal preservation injury by improving several major epithelial characteristics without negatively affecting the brush-border enzymes or promoting edema.

O16-0031 IMPACT OF PRESERVATION SOLUTION ON GRAFTS AND POSTOPERATIVE ORGAN FAILURES FOLLOWING LIVER TRANSPLANTATION

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Introduction: During Liver Transplantation Ischemia/reperfusion injury (IRI) is a major concern. After unclamping of portal vein, cold and acidotic mediators, nitric oxide and cytokines are released in systemic circulation and can lead to a systemic inflammatory response which could result in multiorgan failure. The preservation solution is one parameter influencing IRI.

The aim of this study was to assess the impact of 2 preservation solutions on IRI assessed by cytokine measurement, and on post liver, heart, lung and kidney functions. We compared a fourth generation solution (extracellular): Solution de Conservation des Organes et des Tissus (SCOT) vs a second generation solution (intracellular): University of Wisconsin solution (UW).

Methods: This was a longitudinal prospective study.
Cytokines were measured before transplantation in systemic blood; in the ''wash-out'' fluid; 30 min after unclamping the portal vein: in the sus-hepatic vein; and after surgery in systemic blood. Clinical and biological data were collected pre-, per- and post-operatively. Heart and lung ultrasounds were daily performed until day 10.

Results: Forty six patients were included: 28 in the UW Group and 16 in the SCOT Group. Patients' characteristics were different. Disease stage, assess by the MELD and CHILD score, was significantly worse in SCOT vs UW group: MELD score was 18 ± 7.2 vs. 13 ± 6.8 ($P = 0.009$) and percent of Stage A/B/C of CHILD Score was 33/17/50% vs. 18/61/21% ($P = 0.013$). The graft characteristics and preoperative data were not statistically different.

In UW group, IL6 et IL8 concentrations were significantly higher in the wash-out fluid and in sus-hepatic vein 30 min after unclamping portal vein.

In the postoperative period, liver function was not different between groups but we found clinical differences regarding cardiovascular, lung and kidney functions. Post reperfusion syndrome was more frequent using UW: 70.4% vs. 38.9%, $P = 0.036$. Length of mechanical ventilation was longer in UW group: 65 (33–123) h vs. 33.5 (23–43), $P = 0.006$. Lung injury and incidence of pneumonia tended to be greater in UW group. Renal failure was more frequent in UW group: 57.7% vs. 27.8%, $P = 0.05$. ICU and hospital length of stay and mortality tended to be shorter in SCOT group.

Discussion and Conclusion: The main result of our observational study is that using SCOT was associated with less systemic release of pro-inflammatory cytokines, reduction of post-operative respiratory complications and better preservation of renal function. These beneficial results might be explained by a better liver preservation with less IRI.

O17-0037 POTENTIAL BENEFITS OF THE SUPPLEMENTATION OF MACHINE PERFUSION SOLUTION WITH *ARENICOLA MARINA* HEMOGLOBIN FOR THE PRESERVATION OF PORCINE KIDNEY GRAFTS SUBJECTED TO WARM ISCHEMIA

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Introduction: Despite the use of machine perfusion (MP), delayed graft function rates remain elevated for kidneys obtained from donors deceased after cardiac death (DCD) suggesting that MP preservation can still be improved. Benefits of the supplementation of static cold storage solutions with the extracellular *Arenicola Marina* hemoglogin (M-101) have been reported in a large animal model of optimal donors. Implementation of this strategy to MP solutions may improve early and late function of DCD kidney grafts. The current preliminary study was aimed at evaluating this hypothesis *ex vivo* in a DCD model.

Methods: For this *ex vivo* study, the renal pedicle of Large White male pigs was clamped for 1 hour *in situ,* removed and preserved for 24 hours in the Lifeport® machine (ORS) containing KPS-1® alone or supplemented with M-101 at either 0.5, 1 or 2 g/l. We quantified in the perfusion fluid, the partial pressure in dissolved oxygen, lactate levels, the release of the injury marker: Aspartate Aminotransferase (ASAT). At the end of preservation, we evaluated the cortical expression of proteins involved in oxidative stress (P67) and its modulation (SOD1).

Results: MP preservation with KPS-1 alone was associated with a time-dependent decrease in dissolved oxygen levels (from 189.0 ± 15.4 to 31.3 ± 1.9 mm Hg) and a concomitant increase in extracellular lactate and ASAT levels. Also, P67 and SOD1 protein expressions were elevated in the cortex. In the M-101 groups, only the 2 g/l dose significantly reduced dissolved oxygen levels (15.1 ± 2.3 mm Hg, $P < 0.05$) and had a tendency to reduce lactate release after 24 hours of preservation. Only the 0.5 g/l dose led to a significant 3.6-fold reduction in ASAT release during preservation ($P < 0.05$). M-101 supplementation at all doses normalized P67 and SOD1 protein expressions.

Conclusions: This pilot study reported that M-101 supplementation at 0.5 g/l significantly protected kidney cells during MP preservation and supports the performance of a complete study in a large animal model. The absence of effect on lactate levels of M-101 supplementation in a non-oxygenated machine suggests that in this paradigm M-101 does not improve oxidative metabolism. The use of MP with active oxygenation may exploit the full capacity of the M-101 oxygen carrier and further improve preservation efficiency.

O18-0064 THE NOVEL MITOCHONDRIA-TARGETED ANTIOXIDANT MITOQ AMELIORATES RENAL ISCHAEMIA REPERFUSION INJURY IN A MURINE MODEL

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Introduction: Renal ischaemia reperfusion injury (IRI) represents a major cause of acute renal failure and renal allograft dysfunction. Considerable evidence exists to support a key role for mitochondrial damage and mitochondrial-derived oxidative stress in the development of renal IRI. We have developed a novel mitochondria-targeted antioxidant MitoQ, with confirmed human safety and efficacy, which concentrates within the mitochondria, preventing mitochondrial oxidative damage. The aim of this study was to investigate the efficacy of MitoQ in protecting against mitochondrial and tissue damage during experimental renal IRI.

Methods: In a murine model of bilateral renal ischemia-reperfusion injury, animals were randomized to 4 groups; sham laparotomy control or 45 min renal ischemia, each with or without MitoQ (20 mg/kg) i.v. 15 min prior to laparotomy. All groups underwent a 24 hours reperfusion period. At sacrifice, renal tissue was taken for analysis of mitochondrial function (respirometry, ATP/ADP) and damage (mtDNA integrity), markers of oxidative stress (protein carbonyl content) and histology. Serum creatinine levels were measured as a marker of renal function.

Results: Renal IRI was associated with a significant rise in serum creatinine and markers of oxidative protein and mtDNA damage compared to controls at 24 hours post-reperfusion. Administration of MitoQ prior to ischemia resulted in a marked reduction in kidney injury as measured by serum creatinine levels compared to the untreated group (88 ± 14 umol/l vs. 23 1 ± 18 umol/l; $P < 0.05$). This was associated with a significant reduction in protein and mtDNA damage.

Conclusion: MitoQ represents a promising novel therapeutic approach to ameliorating renal IRI, with potential application in a variety of clinical settings including transplantation.

4TH SESSION: MACHINE PERFUSION: WHY AND HOW?

O19-0041 ONE-YEAR TRANSPLANTATION RESULTS OF KIDNEYS PRESERVED BY MACHINE PERFUSION – LIFEPORT KIDNEY TRANSPORTER VERSUS WATERS RM3 - PROSPECTIVE STUDY

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Recent studies have shown beneficial effects of Machine Perfusion (MP) on early and long-term graft survival. Histopathological analysis undertaken at our center revealed significantly lower incidence of chronic rejection and interstitial fibrosis in kidneys preserved by MP. A recent pre-clinical study in France demonstrated significantly higher levels of fibrosis at 3 months post-op in kidneys preserved on the RM3 vs. the LifePort. We used two pulsatile perfusion devices: the flow controlled Waters (WM) and the pressure controlled LifePort (LP).The aim of the present study was to assess results of kidneys preserved prior to transplantation on LP versus WM.

Patients and methods: Between August 2009 and January 2011, 50 kidneys retrieved from 25 donors were transported to our center on static storage prior to being placed on MP. One kidney was randomized to the WM ($n = 25$) and the contralateral to the LP ($n = 25$). From the study group 48%($n = 12$ pairs) were from Expanded Criteria Donors (ECD) All kidneys had biopsy taken after MP and then in case of deterioration of graft function. Primary endpoints were: kidney function after transplantation (Delayed Graft Function(DGF), number of Haemodialysis(HD) sessions post-transplantation), kidney function up to 12 months post transplantation (creatinine level, return to HD treatment, 24 hours-proteinuria).

Results: Patient populations didn't differ between the groups regarding age, sex, HLA mismatch, immunosupression regiments, HD treatment prior to transplantation or cold ischemia times. Lactate dehydrogenase (LDH) activity at 4th hours of perfusion was significantly higher in WM group −315 ± 126 vs.212 ± 77 than in LP group ($P = 0.004$). DGF was the same in both groups at 32% (8/25,P = NS). Patients with DGF in WM group underwent mean 4,66 HD sessions versus 2,65 HD sessions in LP group ($P = 0.005$). One-year graft survival was 80% (20/25)vs.96%(24/25) in WM and LP groups, respectively. One-year graft survival of kidneys retrieved from ECD was 66% (8/12)vs.91,6%(11/12) in WM and LP groups, respectively. Chronic 24-proteinuria was observed in 25%(5/20)vs.12.5%(3/24) in WM and LP groups, respectively. Chronic 24-proteinuria of kidneys retrieved from ECD was observed in 37%(3/8)vs.9%(1/11) in WM and LP groups, respectively. Biopsies of kidneys taken after perfusion did not differ between groups regarding ATN and occurrence of chronic vascular changes. During the observation period 20 patients underwent graft biopsy due to medical reasons−9 in LP group; 11 in WM group. Interstitial Fibrosis: Tubular Atrophy (IF/TA) was significantly more often present in WM group vs. LP group − 45% (5/11) vs. 0% (0/9) ($P = 0.03$) respectively. There were no differences in creatinine levels between the groups. Graft survival of kidneys who had CIT-1 > 4.5 hours was 80% (6/30) vs. 100% (0/20) if CIT-1 < 4.5 hours ($P < 0.05$).

Conclusion: MP with the LifePort system is superior to the Waters Machine in preserving kidney function especially in kidneys retrieved from ECD. Initiating perfusion at retrieval site might improve results even further.

O20-0044 COATING OF THE VESSEL WALL WITH MACROMOLECULAR HEPARIN DURING COLD MACHINE PERFUSION

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Background: Endothelial glycocalyx consists of membrane-bound proteoglycans and glycoproteins that cover the endothelial wall. Together they function as a mechanical filter against macromolecules and act as a protective barrier against the coagulation system. During ischemia/reperfusion the glycocalyx is rapidly shed into the blood stream. Macromolecular heparin (CHC; Corline®, Uppsala, Sweden) consists of 70 heparin molecules covalently linked to a carrier which implies capacity to adhere to biological tissues. We hypothesized that CHC could be used to restore disrupted glycocalyx *in vivo* in kidneys from brain dead pigs. Since the binding of CHC occurs even at low temperature continuous perfusion of kidneys with the compound during cold storage was explored.

Materials and methods: Brain death was induced in landrace pigs($n = 7$) by inflating a balloon catheter in the epidural space while continuously measuring the cerebral perfusion pressure. After a stabilization period of 60 min both kidneys were harvested and placed on two separate Lifeport®kidney transporters (Organ Recovery Systems, Chicago, IL, USA) with continuous cold perfusion. Fifty mg CHC (including 25 mg biotinylated CHC) was added to the perfusion fluid (1 l) in one machine and 50 mg heparin (control) was added to the perfusion fluid to the other machine. The binding of CHC to the kidney was detected by immunofluorescence and confocal microscopy, and indirectly by an *in vitro* assay with toluidine blue dye.

Results: CHC was detected by immunofluorescence not only on the endothelium but also throughout the intima and media of the vessel walls. The binding of CHC in the kidney was confirmed indirectly by consumption of CHC from the perfusion fluid. Heparin did not bind to the vessels in the control kidneys. No consumption of CHC was seen when perfusing the Lifeport®kidney transporter without the presence of a kidney, indicating that CHC does not adhere to the Lifeport® perfusion system. Seperate *in vitro* tests with blood vessels from pigs confirmed that CHC binds to the vessels at both 4 °C and room temperature.

Conclusion: We show the possibility of adding an exogenous substance, in this case CHC, to the perfusion fluid in the Lifeport®kidney transporter without the loss of substance in the perfusion system. The vessel walls of perfused kidneys are coated with CHC, an approach that might become useful in restoring endothelial glycocalyx and thereby minimizing ischemia/reperfusion injuries of kidneys from brain dead donors.

O21-0030 PNEUMATIC CONTROL AND MONITORING FOR KIDNEY PERFUSION IN A NMR COMPATIBLE SYSTEM

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Introduction: Improving viability of kidneys originating from Donation after Cardiac Death (DCD) using *ex-vivo* hypothermic oxygenated perfusion has become absolutely necessary for organs having suffered from warm ischemic lesions. To date, vascular resistance is the only functional parameter for predicting the kidney's function. We have developed a system using Nuclear Magnetic Resonance (NMR) imagery and spectroscopy that would allow evaluation of such kidneys and could establish their viability. To be closely similar to the morphologic parameter of perfusion, the system injects a bolus of solution (KPS-1) until a systolic pressure is reached and then, lets the system reduce to a diastolic pressure. It is thereby the kidney itself which pilots its own perfusion avoiding the risk of injury after high pressure perfusion.

Methods: The system is pneumatically controlled and is able to perfuse simultaneously two kidneys under specific conditions related to NMR. That induces the kidney-box to separate from the electronic command, which must stay out of the high magnetic field. Both are connected together with a 7 m long connexion called ''umbilical cord''. Consequently, the pneumatic signal is picked up close to the renal artery and then treated by the electronic command seven meters away. The average signal of pressure, between the command module and the organ, depends on the total closed volume which interacts with the other elements in our construction. Pressure Signals were measured with ''keller'' pressure sensor Dv-22-PP and its interface software at both places (organ and electronic module).

Results: Pulsatile flow (between 50 and 15 mmHg pressure) is supplied thanks to a pneumatic pump fuelled with oxygen. A partial pressure of oxygen (about 100 [kPa]) is maintained with a hollow fibre oxygenator which maintains the cellular metabolism as ATP production. As the pressure signal is taken from the organ and treated in the electronic command 7 m away, the loss of signal between them corresponds to the following relation:

Conclusion: The loss of signal is processed and integrated into the interface in order to fit into the real perfusion parameter. That makes this system of perfusion safe and reliable regarding the real rate of pressure at the organ's location. We have incorporated the possibility of a NMR diagnosis under controlled perfusion parameters and thereby enlarging the criteria of acceptability for marginal organs as well as improving their postoperative functions.

O22-0040 THE FINAL SHOWDOWN: PRECLINICAL COMPARISON BETWEEN PERFUSION MACHINES ORS LIFEPORT AND WATERS RM3

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Background: Machine preservation has demonstrated clear benefits for organs in terms of reducing DGF rate and improving one year outcome. However, there is an ongoing debate about which perfusion technology provides the highest level of protection. We used a highly reproducible porcine kidney autotransplantation model to compare them.

Methods: Kidneys were subjected to 60 min warm ischemia prior to machine preservation on either the Waters RM3 (RM3 group) or the ORS Lifeport (ORS group). Perfusion parameters were identical to those used in the clinic. Pigs were followed for 2 weeks, endpoints were kidney function and urinary enzymes excretion.

Results: Kidney function recovery was identical between the 2 machines in terms of diuresis, creatininemia, calciuria and free water clearance. There was a slight superiority of the RM3 group compared to the ORS regarding sodium excretion, osmolarity ratio and glycosuria. However, the RM3 group displayed

a higher level of urinary alanine aminoperoxydase at days 3 and 5 compared to the ORS group.

Conclusions: Our porcine autotransplantation model allows for a clear evaluation of the benefits of machine perfusion against ischemia reperfusion injury. The high reproducibility of the model permits us to state that all other parameters being equal, there were minimal differences between the two groups in terms of early recovery of function. However, these parameters are unlikely to keep their discriminating power in patients. Thus, both machines appear to perform at a similar level of quality in the first two weeks. Chronic evaluation is ongoing to evaluate the benefits of each technology on chronic inflammation and fibrosis.

5TH SESSION: MANAGEMENT OF BRAIN DEAD DONORS

O23-0029 SECRETORY LEUKOCYTE PROTEASE INHIBITOR DECREASES RENAL ISCHEMIA REPERFUSION INJURY

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Introduction: Ischemia reperfusion injury (IRI) remains one of the major problems in solid organ transplantation. The aim of this study was to evaluate the effect of recombinant human secretory leukocyte protease inhibitor (SLPI) in a model of rat IRI.

Materials and methods: Bilateral ischemic procedures were performed in male Sprague-Dawley rats of 200–300 g. Renal artery and vein were clamped for 40 min. After 24 h of reperfusion, the animals were sacrificed. There were four experimental groups: i) Sham (no IRI); ii) Control (IRI + control buffer); iii) SLPI (IRI + SLPI); iv) dSLPI (IRI + SLPI without anti-serine protease activity). Animals were treated (250 μ g / kg ip) with control buffer, SLPI or dSLPI, 24 h pre-ischemia, during the ischemia and 6 h post-ischemia.

Results: Animals treated with SLPI or dSLPI showed lower levels of serum creatinine (control: 2.6 ± 1.0; SLPI: 1.2 ± 0.8; dSLPI 0.45 ± 0.41 mg/dl, $P < 0.05$ for SLPI and dSLPI) and blood urea nitrogen (control: 143 ± 9; SLPI: 63 ± 27; dSLPI: 46 ± 23 mg/dl, $P < 0.05$ for SLPI and dSLPI) compared to control animals. Acute tubular necrosis was less severe in animals treated with SLPI or dSLPI (Control: 63 ± 6; SLPI: 20 ± 3; dSLPI: 38 ± 7, $P < 0.01$ for SLPI; $P < 0.05$ for dSLPI). Furthermore, animals treated with SLPI or dSLPI showed lower levels of myeloperoxidase than control rats (Control: 370 ± 34; SLPI: 230 ± 23; dSLPI: 260 ± 22 EU/mg prot., $P < 0.001$ for SLPI and dSLPI). Finally, qRT-PCR showed that the increased expression of genes for TNF-α, ED-1, MCP-1, CD86, CD14 and IL-10 produce by IRI was reduced by treatment with SLPI or dSLPI ($P < 0.05$).

Conclusion: overall this study demonstrates that treatment with SLPI or dSLPI reduces the IRI and improves the kidney function. Moreover, this effect is not dependent on the inhibitory serine protease activity of SLPI.

O24-0066 DONOR PRETREATMENT WITH METHYLPREDNISOLONE REDUCES BRAIN DEATH-INDUCED RENAL INJURY BEFORE ORGAN RETRIEVAL

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Introduction: Renal allografts retrieved after donor Brain Death (BD) show inferior transplant outcomes compared to living donor grafts. BD is associated with activation of the immune system and it is well known that steroids have anti-inflammatory capacities. Therefore, this study investigated whether Methylprednisolon (MP) pre-treatment reduces inflammation and renal injury in brain-dead organ donors.

Methods: BD was induced in rats by inflating a subdurally placed balloon catheter. Animals were treated with saline or MP (22.5 mg/kg) 1 h before BD. After 4 h of BD, serum and kidneys were collected. Sham-operated rats treated with saline or MP served as controls. Tissue gene expression was measured by Real Time qPCR. Tissue protein expression was detected by immunohistochemical analyses.

Results: Pretreatment of brain-dead donors with MP significantly reduced expression rates of pro-inflammatory genes and influx of polymorphonuclear cells in the kidney. Importantly, MP treatment almost fully recovered renal function in brain-dead donors to the level of a sham-operated animal. In addition, BD increased the IL-6 level in plasma which was significantly recovered to sham level after MP pre-treatment

Conclusions: This study shows that pre-treatment of brain-dead donors with MP significantly reduces renal inflammation and improves renal function before organ retrieval.

O25-0069 ANGIOPOIETIN IN LIVING AND DECEASED BRAIN DEAD KIDNEY DONORS

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Introduction: Organs derived from deceased brain dead (DBD) donors show worse organ function and more acute rejection episodes than organs derived from living donors. Human studies have provided evidence that DBD donors suffer from bacterial translocation and higher endotoxin load. A link between endotoxemia and Angiopoietin 1 (Ang1) and Angiopoietin 2 (Ang2) is established. Use of Ang1 and Ang2 as biomarkers of endothelial integrity has gained much attention. Ang1 and Ang2 are antagonictic ligands that bind to Tie2 receptors. Angiopoietins reflect the immunogenic state of the organ and could be used as predictors of organ quality and survival.

Methods: We measured serum Ang1 and Ang2 by ELISA in 100 DBD and 220 living donors (LD). Serum was obtained immediately after the determination of brain death (T0) and just before organ retrieval (T1). Serum from living kidney donors retrieved after start of the operation (T0) and just prior to organ retrieval (T1) was used as control.

Results: In LD and DBD, Ang1 levels increased 4-fold between T0 and T1. Serum Ang2 levels in DBD donors are higher at T0 and T1 (T0: 1925 ± 198.9 pg/ml and T1: 2418 ± 305.2 pg/ml) compared to living donors (T0: 690.7 ± 47.60 pg/ml and T1: 1384 ± 102.9 pg/ml). In LD, Ang2 levels increased at T1 compared to T0 ($P < 0.05$). The Ang1/Ang2 ratio in LD is increased at T1 compared to T0 ($P < 0.05$). This ratio is decreased at T1 in DBD donors compared to T1 in LD ($P < 0.05$). In the LD, T1 Ang2 levels are associated with glomerular filtration rate (GFR) 12 months after transplantation (Spearman's ρ - 0.193 $P = 0.019$). In DBD donors, T0 Ang2 levels are associated with serum creatinin 14 days after transplantation (Spearman's ρ - 0.333 $P = 0.017$). The Ang1 levels at T1 are correlated with acute rejection of the kidney graft (HR 1,000 $P < 0.05$).

Conclusion: These preliminary results show increasing Ang1 levels from T0 to T1 in living and DBD donors indicating a vascular response in the donor during organ retrieval. At both time points, Ang2 levels in DBD are elevated compared to LD illustrative of the inflammatory response. In both donor types, the Ang1/Ang2 ratio increases from T0 to T1. In LD, T1 Ang2 levels are associated with worse GFR 12 months after transplantation. In DBD donors, T0 Ang2 levels are associated with higher serum creatinin 14 days after transplantation. Acute rejection is predicted by Ang1 levels at T1.

O26-0061 EFFECT OF PRECONDITIONING WITH TRIIODOTHYRONINE ON RENAL ISCHEMIA-REPERFUSION INJURY

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Introduction: Ischemia-reperfusion (I/R) model in rats allows pharmacological investigation of the protective renal effect of certain agents and thereby diminish the incidence of delayed graft function (DGF). The aim of this study was to determine the effects of preconditioning with triiodothyronine (T_3) on renal function and oxidative status in renal I/R injury.

Methods: 60 male Wistar rats were preconditioned with triiodothyronine (T_3) (100 μg/kg) or Placebo (normal saline) 24 h prior to 45 min of renal ischemia, followed by a 4 h (groups P-4 h and T_3-4 h), 24 h (groups P-24 h and T_3-24 h) or 48 h (groups P-48 h and T_3-48 h) reperfusion period.

Results: We determined renal function parameters (urea, creatinine and proteinuria), oxidative stress biomarkers in plasma (MDA and GSH), urine (H_2O_2 and isoprostanes), and renal tissue (GSH and MDA). Proteinuria was significantly lower in the T_3-treated groups (4 h: 4.12 ± 0.59 vs. 5.12 ± 0.62, 24 h: 1.81 ± 0.44 vs. 3.19 ± 0.43 and 48 h: 1.21 ± 0.12 vs. 2.78 ± 0.26 mg/ml/100 g body wt). Pretreated rats had lower levels of plasma and tissue MDA and urine isoprostanes (4 h: 1422 ± 71.77 vs. 1956 ± 83.87, 24 h: 2025 ± 73.89 vs. 2543 ± 63.01 and 48 h: 1155 ± 54.39 vs. 1858 ± 167.28 pg/ml). The T_3 treatment was associated with a lower post-ischemia GSH concentration at 4 h (3.82 ± 1.16 vs. 4.89 ± 0.68) but that increase significantly at 48 h (5.41 ± 0.26 vs. 4.40 ± 0.40 nmol/mg protein). Preconditioning with the hormone also reduced significantly urine H_2O_2 at 48 h ($P < 0.05$).

Conclusion: These findings suggest that preconditioning with T_3 reduces proteinuria, improves lipid peroxidation biomarkers, and increases antioxidant enzyme levels in renal I/R injury.

O27-0017 DIAZOXIDE ATTENUATES ISCHEMIA/REPERFUSION INJURY VIA UPREGULATION OF HEME OXYGENASE-1 AFTER LIVER TRANSPLANTATION IN RATS

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Objective: Recent studies have shown that the selective opening of mitochondrial ATP-sensitive potassium channels with diazoxide (DZ) plays a key role in cardioprotection and neuroprotection against ischemia/reperfusion (I/R) injury. In the present study, we evaluated the effects of diazoxide on I/R-injured hepatocytes and further elucidated its underlying mechanisms.

Methods: Male Sprague–Dawley (SD) rats were randomized (8 for donor and recipient per group) into five groups: I/R group (4 h of liver cold ischemia followed by 6 h of reperfusion), Heme oxygenase-1 (HO-1) small interfering RNA (siRNA) group (injection of siRNA via donor portal vein 48 h prior to harvest), DZ group (injection of DZ via donor portal vein 10 min prior to harvest), HO-1 siRNA + DZ group, and siRNA control group. Blood and liver samples were collected at 6 h after reperfusion. The mRNA expressions and protein levels of HO-1 were determined by RT-PCR and Western blot, and tissue morphology was examined by light and transmission electron microscopy. Serum transaminases level and cytokines concentration were also measured.

Results: We observed that a significant reduction of HO-1 mRNA and protein levels in HO-1 siRNA and HO-1 siRNA + DZ group when compared with I/R group, while the increases were prominent in DZ group. Light and transmission electron microscopy indicated severe disruption of tissue with lobular distortion and mitochondrial cristae damage in HO-1 siRNA and HO-1 siRNA + DZ group as compared with DZ group. Serum alanine aminotransferase (ALT), aspartate transaminase (AST), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels increased in HO-1 siRNA and HO-1 siRNA + DZ group and decreased in DZ group.

Conclusion: DZ pretreatment provided significant protection against I/R injury to liver grafts. The protective effect of DZ may be induced by upregulation of HO-1. Inhibiting the expression of HO-1, DZ has not the protective effect.

O28-0007 EFFECTS OF ANAESTHETIC PRECONDITIONING PLUS POSTCONDITIONING WITH SEVOFLURANE IN WARM LIVER ISCHEMIA/REPERFUSION INJURY IN RATS

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Background: Preconditioning is a therapeutic strategy aimed to increase ischemic tissue tolerance against ischemia/reperfusion (IR) injury. Recent studies demonstrated that volatile anaesthetics may improve postischemic recovery by an ischemic preconditioning-like mechanism. Postconditioning is a new concept that may have hepatoprotective effect. We hypothesized that sevoflurane preconditioning combined with postconditioning may reduce the hepatocellular damage in a rat model of warm liver IR.

Methods: Ten Wistar rats under mechanical ventilation were divided into 2 groups of 5; 1) IR: rats subjected to 45 min of warm liver ischemia of left and median lobes, followed by resection of non-ischemic lobes at early reperfusion; and 2) SEVO + IR: rats were exposed to sevoflurane 2.5% for 15 min, followed by 5 min washout, before ischemia, plus sevoflurane 2.5% for 15 min at reperfusion. Carotid artery was cannulated for mean arterial pressure (MAP). Mean portal flow (MPF) was assessed by perivascular flowprobe. MAP and MPF were recorded at baseline, pre-repefusion and 4 h postreperfusion. Liver transaminases, creatinine, pH, bicarbonate (BIC) and base excess (BE), potassium (K), glucose and lactate were measured at 4 h postreperfusion.

Results: AST and ALT were decreased in SEVO + IR group (12.118 ± 3.611 and 7.870 ± 1.586 U/L) compared to IR group (16.890 ± 1.630 and 13.418 ± 1.088 U/L), $P < 0.05$. BIC, and K were increased in SEVO + IR group (11.20 ± .86 mmol/l and 6.1 ± 1.3 mEq/dl) compared to IR (6.70 ± 3.32 mmol/l and 4.7 ± 0.7 mEq/dl), $P < 0.05$. There were no differences in MAP, MPF, creatinine, glucose, lactate, pH and BE; however glucose tended to be higher in SEVO + IR group (50.8 ± 26.0 mg/dl) compared to IR (35.0 ± 18.4 mg/dl).

Conclusions: In experimental warm liver ischemia/reperfusion, sevoflurane preconditioning plus postconditioning reduced the hepatocellular injury demonstrated by lower levels of transaminases with a better behaviour of acid–base variables and good hemodynamic recovery. These preliminary results are encouraging because postconditioning may have the advantage of being implemented at the moment of reperfusion, what is more feasible to be applied during liver transplantation surgery. (FAPESP 2011/05214-3)

O29-0047 PROTECTIVE EFFECTS OF REMOTE AND CLASSICAL POST-CONDITIONING ON ISCHEMIA/REPERFUSION-INDUCED ACUTE KIDNEY INJURY

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Objective: There are some studies in recent years on the successful application of ischemic postconditioning (POC) in the reduction of organ injuries after a very prolonged ischemic assault. The objective of the present study was to analyze and compare the effects of classical and remote POC on the rat renal ischemia/reperfusion-induced acute kidney injury.

Methods: After right nephrectomy, male Sprague–Dawley rats were randomly assigned into four groups ($n = 6$). In the IR group, 45 min of left renal artery was induced followed by 24 h of reperfusion. In the sham group, all of the above surgical procedures were applied except that IR was not induced. In classical POC group, after induction of 45 min ischemia, four cycles of 10 s of intermittent ischemia and reperfusion were applied to the kidney before complete restoring of blood to the kidney. In remote POC group, four cycles of 5 min intermittent ischemia and reperfusion of left femoral artery were applied after ischemia and right at the time of restoring the blood to the kidney.

Results: In the IR group, there was a reduction in renal function demonstrated by an increase in BUN and serum creatinine. Application of both forms of POC prevented the IR-induced reduction in renal function. There were also significant improvements in kidney oxidative stress status in POC groups demonstrated by a reduction in MDA formation and preservation of antioxidant levels comparing to IR group. Remote POC was more protective than classical model in preservation of antioxidant contents, namely SOD and GSH levels accompanying with a lower MDA concentration and a higher FRAP levels. In histological evaluation, induction of classical POC prevented the structural changes and there was no detectable cellular necrosis, less cast formation in the tubules so the majority of the nephrons were still open. The same conditions were seen in remote POC.

Conclusion: We concluded that both methods of POC have protective effects on renal function and histology possibly by a reduction in IR-induced oxidative stress.

6TH SESSION : DONORS AFTER CARDIOCIRCULATORY DEATH

O30-0021 RANDOMISED CONTROLLED TRIAL OF THE EFFECTS OF HYDROGEN SULPHIDE IN RENAL ISCHAEMIA REPERFUSION INJURY.

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Aims: Renal ischaemia reperfusion injury (IRI) is a major cause of acute renal failure and renal transplant dysfunction. The aim of this study was to investigate the efficacy of the endogenous gaseous signalling molecule hydrogen sulphide (H_2S) in protecting against renal IRI.

Methods: Large white, female pigs underwent laparotomy and cross clamping of the left renal pedicle for 60 min. Animals were randomly allocated to treatment with an i.v. infusion of either H_2S ($n = 6$) or saline control ($n = 6$) 10 min prior to clamp release and then underwent a right nephrectomy. Veterinary and surgical staff were blinded to treatment allocation. Animals were recovered for 7 days and serum creatinine was measured as an index of renal function.

Results: Administration of H_2S resulted in a marked reduction in kidney injury with reduced serum creatinine on days 1, 3 and 5; reduced area under the curve of creatinine and halving of the time to creatinine < 250 μmol/l (table 1.). H_2S also preserved tubular function as shown by urinary protein:creatinine ratio which, compared to baseline, increased at day 1 and 3 in the control group (3.22 ± 2.86; $P = 0.01$ and 2.585 ± 1.267; $P = 0.031$) but not the treatment group (0.255 ± 0.185; $P = 0.19$ and 1.062 ± 0.4365; $P = 0.11$). TNF-α levels at 6 h post reperfusion increased in the control animals (57 ± 15 vs. 109 ± 51; $P = 0.026$) but not in the H_2S treated animals (62 ± 18 vs. 80 ± 27; $P = 0.46$). Renal leukocyte infiltration at 30 min (Myeloperoxidase staining) was also significantly reduced by treatment with H_2S ($P = 0.016$). H_2S did not lead to any adverse postoperative events.

Conclusion: H_2S offers a promising novel approach to ameliorating renal IRI with potential translation into a number of clinical settings including renal transplantation.

Variable	H_2S	Control	p-value
Creatinine			
Pre	116 ± 11	107 ± 7	0.097
Day 1	394 ± 108	535 ± 76	0.012
Day 3	245 ± 45	434 ± 408	0.004
Day 5	187 ± 26	249 ± 370	0.037
Day 7	160 ± 26	186 ± 109	0.390
Area under curve (AUC)	2195 ± 260	3009 ± 1867	0.026
Time to Cr <250 (days)	3 ± 1	6 ± 1	0.007

O31-0027 NON-HEART-BEATING DONORS: PRELIMINARY STUDY ON AN ISOLATED PERFUSED SWINE HEART HARVESTED AFTER 20 MIN OF NORMOTHERMIC ISCHEMIA

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Objective: In order to assess the feasibility of using non-heart-beating donors in heart transplantation, we have measured the functional and metabolic status of hearts submitted to normothermic ischemia before preservation using an *ex-vivo* pig heart model.

Methods: 10 pigs were separated in 2 groups: Control ($n = 6$, brain-dead group) and NHBD ($n = 4$, Non-Heart-Beating Donor). In Control group, hearts were harvested 20 min after the brachiocephalic trunk cross-clamping and were immediately reperfused. In NHBD group, hearts were harvested 20 min after exsanguination and asphyxia, stored in the CRMBM solution for 2 h and then reperfused. Cardioplegic arrest was induced using 1 l of CRMBM cardioplegic solution (4 °C) and the heart was reperfused for 60 min using an *ex-vivo* perfusion system with normothermic autologous blood. During reperfusion, functional parameters were analyzed. CK, CK-MB, inorganic phosphate and lactate were determined in myocardial effluents. At the end of the protocol, hearts were freeze-clamped for biochemical assays. Adenine nucleotides, phosphocreatine and malondialdehyde (MDA) contents were measured by HPLC. Caspase-3 expression was studied as an index of apoptosis. Nitric oxide (NO) pathway was evaluated by NO synthase (NOS) isoforms expression and total nitrate concentration (NOx).

Results: No electromechanical activity was found in NHBD compared with Control. CK and CK-MB were significantly higher ($P < 0.05$) in NHBD vs. Control (19 ± 6 vs. 12 ± 9 ng/ml and 0.53 ± 0.4 vs. 0.15 ± 0.07 ng/ml, respectively). ATP (μmol/g proteins) was lower in NHBD (6 ± 3) vs. Control (34 ± 4) ($P = 0.002$). MDA content, used as a marker of oxidative stress, was measured only in NHBD (0.070 ± 0.04 μmol/g proteins). Myocardial caspase-3 was not expressed in both groups. NO pathway was significantly impaired in NHBD compared with Control with lower eNOS expression ($P < 0.0001$) and NOx content ($P = 0.04$).

Conclusion: We reported no functional and metabolic recovery in the non-heart beating donor group after normothermic ischemia and reperfusion indicating that a single immersion of the cardiac graft during storage does not provide an optimal protection. New strategies in heart preservation are necessary for recruiting non-heart-beating donors.

O32-0056 CARDIAC TOLERANCE TO WARM, GLOBAL ISCHEMIA IS IMPROVED WITH MILD HYPOTHERMIA FOLLOWING ISCHEMIC ARREST

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Background: Lack of donor organs currently limits cardiac transplantation. Although use of non-heartbeating donors (NHBDs) could increase cardiac graft availability, it has not been widely adopted given concerns about irreversible injury provoked by inevitable warm cardiac ischemia. In this context, clinically applicable approaches to limit warm ischemic injury have not yet been characterized. Therefore, we investigated the effects of slightly lowering heart temperature after ischemic onset on recovery of hemodynamic function.

Methods: Hearts isolated from male Wistar rats were perfused aerobically with Krebs–Henseleit buffer in working mode, subjected to global, no-flow ischemia and reperfused for 60 min. Hearts were initially reperfused in an unloaded mode, and then switched to loaded mode. Five ischemic groups were investigated: 37 °C for 20 ($n = 5$) or 30 min ($n = 5$), and 32 °C for 40 ($n = 6$), 50 ($n = 6$) or 60 min ($n = 6$). Hemodynamic parameters and markers of metabolism and necrosis were measured.

Results: At 37 °C, hearts recovered almost completely after 20 min ischemia; however, no recovery was detectable after 37 °C/30 min ischemia. At 32 °C, hearts also recovered well after 40 and 50 min ischemia, but not after 60 min. Following 60 min reperfusion, percent recovery of rate-pressure product (RPP) after was: 97 ± 9% for 37 °C/20 min ischemia, 86 ± 4% for 32 °C/40 min ischemia, 76 ± 15% for 32 °C/50 min ischemia versus < 20% for any heart in 37 °C/30 min or 32 °C/60 min groups. Coronary perfusion and markers of metabolism and necrosis were significantly improved in 32 °C vs. 37 °C groups despite substantially longer periods of ischemia.

Conclusions: Slightly reducing cardiac temperature after ischemic arrest improves ischemic tolerance by approximately two-fold. Cardioprotection afforded by mild hypothermia during ischemia likely results from better microvascular protection, reduced necrosis, and improved metabolic recovery. Mild hypothermia is a promising approach towards the use of hearts from NHBDs for transplantation.

O33-0071 OUTCOME AFTER LIVER TRANSPLANTATION USING CONTROLLED DONATION AFTER CARDIAC DEATH DONORS: A SINGLE-CENTER EXPERIENCE

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Introduction*:* Liver Transplantation (LTx) using Donation after Cardiac Death (DCD) donors are increasingly used but considered a risk factor for poor outcome. Therefore we reviewed the results of controlled DCD-LTx at our center.

Patients & Methods*:* Between 2003 and 2010, 30 DCD-LTx were performed (6% of all LTx). Medical records after DCD and Brain Dead Donors (DBD)-LTx were retrospectively reviewed. Donor demographics, post-LTx peak aspartate amino transaminase (AST), biliary strictures, re-transplantation rate and patient/graft survival were analyzed.

Results: 80% of DCD-LTx were procured locally vs. 39% for DBD; $P < 0.0001$. Median donor age was similar for DCD and DBD-LTx {51yo (IQR: 38–59) vs. 53yo (IQR: 42–64); $P = 0.244$}. Mean donor warm ischemia time was 23 ± 11 min. Median cold ischemia time was shorter for DCD {6 h 54 min (IQR: 5 h 25 min – 7 h 51 min)} compared to DBD-LTx {8 h 40 min (IQR: 7 h 13 min – 10 h 06 min)}; $P < 0.0001$. Median recipient age was 60yo (IQR: 53–65) for DCD, and 58yo (IQR: 49–64) for DBD-LTx; $P = 0.25$. Mean labMELD was 15 (IQR: 12–17) for DCD, and 16 (IQR: 11–23) for DBD-LTx; $P = 0.59$. Median post-LTx AST peak was higher after DCD {1178 IU/L (IQR 560–1997)} vs. DBD-LTx {651 IU/L (IQR 333–1243)}; $P = 0.005$. The incidence of biliary strictures was not statistically different (30% for DCD vs. 20% after DBD-LTx; $P = 0.24$). The re-transplantation rate within one year post-LTx was 3% after both DCD and DBD-LTx; $P = 1.$ 1, 3, and 5-year patient survival was 92, 83 and 83%, and 1, 3, and 5-year graft survival was 89, 79, and 79% after DCD-Tx and not different from DBD-LTx (1, 3 and 5-year patient survival of 90, 82 and 75%, $P = 0.846$ and graft survival of 88, 79 & 73%, $P = 0.707$).

Conclusion: Despite substantial ischemic injury (e.g. high peak AST) short & long term survival after DCD-LTx is comparable to DBD-LTx. Rapid donor surgery, careful donor and recipient selection, short warm and cold ischemia times are key factors to optimize outcome after DCD-LTx. However, strategies to reduce ischemic injury and biliary complications remain warranted.

O34-0024 KIDNEY GRAFT QUALITY AFTER DONATION FROM UNCONTROLLED DECEASED DONORS AFTER CARDIAC ARREST

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Introduction: Kidney grafts from uncontrolled deceased donors after cardiac arrest (uDDCA) have recently been used in France to counteract organ shortage. The quality of these kidneys remains debatable. The aim of our study was to compare the outcomes and the quality of uDDCA kidneys with that of kidneys from optimal donors such as simultaneous kidney and pancreas (SPK) donors and extended-criteria donors (ECD).

Methods: 27 kidney grafts from uDDCA (mean donor age, 41) were compared with 24 kidney grafts from SPK donors (mean donor age, 26), and 30 kidney grafts from ECD (mean donor age, 66). After kidney retrieval, preservation protocol consisted in hypothermic pulsatile perfusion (RM3, Water's medical) and the organ preservation solution was UW solution. Kidneys with intrarenal vascular resistance above 0.35 mmHg/ml/mn after 6 h of perfusion were discarded. All three patient groups were non-sensitized and received the same induction and maintenance immunosuppressive therapy.

The quality of the grafts was assessed by renal function and histology. GFR was estimated by MDRD formula (eGFR) at M1 ($n = 80$), M3 ($n = 80$), M6 ($n = 79$), M12 ($n = 74$), M24 ($n = 70$) and M36 ($n = 51$) and measured by inuline clearance (mGFR) at M12 ($n = 66$) and M36 ($n = 46$). Vascular lesions were analyzed in systematic kidney biopsies at M3 ($n = 54$) and M12 ($n = 50$) with the Banff 2007 classification. Interstitial fibrosis (IF) was quantitatively measured by colour image analysis.

Results: Mean renal blood flow increased from 42 to 83 mL/min/100 g. Mean intra-renal vascular resistance decreased from 0.85 to 0.3 mmHg/ml/min/100 g. The warm ischemic time in the uDDCA group was higher than the two others groups (106 min, $P < 0.001$). In the short term, no PNF was observed and the DGF in the uDDCA group was significantly higher than in the ECD group (81 vs. 28%, $P < 0.001$). In the uDDCA group renal function was initially poorer but improved during the first year. However on the long term, renal function (mGFR at M36, 41.2 vs. 33.7 ml/min/1.73 m^2, $P = 0.09$) and interstitial fibrosis score was not different in uDDCA vs ECD group (IF score at M12, 36 vs. 34% $P = 0.47$).

Conclusion: Our study suggests that the quality of kidneys from uDDCA donors is similar to that of ECD and that these kidneys should be attributed to the same recipient population.

O35-0078 IMPACT OF THE DELAYED GRAFT FUNCTION ON KIDNEY GRAFT SURVIVAL IN RECIPIENT WITH GRAFT COMING FROM DONATION AFTER CARDIOCIRCULATORY DEATH

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Introduction: The aim of our study was to compare the impact of delayed graft function (DGF) on kidney graft survival after Donation after Cardiocirculatory Death (DCD) or donation after Brain Death (DBD) either extended criteria donors (ECD) or standard criteria donors (SCD).

Method: All recipients of a solitary deceased-donor graft between 1/1/2008 to 31/12/2011 were included. Data are recorded in a database and checked prior to analysis.

Results: Two hundred and fifty-eight patients were included, 53 DCD recipients and 205 DBD recipients. Results are shown in table 1.

Graft survival in DBD was significantly better if no DGF occurred (Log rank Test, $P = 0.03$). Despite a higher rate of DGF in DCD, graft survival was not different according to DGF occurrence (Log rank $P = 0.67$). Creatinine Clearance at 2 years was 56 ± 25 ml/min for DBD-SCD, 50 ± 13 ml/min for DCD and 38 ± 17 ml/min for DBD-ECD, significantly lower for ECD than for the two others ($P < 0.01$).

Conclusion: DGF is a major prognostic factor for long term graft survival of DBD grafts, but not for DCD grafts. DGF could have a different impact on DCD and DBD outcome. One explanation could be that organs from DCD are retrieved much sooner after the cytokine storm induced by brain death. This has to be established on animal models.

	DCD ($n = 53$)	DBD ($n = 205$)	P
Donor age	41.5 ± 11 years	50 ± 15 years	0.0004
Recipient age	45.8 ± 11 years	50 ± 13 year	0.017
Donor sex	H = 85%	H = 53%	0.045
Donor creatinine	120 ± 30 μmol/l	84 ± 48 μmol/l	< 0.00001
Cold ischemia	13.3 ± 4 hours	15.3 ± 4.4 hours	0.002
Time on waiting list	14 ± 11 months	23 ± 21 months	0.00005
Day of diuresis	2.98 ± 5.1 days	0.3 ± 0.9 days	0.0016
DGF	63.5%	21%	< 0.0001
Nb of hemodialysis	4.7 ± 2.4	3.7 ± 3.6	0.14
Nb day creatinine < 300	21.3 ± 14.3 days	6.2 ± 6 days	<0.00001

O36-0054 NEW MODEL OF KIDNEY DONATION FROM UNCONTROLLED DONORS

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Introduction: Inevitable injury due to warm ischemic time (WIT) prevent wide acceptance of the practice of uncontrolled organ donation after circulatory determination of death (UDCDD).

Methods: Between 2009 and 2010, organ procurement service of St Petersburg, Russia performed the transplantation of organs from 17 UDCDD donors with warm ischemic time from 45 – 91 min. For resuscitation of the kidneys after ischemia, the subnormothermic extracorporal abdominal perfusion (SNECP) with leukocyte depletion *in situ* was employed.

Results: Immediate functioning of kidney grafts was observed in 11 of the 34 recipients, with no rejection observed. The 1 year graft survival was at 97% ($n = 33$) with rejection rate at 5.8% ($n = 2$). At the end of first year of observation, the average creatinine levels were 1.81 ± 0.05 mg/dl.

Conclusion: In our opinion, the SNECP could be considered as a resuscitation practice for donor organs from uncontrolled donors which had critically expanded warm ischemia time. The protocol for isolated abdominal SNECP provides the organ procurement service with up to 1 h of a time reserve between the declaration of death and the initiation of perfusion. In our opinion, this protocol could substantially expand the pool of the potential donors.

O37-0070 KIDNEY DONATION AFTER CIRCULATORY DEATH HAS NOT INCREASED KIDNEY TRANSPLANT ACTIVITY IN BELGIUM – A COMPARATIVE ANALYSIS WITH THE NETHERLANDS BETWEEN 1995 AND 2010

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Aim: Did the (re)introduction of controlled kidney donation after circulatory death (DCD) in Belgium in the 2000 increase the number of kidney transplants (KT) as we hoped?

Method: We studied the evolution of effective kidney donation/KT in Belgium vs. the Netherlands between 1995 and 2010. Data were obtained from the Eurotransplant registry and adjusted for number of inhabitants using Eurostat population data (variable [quartile]). 3 eras (1995–1999, 2000–2005, 2006–2010) were compared by Kruskal–Wallis test. ($P < 0.05$ significance).

Results: Between 1995 and 2010, Belgium mainly had deceased donors (DD) [20.6 pmp (19.0–22.4)], mostly donation after brain death (DBD) [19.4 pmp (18.3–20.9)] and some DCDs [0.4 pmp (0.2–2.8)]. Living donation (LD) [2.2 pmp (1.5–3.8)] increased total kidney donors to 23.0 pmp (21.1–26.0). KT rates were similarly distributed: a DD KT-majority [37.9 pmp (31.9–38.8)]; mainly DBDs [33.5 pmp (30.3–37.1)] and some DCDs [0.7 pmp (0.3–4.8)]. LD [2.5 pmp (1.5–4.0)] increased total KT to 39.2 pmp (34.7–42.8). Between 2000 and 2005, only 1.5% (0.75–4.25) of DD KT were DCDs, this percentage increased to 16% (12–16.5; $P = 0.04$) in 2006–2010. However, increased DCD and LD KT did not result in a significant increase of total KT activity ($P = 0.1$). In the Netherlands, kidney donation rates reached 25.0 pmp (19.9–34.9) with equal distribution between LD [12.2 pmp (7.3–20.8)] and DD [12.5 pmp (12.0–13.6)]: DBDs [8.1 pmp (7.4–10.2)] and DCDs [4.1 pmp (2.2–5.5)]. KT were mainly DDs [23.2 pmp (22.1–24.9)], both from DBDs [14.7 pmp (13.7–19.1)] and DCDs [7.6 pmp (3.7–10.0)]. LD KT [12.4 pmp (7.3–20.8)] increased total KT to 35.4 pmp (31.3–44.6). LD increased kidney donation rates ($P < 0.01$). DD activity remained stable ($P = 0.6$), but DBD activity decreased ($P = 0.01$) while an exponential increase in DCDs was simultaneously observed ($P = 0.01$). KT rates also increased ($P < 0.01$), mainly due to increased LDs (in 2010, 57% KT were LD). DD KT rates remained stable ($P = 0.6$), but with increasing use of DCDs ($P = 0.01$) and decreasing use of DBDs ($P = 0.01$).

Conclusion: Increased DCD donations/KT in Belgium did not substantially increase DD KT, suggesting a shift from DBD to DCD, a phenomenon clearly observed in the Netherlands. These data should alert the transplant community and donor management teams; DCD program development is only justified when it concerns potential donors who will otherwise not progress to brain death.

7TH SESSION : ORGAN *EX VIVO* REPAIR

O38-0036 TISSUE ENGINEERING FOR *EX VIVO* REPAIR OF ISCHEMICALLY DAMAGED TUBULE EPITHELIUM

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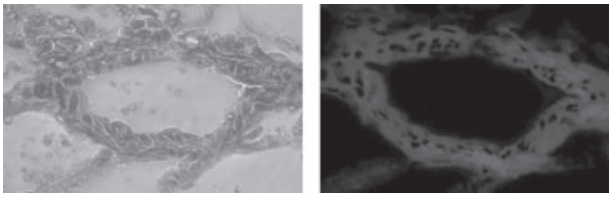
Introduction: The ability to repair warm ischemically damaged renal tubule epithelium prior to transplantation would provide the potential to expand organ donor criteria to include uncontrolled DCD (uDCD) patients. We have demonstrated that an *ex vivo* acellular, near-normothermic perfusion can resuscitate oxidative metabolism after warm ischemia sufficiently for there to be new synthesis that restores cytoskeletal integrity. However, during the period of *ex vivo* perfusion, the tubule epithelium is not regenerated because rather than repair, cell replacement is needed. We now describe the ability to deliver progenitor renal epithelial cells (REC) during *ex vivo* perfusion to the renal tubule epithelium with homing to the sites of damage.

Methods: Human REC were fluorescently labeled with PKH26 red fluorescent cell linker (Sigma-Aldrich, St. Louis, MO). Porcine kidneys were damaged by 60 min of postmortem warm ischemia. The damaged kidneys were then flushed and placed on exsanguinous metabolic support perfusion at 32 °C for 60 min to restore oxidative metabolism and normalize perfusion pressures and vascular flow rates. 5.0 X 10^7 labeled human REC were then infused into the renal artery at the rate of 0.5 X 10^6 per min. The perfusion was then continued for an additional 8 h.

Results: During the administration of the REC there were no adverse vascular reactions in terms of there being no rise in perfusion pressures nor diminution in the vascular flow rate. Post-perfusion the labeled human REC were only detected within the renal tubule epithelium. By using an *ex vivo* closed perfusion system we were able to determine the number of human REC that were introduced intra-arterially, the number of REC remaining in the perfusate post-perfusion and the number of cells removed from the vascular space by copious flushing post-perfusion. More than 90% of the fluorescently labeled human REC were taken up by the kidney and could be detected predominantly in the tubules of the outer medulla (Fig. 1a and b). The human REC were not found in the vascular compartment.

Figure 1 (a).H&E Staining of a Renal Tubule (b). Immunofluorescence of the Same Tubule.

(a) H&E Staining of a RenalTubule **(b) Immunofluorescence of the SameTubule**



Conclusions: These results demonstrate the ability to deliver REC to the renal parenchyma during an *ex vivo* perfusion with homing to the site of damaged tubule epithelium and the corresponding ability to quantify the number of REC retained within the renal tissue. The ability to target progenitor cell delivery with corresponding determination of the number of cells deposited could provide opportunities for enhanced *ex vivo* repair of ischemically damaged kidney allografts.

O39-0019 THE FIRST CLINICAL SERIES OF NORMOTHERMIC PERFUSION IN MARGINAL DONOR KIDNEY TRANSPLANTATION

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Introduction: Delayed Graft Function (DGF) is a common problem in kidneys from donation after circulatory death (DCD) and extended criteria donors (ECD). A period of *ex-vivo* circulatory support prior to transplantation may allow the reversal of some of the detrimental effects of hypothermic storage and improve early graft function. Here we report the first clinical series of normothermic perfusion in marginal kidneys.

Methods: A total of 17 kidneys from marginal donors underwent normothermic perfusion (NP) after a period of static cold storage (CS). Kidneys were perfused on an isolated perfusion system adapted from paediatric cardiopulmonary bypass technology with one unit of compatible cross matched packed red blood cells. This was supplemented with a priming solution, nutrients, multivitamins and a vasodilator. Kidneys were perfused for an average of 65 ± 15 min at 34.5 ± 1.1 °C. After NP kidneys were flushed with preservation solution and transplanted.

Results: The mean donor age was 57 ± 11.5 years, cold ischaemic time 12.5 ± 10.3 h and total preservation time 13.7 ± 4.7 h (range 6.11 – 21.10 h). Renal function was restored in all kidneys during perfusion and they produced a mean of 199 ± 123 ml of urine. There were no complications during NP and all kidneys were transplanted successfully. The mean recipient age was 58 ± 10.3 years and anastomosis time 26 ± 6 min. One patient had delayed graft function (6.3%) requiring dialysis within the first week of transplantation. Four patients were treated for acute rejection within the first month (25%). The mean serum creatinine levels at day 7, 1, 3 and 6 months post transplant were 217 ± 163 μmol/l, 160 ± 57 μmol/l, 141 ± 28 μmol/l, 160 ± 56 μmol/l respectively.

Conclusions: The novel application of cardiac bypass technology to restore circulation to the kidney after a period of hypothermic storage and before implantation appears to be a safe and feasible method of preservation. Furthermore, the high rate of initial graft function in this series of marginal kidneys is notable but further comparative studies are required to assess the effects on DGF. The technique also has potential in the delivery of pre transplant *ex-vivo* therapies such as stem cells or gene transfer.

O40-0020 THE BIOLOGICAL EFFECTS OF NORMOTHERMIC KIDNEY PERFUSION ON ISCHAEMIA REPERFUSION INJURY

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Introduction: A short period of isolated normothermic perfusion (NP) can be used to resuscitate the kidney after periods of warm and cold ischaemic injury. However, the biological effects of NP on reperfusion injury remain undetermined.

Methods: Porcine kidneys were retrieved after 10 min of warm ischaemic injury and stored by either static cold storage (CS) for 24 h or CS for 23 h followed by 1 h of NP at 38 °C with leukocyte depleted autologous blood ($n = 6$) on an isolated organ perfusion system. After preservation kidneys in both groups underwent 3 h of *ex-vivo* reperfusion with whole autologous blood to assess the renal function and injury.

Results: NP kidneys had significantly lower intra-renal resistance (NP 2.28 ± 1.1 vs. CS 3.86 ± 1.2 mmHg/ml.h; $P = 0.040$), maintained their acid base homeostasis, had higher levels of oxygen consumption (NP 42.6 ± 19.5 vs. 20.8 ± 5.7 ml/min/ g; $P = 0.026$) and reduced tubular injury ($P = 0.008$) compared to kidneys in the CS group during reperfusion. There was no significant difference in the levels of inflammatory cytokines (IL-1β, IL-8 or TNFα; $P > 0.05$) or in renal function (Creatinine clearance NP 2.6 ± 1.3 vs. CS 3.0 ± 1.5 ml/min/100 g; $P = 0.070$). However, levels of IL-6 were significantly raised in the NP group after reperfusion ($P = 0.026$). Levels of heat shock protein 70 (HSP 70) were up-regulated after 1 h of NP and expression increased during reperfusion to a significantly higher level than the CS group ($P = 0.045$).

Conclusion: Kidneys undergoing a short period of NP had improved metabolic function and less tubular injury compared to CS kidneys. The increased expression of HSP 70 suggests that NP may up-regulate mechanisms that condition the kidney in preparation for reperfusion. Furthermore, the elevated levels of IL-6 suggest that NP may also have a role in promoting repair.

O41-0035 POTENTIAL FOR *EX VIVO* RENAL REPAIR

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Introduction: Treatments that could be used to repair ischemically damaged kidneys resulting in accelerated cellular recovery and amelioration of reperfusion injury would have major a impact on the availability of cadaveric renal allografts. An acellular, near-normothermic perfusion technology referred to as Exsanguinous Metabolic Support (EMS) was used to treat ischemically damaged kidneys. We evaluated the feasibility of resuscitating oxidative metabolism of sufficient magnitude to support the new synthesis requisite for modulation of ischemic injury and repair of the cytoskeleton.

Methods: Kidneys with 120 min of warm ischemic injury were recovered, flushed of blood and placed on EMS perfusion at 32 °C for 24 h. During the perfusion period the respiratory gasses, pH and temperature were maintained. Biopsies were taken at 6, 12, 18 and 24 h. The sections were fixed in 2% paraformaldehyde with 75 mM L-lysine and 10 mM sodium periodate for 24 h at 4 °C and then stored in phosphate buffered saline. Frozen sections 4-μm thick were cut and histologic evaluations were made from five representative sites and tested in duplicate. Indirect immunohistochemistry assays were performed to evaluate restoration of the cytoskeleton integrity over time using the injury marker zonal occludens (ZO-1) and new synthesis requisite for cellular repair was evaluated by measuring proliferating cell nuclear antigen (PCNA).

Results: 120 min of warm ischemia resulted in widespread cytoplasmic staining for ZO-1 in approximately 80% of the tubule epithelium. A normalizing trend in the cytoskeleton was observed with reduced positive ZO-1 staining of 32% by 18 of warm perfusion. By 24 h of EMS ZO-1 staining was equivalent to that of undamaged kidneys with approximately 8% of cells staining positive. An up-regulation in positive staining for PCNA was first observed at 6 h of warm perfusion. Increased expression of the repair marker was observed in a time dependent manner with widespread positive staining at 24 h.

Conclusion: During EMS perfusion of severely ischemically damaged kidneys it appears that oxidative metabolism was sufficiently recovered to support the up-regulation of cellular processes that forms the basis for modulation of both injury and repair proteins. The ability to support cellular reparative processes in ischemically damaged kidneys during an *ex vivo* warm perfusion could enable an expansion of the existing cadaveric kidney pool by recovering uDCD kidneys with prolonged ischemia.