POSTERS

NEW ASPECTS OF CELL METABOLISM DURING IRI

P1-0026 MICRORNA-21 UP-REGULATE HIF-1 ALPHA EXPRESSION IN HEPATOCYTES DURING ISCHEMIA INJURY BY TARGETING PTEN

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Background: MicroRNAs (miRs) are non-coding RNAs that could regulate gene expression. Until now, very little is known about miRs' role during ischemia/reperfusion (I/R). It was reported that microRNA-21 (miR-21) could promote tumor angiogenesis and HIF-1 alpha expression in cancer cells, but its role in I/R in normal cell remains unknown. Here, we investigated the role of miR-21 during hypoxia in hepatocytes.
Methods: We employed real-time RT-PCR to investigate the expression pattern of miR-21 after different durations of hypoxia in L02 cell line. To investigate the role of miR-21 during hypoxia, L02 cells were transfected with miR-21 inhibitor and mimic respectively. Transfected cells were harvested after 4 hours of hypoxia, and HIF-1 alpha expression in each group was detected by real-time RT-PCR. As PTEN is a putative target of miR-21 and can regulate HIF-1 alpha expression via AKT signaling, PTEN expression in each group was detected by western blot.
Results: Real-time RT-PCR analysis revealed that mature miR-21 levels was increased by approximately fourfold after 4 hours of hypoxia, decreased to twofold after 8 hours of hypoxia, and kept this expression level after 16 hours of hypoxia. In loss-of-function and gain-of-function study, HIF-1 alpha mRNA expression was inhibited in miR-21 inhibitor group (I group) and up-regulated in miR-21 mimic group (M group) compared to negative control (C group) after 4 hours of hypoxia (C:I:M group $P = 1:0.8:1.5$), and western blot results showed PTEN was significantly decreased when miR-21 was over-expressed.
Conclusion: MiR-21 was up-regulated at early stage of hypoxia in hepatocytes; it can help to up-regulate HIF-1 alpha expression by target PTEN. Over-expression of miR-21 might protect hepatic cells from ischemia injury by enhancing HIF-1 alpha expression. These findings suggested that over-expression of miR-21 might have the therapeutic potential for the better management of liver I/R injury.

P2-0045 DOWNREGULATION OF MIR-20A DECREASES HIF-1 ALPHA AND ACCELERATES APOPTOSIS IN HYPOXIA

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Background: Hypoxia inducible factor-1 alpha (HIF-1 alpha) is widely considered to be one of the key nuclear transcription factors. It plays an important role in ischemia/reperfusion through regulating the expression of the downstream genes. However, the upstream regulation of HIF-1 alpha has not been fully clarified. This study was undertaken to determine if miR-20a is a regulator of HIF-1 alpha in hypoxia of normal cell.
Methods: We employed real-time PCR to investigate the expression pattern of miR-20a and western blot to detect HIF-1 alpha expression after hypoxia in human liver cell line L02 and human umbilical vein endothelial cells (HUVEC). To investigate the role of miR-20a during hypoxia, L02 cells and HUVEC were transfected with miR-20a inhibitor respectively. Transfected cells were harvested after hypoxia, and HIF-1 alpha expression in each group was measured by real-time PCR and western blot. Apoptosis was also detected with AnnexinV by fluorescence microscope.
Results: We found miR-20a was significantly increased after 4 hours of hypoxia in L02 by real-time PCR, decreased in following hypoxia. Interestingly, the obvious up-regulation was observed after 16 hours of hypoxia in HUVEC. Western blot results showed the protein level of HIF-1 alpha was increased in cells hypoxia. In miR-20a inhibitor groups, the protein and mRNA levels of HIF-1 alpha were decreased in L02 following 4 hours of hypoxia and HUVEC following 24 hours of hypoxia. In groups of L02 and HUVEC following 16 hours of hypoxia, miR-20a inhibitor treatment accelerated hypoxia-mediated apoptosis.
Conclusions: Taken together, our findings suggested miR-20a was upregulated in cell hypoxia, and the expression pattern of miR-20a after hypoxia was differential in HUVEC and L02. During cell hypoxia, miR-20a participated in the regulation of HIF-1 alpha, and accelerated the hypoxia-mediated apoptosis. Thus, our results suggested that further delineating the mir-20a regulatory pathways may provide new insight into mechanism of ischemia/reperfusion.

P3-0077 RENAL PKC EPSILON DEFICIENCY ATTENUATES ISCHEMIA REPERFUSION INJURY VIA IMPAIRED NEUTROPHIL INFILATRATION AND TNF ALPHA DEPENDENT APOPTOSIS INHIBITION

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Introduction: Prolonged cold ischemia time is a major determinant of acute rejection and renal allograft survival. In this study we investigated the effect of PKC epsilon deficiency on ischemia-reperfusion injury and ischemic allograft damage after kidney transplantation.
Methods: IR injury was induced by bilateral renal pedicle clamping for 35 min. PKC epsilon deficiency resulted in markedly improved survival and attenuated loss of kidney function compared to wild type (WT) controls. Acute tubular necrosis and neutrophile infiltration was markedly reduced. To rule out whether the resistance to IR injury is mediated by local renal cells we studied a life supporting renal transplant model with ischemic graft injury. We transplanted kidneys from H2b PKC epsilon-deficient mice and their corresponding WT littermates into MHC-incompatible H2d recipients (BALB/c) and induced ischemic graft injury by prolonged ischemia time.
Results: All recipients of WT allografts died within 10 days after transplantation and developed severe renal failure. In contrast, recipients of PKC epsilon deficient allografts showed improved renal allograft survival and had significantly reduced s-creatinine elevation at day 6 after transplantation (77 ± 8 vs. 160 ± 30 μmol/l in PKC epsilon -/- vs. WT allograft recipients, $P < 0.05$). PKC epsilon deficiency of the allograft caused decreased radical generation; reduced TNF-alpha up-regulation resulting in reduced inflammation and tissue damage. Consecutively, PKC epsilon deficient allografts showed reduced up-regulation of the adhesion molecule ICAM-1 and attenuated inflammatory cell infiltration six days after transplantation.
Conclusion: The data suggests that local renal PKC epsilon expression mediates the up-regulation of pro-apoptotic and pro-inflammatory signaling molecules mediating acute ischemia reperfusion injury.

P4-0043 EFFECTS OF HEPATIC REPERFUSION INJURY ON THE GENE EXPRESSION OF SOME CYTOCHROME P_{450} ISOFORMS IN RAT LIVER

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Introduction: The hepatic ischemia/reperfusion injury (IRI) has been shown to be the major problem associated with stroke, shock, cirrhosis, liver surgery and transplantation. Liver is known as an important metabolizing organ and cytochrome P450s (CYPs) as superfamily enzymes play an important role in the metabolism of different compounds. This study aimed to investigate the effects of IRI on the gene expression of the major cytochrome P_{450} isoforms in rat liver
Methods: Four groups ($n = 5$) of male Sprague-Dawley rats underwent 60 min lobar hepatic ischemia followed by 1, 6, 12 or 24 hours reperfusion and a sham-operated group was selected as control. Blood samples were collected at different time intervals to test hepatic enzyme alterations induced by IRI. At the end of each reperfusion period, the animals were euthanatized and tissue samples were taken for gene expression. Total RNA was isolated from the tissues and then cDNA was synthesized from an mRNA template. The level of mRNA expression in liver was analyzed by real-time PCR using specific primers for CYP450 isoforms.
Results: The release of ALT, AST and ALP in the groups subjected to IRI was markedly ($P < 0.001$) increased during different times of reperfusion. However, the level of CYP1A1, CYP3A1 and CYP2E1 mRNA significantly ($P < 0.05$) decreased during 6, 12 and 24 hours reperfusion ($P < 0.05$).
Conclusion: The results of this study suggest that IRI injury may induce down regulation of the individual CYP450 isoforms, which can affect CYP-mediated drug-metabolizing activities by the liver.
Keywords: Ischemia/ reperfusion injury, Liver, Cytochrome P_{450}

P5-0025 OAT1/3 AND RENAL ISCHEMIA AND REPERFUSION: EVIDENCE FOR A ROLE OF PROXIMAL TUBULAR TRANSPORTER EXPRESSION ON RENAL OUTCOME

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We have shown that expression of proximal tubular organic anion transporters OAT1 and OAT3 is diminished by prostaglandin E2 (PGE2) or after renal ischemia and reperfusion (I/R). Indomethacin applied at a low dose after ischemia (1 mg/kg) inhibits I/R induced down regulation of OAT1/3 and improves renal outcome. Indomethacin is taken up into the proximal tubules by OAT1/3 and accumulates. We applied probenecid to block indomethacin uptake into proximal tubules and abolish its effect on OAT1/3 regulation and renal outcome.

iAKI was induced in rats by bilateral clamping of renal arteries for 45 min. Indomethacin (1 mg/kg) was given i.p. as soon as reperfusion started. Probenecid (50mg/kg) was applied i.p. 10 min later. The reperfusion period was 24 hours.

Indomethacin restored the expression of OAT1/3, PAH net secretion and PGE2 clearance. Additionally, indomethacin improved kidney function as measured by GFR, renal cortical apotosis and PAH clearance. Notably, low-dose indomethacin did not affect inflammation parameters in the kidneys (e.g. MCP-1, ED1+-cells). Probenecid blocked the restoration of OAT1/3 expression induced by indomethacin and moreover abrogated all beneficial effects of indomethacin.

Our study indicates: (i) The beneficial effect of low dose indomethacin is not due to its anti-inflammatory potency, but (ii) due to its effects on regulation of expression of OAT1/3. Probenecid competitively inhibits the uptake of indomethacin into the proximal tubular cells, (iii) thereby restoring PGE2 induced down regulation of OAT1/3 after I/R and as a cause re-establishing renal damage. This is (iv) evidence for a mechanistic effect of OAT1/3 on renal outcome after ischemia and reperfusion.

Moreover our data indicate: (v) The thought of prolonged renal perfusion impairment during reperfusion after ischemia may be an overestimation due to determination of renal perfusion by PAH clearance (which is highly dependent on expression of OAT1/3).

P6-0009 CANNABIDIOL TREATMENT AMELIORATES ISCHEMIA/REPERFUSION RENAL INJURY IN RATS

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The nephroprotective effect of cannabidiol, the major non-psychotropic Cannabis constituent, was investigated in rats exposed to renal ischemia/reperfusion injury. Bilateral renal ischemia was induced for 30 min followed by reperfusion for 24 hours. Cannabidiol (5 mg/kg, i.v.) was given 1 hour before and 12 hours following the procedure. Ischemia/reperfusion caused significant elevations of serum creatinine and renal malondialdehyde and nitric oxide levels, associated with significant decrease in renal reduced glutathione. Cannabidiol significantly attenuated the deterioration in the measured biochemical parameters induced by ischemia/reperfusion. Histopathological examination showed that cannabidiol ameliorated ischemia/reperfusion-induced kidney damage. Immunohistochemical analysis revealed that cannabidiol significantly reduced the expression of inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-α, nuclear factor-κB, Fas ligand and caspase-3, and increased the expression of survivin protein in ischemic/reperfused kidney tissue. It was concluded that cannabidiol represents a potential therapeutic option to protect against ischemia/reperfusion renal injury.

P7-0010 EFFECT OF METHYLENE BLUE ON THE HEMODYNAMIC INSTABILITY RESULTING FROM LIVER ISCHEMIA AND REPERFUSION IN RABBITS

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The experimental investigation was performed to study the effects of methylene blue (MB) on hemodynamic, biochemical, and tissue changes among rabbits undergoing liver ischemia and reperfusion (IR). Twenty-four rabbits were randomized into 5 groups: 1, SHAM, control; 2, MB infusion bolus (3 mg/kg); 3, IR, hepatic ischemia for 60 min followed by 120 min of reperfusion; 4, MB-R, undergoing ischemia that had received an MB bolus infusion (3 mg/kg) prior to reperfusion; 5, R-MB, undergoing ischemia and MB bolus infusion after hemodynamic instability caused by reperfusion. The analysis included continuous recording of vital signs. Blood samples were collected at 0, 60, and 180 min of IR to determine blood gases as well as biochemical markers of liver function, nitric oxide, lipid peroxidation, and neutrophil activity. At the end of each experiment, liver tissue samples were collected for histological evaluation of parenchymae markers. Statistical analysis used two-way analysis of variance (ANOVA) tests with significance set at $P < .05$. Vital signs significantly improved with MB infusion, irrespective of whether it was applied before or after reperfusion. Blood gas data revealed different patterns among the SHAM, MB, IR, MB-R, and R-MB groups, without statistical significance, except for favorable lactate results in the R-MB group ($P < .01$), which displayed greater survival. Biochemical tests did not show significant differences among the groups, whereas histological analysis revealed favorable appearances for the MB-R and R-MB groups. The MB effect lasted long after reperfusion, suggesting that improvement in the hemodynamic parameters was not based on liver integrity, but rather was possibly related to endothelial function.

P8-0057 BEST TEMPERATURE FOR STATIC LIVER GRAFT STORAGE IS 1 °C. AN EXPERIMENTAL STUDY

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Background: The best storage temperature for liver grafts is unknown. Methods. Two groups of rat livers were stored for 24 hours in UW solution at +4 °C, +1 °C or -0.5 °C. One was subjected to 15 min. of warm ischemia, rinsed with Ringer lactate and later reperfused with oxygenated Krebs-Henseleit buffer. Results. After warm ischemia, in livers stored at +4 °C, creatine kinase (CK) peaked at 21 ± 5 IU.g-1.h-1, hepatic resistance at 34700 ± 1500 dyn.s.cm 5, bile flow reached 18 ± 4 µl.g-1.h-1 after 10 min, and oxygen consumption stabilized at about 25 µmol.g-1.h-1 after 20 min. After storage at +1 °C, CK and hepatic resistance were lowered, bile production was 33 ± 6 µl.g-1.h-1 ($P < 0.05$ vs. +4 °C) and oxygen consumption was 105 ± 10 µmol.g-1.h-1 ($P < 0.001$). After storage at -0.5 °C, results did not differ statistically from those in the +1 °C group, except that bile flow was significantly lower. Without warm ischemia, peak CK ($P = 0.015$) and peak hepatic resistance ($P < 0.001$) in the +4 °C group were significantly higher than in the +1 °C or -0.5 °C groups, but no difference in bile flow or oxygen consumption was observed. Number of trypan blue-positive non-parenchymal cells ($P = 0.003$) and liver weight gain during reperfusion ($P = 0.015$) were minimal after storage at +1 °C. Conclusion. Liver function was better after storage at +1 °C than at +4 °C or -0.5 °C, mainly in parameters reflecting sinusoidal cell injury.

P9-0001 ATP AND OTHER ADENINE NUCLEOTIDE PRECURSORS MEASURED IN KIDNEYS FROM DCD BY ^{31}P NMR SPECTROSCOPY, DURING HYPOTHERMIC-OXYGENATED-PULSATILE PERFUSION, TO EVALUATE THEIR VIABILITY

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Introduction: The problem with organs from DCD (Donation after Cardiac Death) presenting long warm ischemia (WI) is the risk to transplant an organ that won't function. As shown, the pretransplant energetic level (ATP and precursors) is indicative of viability. After Bretan et al. 3 levels of energy correspond to immediate or no function and intermediate states where DGF is expected after transplantation.

Mitochondria is the site of energy production. Oxygen allows Mitochondria to regulate the production of electrons necessary for the Krebs' cycle. WI is deleterious to Mitochondria which is the principal inductor of apoptosis.

Using ^{31}P NMR spectroscopy, the precursors are PME, the phosphomonoester, a peak that contains the resonance of cell membrane precursors, sugar phosphates and adenosine monophosphate (AMP).We can observe NAD, and γ ATP peaks.

The aim of this experimental study was to determine, during oxygenated hypothermic pulsatile perfusion (O_2+HPP), the content of ATP and of other energetic Adenine Nucleotides in kidneys from DCD.

Methods: In a porcine model of DCD, organs presenting WI, up to 60 min. have been tested. In line acquisition of ATP and of precursors' content in the kidneys were obtained by NMR spectroscopy. The Chemical Shift Imaging (CSI) was obtained on the multi-nuclear MRI 3-T (Siemens Trio) apparatus.

Perfusions were realized with the HUG's HPP machines, compatible with the MRI (magnetic fields and bore's size). The perfusion' machines were equipped with a specific coil for ^{31}P resonance induction and CSI acquisition.

After 8 or 20 hours of perfusion, the organs were analyzed following the histological protocol of Goujon et al to observe a correlation between histology and viability by assessment of energetic level.

Results: As the duration of WI increases, ATP content decreases. In situations induced by WI, only the presence of precursors was observed. The absence of Adenine Nucleotide was observed in some specific situations (intoxication, vasoconstriction…). The histological scores correlated with the energetic levels.

Conclusions: This study shows that the spectroscopy can be realized during O_2+HPP perfusion. Consequently it is possible to see a difference between VIABLE (ATP), risk of DGF (only presence of precursors) or NOT VIABLE (absence of Adenine Nucleotide). That is in accordance with the Britan's observations. Cuts-off are still in study. That could in the future prevent any risk of grafting no viable organs.

IMMUNOLOGICAL CONSEQUENCES OF IRI

P10-0016 INCREASED INFLAMMATION CAUSED BY HYPEROXALURIA LEADS TO LARGER RENAL DISFUNCTION IN AN EXPERIMENTAL MODEL OF ISCHEMIA AND REPERFUSION

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Introduction: Acute kidney injury (AKI) is defined as a rapid loss of renal function due to damage to the organ, resulting in the retention of products of metabolism such as uremic toxins. In renal transplantation, AKI is largely responsible for long term lower graft survival. AKI caused by ischemia and reperfusion (I/R) induces renal dysfunction associated with specific markers of inflammation such as TNF-α. On the other hand, I/R may contribute to crystal deposition of calcium oxalate (CaOx) in renal tubules, causing additional damage in tubular epithelial cells, inducing necrosis and leading to progressive tubular atrophy and interstitial fibrosis. Objective: To assess whether the deposition of CaOx increases renal damage in rats with AKI and to analyze how animals prior exposed to I/R evolve when subjected to an overload of CaOx.

Material and Methods: Rats received a solution with 0.8% ethylene glycol (EG) and 1% ammonium chloride (NH4Cl) in drinking water, for a period of 4 weeks. Then, they were submitted to 60 min of renal ischemia. The reperfusion injury was analyzed 24 hours after the re-establishment of renal blood flow. Serum creatinine, urea, renal tissue histology and gene/protein expression were evaluated. Results: Addition of EG increased urine volume and led to reduced urine pH. Serum creatinine and urea levels in animals subjected to increased renal dysfunction as compared to control group. EG treatment shows a further increase in these levels, with a significant increase compared to group I/R. They also showed higher expression of pro inflammatory cytokines. IL-1b, CINC-2, CINC-3 and IL-6 protein expression was in this group. We also observed glomerular alteration and increased crystals in tubules after I/R, characteristic of calcium oxalate deposition.

Conclusions: Renal I/R is increased after deposition of crystals in renal tubule, also increasing the inflammation in acute kidney injury. FAPESP, CNPq, Complex Fluids INCT.

P11-0034 ANTI-RAT ANTI-THYMOCYTE GLOBULIN ATTENUATES RENAL ISCHAEMIA-REPERFUSION INJURY IN RATS

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Introduction: Ischaemia-reperfusion injury (IRI) is a complex process. Anti-thymocyte globulin (ATG) is a polyclonal antibody that is known to diminish t-lymphocyte count, and decrease leukocyte adhesion to the endothelium following IR. A beneficial effect of ATG on renal IRI has been suggested.

Methods: Adult male Lewis rats were subjected to left renal ischaemia for 40 min and subsequent reperfusion. Either rATG (10mg/kg) or isotype IgG control were administered intravenously prior to clamping the kidney. Kidneys were retrieved at 48 hours H&E stained kidney samples were analysed, and IRI changes were scored. CD3 lymphocyte counts were measured pre-ATG, and at 48 hours.

Results: The untreated IRI group (A: $n = 10$) and isotype control IgG group (B: $n = 6$) were compared with the ATG group (C: $n = 6$). CD3 counts were significantly depleted in the ATG group.

The mean IRI scores obtained were: A=8.5; B=4.6 and C=1.5.

Student's t-test showed a beneficial effect of rATG compared to the isotype IgG ischaemic control group (P=0.005)

Discussion: r-ATG offered renal protection from IR injury. Further research is underway to characterise the mechanisms of IRI attenuation in this model

P12-0053 COMPARISON OF LONG TERM EFFECT OF THYMOGLOBULIN TREATMENT IN PATIENTS WITH HIGH RISK OF DELAYED GRAFT FUNCTION

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T lymphocyte depletion is one strategy that could reverse the impact of IRI in progression to chronic allograft dysfunction, especially in patients at high risk for DGF. The present work assessed the effect of Thymoglobulin in a population with a high incidence of DGF. A total of 209 transplanted patients were analyzed: 97 in the Thymoglobulin group and 112 in the control group. The main complication was DGF (59.3%), with a similar incidence in both groups (63.9% vs. 55.3%, $P = 0.36$). Acute rejection (AR) incidence was decreased with Thymoglobulin (8.2% vs. 28.5%,$P < 0.001$), but CMV viremia was 3.4 times more frequent in these patients (58.3% vs. 17.1%, $P < 0.001$). One year graft function was significantly better in the Thymoglobulin group (59.2 ± 17.2 vs. 51.8 ± 15.3 ml/min, $P = 0.004$), even when censored by AR (59.7 ± 17.5 vs. 53.3 ± 14.4, $P = 0.023$). The same difference was observed at the 2-yr follow up ($P = 0.024$), even when censored by AR ($P = 0.045$). The multivariate analysis showed Thymoglobulin to be a factor strongly associated with graft function protection ($P = 0.039$). Despite not reducing the incidence of DGF, induction with Thymoglobulin significantly lowered the incidence of AR and showed a long-term profile of protection for renal graft function, regardless of reduction in AR.

ADDITIVES TO PRESERVATIONS SOLUTIONS

P13-0075 COMPARATIVE STUDY BETWEEN SCOT-15® AND PERFADEX® AS LUNG PRESERVATION SOLUTIONS

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Objectives: SCOT-15® is a low K+ solution clinically used in kidney, pancreas and liver transplantation. SCOT-15* is including polyethylene glycol (PEG) as a colloid for protection of vascular endothelium. PEG was demonstrated to have ''immunocamouflage'' properties. The aim of this study was to assess the properties of SCOT-15® for lung preservation in comparison with Perfadex® as golden standard solution.

Methods: Two groups with 6 pigs each were compared. After 2L cold pulmoplegia with either Perfadex® [P] or SCOT-15® [S], lungs were stored cold for 4 hours. Peripheral lung biopsies were taken before and after pulmoplegia for High Resolution Magic Angle Spinning (HRMAS) detection of colloid diffusion in the lungs. Left lung function was assessed in an ex vivo lung perfusion and ventilation model. Pulmonary artery flow and pressure were recorded to calculate pulmonary vascular resistance (PVR). Mean airway pressure (mAwP) was monitored as a surrogate for lung compliance. Blood gases were taken on the perfusion outflow line to measure partial oxygen pressure (PO_2). Wet-to-dry weight ratio (W/D) was recorded as a marker of lung edema.

Results: PVR was significantly lower in [S] compared to [P] (846 ± 70 vs. 2063 ± 633 Dynes.sec.cm-5, $P = 0.04$). There were no differences in PO_2 (232 ± 24 [S] versus 258 ± 18 mmHg [P]; $P = 0.13$), mAwP ($P = 0.24$), and W/D ($P = 0.06$). HRMAS spectra of lung biopsies collected before and after flushing showed presence of PEG in end-peripheral lung tissue in [S] while dextran was never detected in [P].

Conclusion: Lungs preserved for 4 hours with SCOT-15® have lower vascular resistance with comparable oxygenation capacity and compliance reflecting well preserved endothelial function. This could be explained by a better diffusion of PEG in the lung tissue. Further experiments with longer cold ischemia are needed to assess the clinical relevance of this new solution.

P14-0068 NAKED CASPASE-3 SIRNA IS EFFECTIVE IN COLD PRESERVATION, BUT NOT IN AUTO-TRANSPLANTATION OF PORCINE KIDNEYS

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Background: Caspase-3 is associated with apoptosis and inflammation and is up-regulated by ischemia reperfusion injury, which plays a key role in renal allograft survival. The efficacy of caspase-3 siRNA administered into the porcine kidney and hemoperfusate during cold preservation has been proved in an isolated organ perfusion system, but not in an auto-transplant model.

Methods: Left kidneys were retrieved from mini pigs with minimal ischemia and flushed with Ringer's solution followed by University of Wisconsin (UW) solution. UW solution with or without 0.3 mg caspase-3 siRNA was infused into the renal artery with the renal artery and vein clamped during 24 hours cold storage (CS). After right nephrectomy, the preserved left kidney was autotransplanted into the right renal bed for 2 days without siRNA systemic treatment.

Results: Caspase-3 mRNA was down-regulated in post-CS kidneys by siRNA, but up-regulated in post-transplant kidneys (both $P < 0.05$). Caspase-3 precursor was down-regulated by 52% in post-CS kidneys preserved with siRNA ($P < 0.01$), whereas 17 kD active caspase-3 was raised 1.5-fold in post-transplant kidneys ($P < 0.05$) with further decreased precursor ($P < 0.01$). In addition, active caspase-3+ cells, apoptotic cells, and myeloperoxidase+ cells were raised 1.3, 2 and 10 folds respectively in siRNA treated transplant kidneys. Moreover, serum creatinine and blood urea nitrogen were not significantly changed by siRNA, but renal tissue damage was significantly aggravated by 20%.

Conclusions: Naked caspase-3 siRNA infused into the kidney was effective in preservation, but not enough to improve post-transplant renal function. These findings may be due to systemic complementary responses overcoming the local effects of short-term siRNA.

P15-0055 ORGAN PRESERVATION REVISITED: ANTICOAGULATION RESCUES BORDERLINE KIDNEY GRAFTS

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Static organ preservation needs to be improved for optimal use of marginal donors. We previously demonstrated the role of the coagulation pathway and specific anticoagulants in ischemia reperfusion injury (IRI). We tested a new inhibitor with a unique anticoagulant profile (Compound 1) for its effectiveness in organ preservation.

Compound 1 was evaluated *in vitro*, mimicking organ preservation using endothelial cells, the first cells affected by IRI, and *in vivo* in an auto-transplanted pig kidney model. Kidneys were subjected to 60 min warm ischemia prior to collection and preservation during 24 hours in static conditions, using University of Wisconsin (UW) solution with standard heparin (UW+UFH) or Compound 1.

In vitro, addition of Compound 1 during hypoxia/hypothermia in UW solution, allowed the cells to have better survival (LDH measurement). *In vivo*, kidneys preserved with UW+UFH showed difficult function recovery, with only 5 animals over 11 recovering function at day 7. No animals were lost in the Compound 1 group, kidneys showed early recovery of function compared to UW+UFH group, with swifter return to pre-transplant levels in regards to serum creatinine. The 3 month follow up showed critical benefits of Compound 1 over UFH: survival was increased from 27% to 100%.

In this model, targeting the coagulation pathway with compound 1 during kidney preservation provided critical benefits for the graft both on the acute and chronic outcomes. The unique anticoagulant profile of Compound 1 makes it highly effective in organ preservation, with a high potential for translation to the clinical situation. Indeed, such a molecule, easily added to a preservation protocol in the clinic, would be invaluable to help face the decreasing quality of transplanted organs.

P16-0039 EFFECT OF CARDIOTROPHIN-1 ON THE TISSUE INJURY ASSOCIATE TO COLD ISCHEMIA AND PRESERVATION OF THE HEART AND LUNG

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Ischemia-reperfusion (I/R) injury occurs when blood flow is restored after prolonged ischemia. Cardiotrophin-1 (CT-1) is a cytokine of the interleukin-6 family. It has been shown that CT-1 administration protected against warm ischemia/reperfusion (I/R)-induced liver and heart damage in rats and mice. Furthermore, CT-1 null mice were more sensitive to this type of damage. In this work, it has been tested if cardiotrophin-1 addition to the perfusion and preservation solution prevented heart and lung damage associate to cold ischemia and preservation.

The study has been performed in Wistar rats. The heart-lung block was perfused and preserved with University of Wisconsin solution (UW) with or without 0.2 μg/ml of cardiotrophin-1 (UW + CT-1). Preservation times were 0, 0.5, 12, 24, and 48 hours at 4 °C. At the end of the preservation time, several parameters of tissue injury and inflammation such as release of tumor necrosis factor alpha (TNF-α) to the preservation solution, superoxide anion (SOA) levels, iNOS and ICAM-1 expression, and NFκB activation were assessed.

Release of tumor necrosis factor alpha (TNF-α) to the preservation solution was lower in UW + CT-1 than in UW groups. Superoxide anion (SOA) levels in lung and heart tissue was lower in UW+ CT-1 than in UW groups. Preservation of lung and heart tissue with UW alone induced a marked increase of inducible nitric oxide synthase (iNOS) and intercellular adhesion molecule 1 (ICAM-1) expression and nuclear transcription factor (NFkB) activation, and this effect was markedly lower when organ were preserved in CT-1containing UW. GP130 expression increased was higher in the CT-1 than in control group, until day 14 after transplantation.

In conclusion, CT-1 may modulate superoxide anion (SOA) production, TNF-α release, iNOS and ICAM-1 expression and NFkB activation and ICAM-1 in both lung and heart during cold preservation.

P17-0042 PRELIMINARY STUDY OF AMNIOTIC FLUID STEMS CELL THERAPY IN KIDNEY TRANSPLANTATION

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Introduction: Interest in stem cell therapy is growing in regenerative medicine, particularly in the field of ischemic pathologies. Among the different sources of stem cells, Amniotic Fluid derived Stem Cells (AFSC) seem to be

promising despite the lack of full characterization. Before investigating the role of AFSC therapy in kidney graft outcome, we explored the impact of Hypoxia/Reoxygenation (H/R) sequence on AFSC's viability, apoptosis and HIF-1α pathway activation.

Materials and methods: The amniotic fluids of two different piglets were recovered from the caesarean of a unique Large White sow. AFSC were isolated in specific medium (Amniochrome II) and characterized using cell surface markers expression. AFSC were cultured until 75% of confluence and incubated with an organ preservation solution (Viaspan®) for 24 hours in wet hypoxia chamber at 4 °C. Then, the cells were reoxygenated for 24 hours at 37 °C in Amniochrome II. Cell viability was evaluated by the XTT test and ATP quantification. Cell death was assessed by extracellular LDH release and AnnexinV staining. HIF-1α pathway was also investigated. AFSC's response to H/R was compared to the Primary Renal Endothelial Microvascular cells (PrEMV) response in the same conditions.

Results: Both isolated-AFSC had different cellular shapes. The first line, called AFSC I, was round shaped, whereas the second, AFSC II, was spindle-shaped. Nonetheless, both had the same pattern of membrane marker expression: CD105-, CD 31- and CD 29+, and expressed at a very low level cKit. Both lines were sensitive to H/R compared to PrEMV, as shown by the striking diminution of XTT cleavage activity and intracellular ATP concentration, the increase of LDH release and AnnexinV staining. AFSC II appeared more sensitive than AFSC I. Moreover, AFSC I exhibited a lower expression of HIF 1α and VEGF compared to AFSC II in basal conditions.

Conclusion: We isolated two cell lines of AFSC from two piglets of the same litter. Despite a high sensitivity to H/R, the AFSC from two different animals had a distinct response to H/R in terms of cell viability, apoptosis and activation of HIF-1α pathway. This characterisation is a major step to determine the best moment to inject AFSC in the transplantation process in order to optimize their therapeutic potential in kidney graft outcome.

P18-0048 A PILOT, RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUPS, CLINICAL TRIAL TO INVESTIGATE THE EFFICACY AND SAFETY OF CARDIOTROPHIN-1 (CT-1) IN KIDNEY TRANSPLANTATION

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Digna Biotech is planning to conduct a clinical trial among 5 Spanish hospitals.

The general objective of this study is to determine the efficacy and safety of CT-1, added to the perfusion solution during cold ischemia, in the prevention of I/R injury, i.e., to determine if CT-1 administration in the kidney transplant graft is able to improve the graft function recovery after kidney transplantation from deceased expanded criteria donors. To this end it is planned to enroll a total of 40 patients (kidney recipients) who will be randomised (1:1) to UW containing CT-1 or placebo. Duration of the study is estimated in 12 months to include all patients and perform all efficacy and safety evaluations. Each patient will be followed-up to one month after transplantation.

Transplantation-associated I/R injury of cadaveric renal allografts drives DGF and results frequently in acute graft loss and/or chronic rejection. Renal transplants with DGF and acute rejection have a greater incidence of chronic dysfunction later. A way to limit kidney damage could be to ameliorate injury from cold storage through donor graft pre-treatment. Preclinical studies performed by Digna in different models of kidney damage provide evidence that kidney pre-treated with CT-1 could benefit the graft in the short-term and, consequently, in the overall survival of the transplanted kidney. The development program in kidney transplantation is based in the extracorporeal administration of CT-1 to graft, ameliorating any risk related with CT-1 patient exposure.

Primary and secondary objectives are as follows:

Primary objective: Assess the effect of CT-1 on kidney function after transplant through the incidence of Delayed Graft Function (DGF), measured as a failure of the serum creatinine to decrease by at least 10% daily on 3 consecutive days during the first week after transplant, irrespective of dialysis requirements, but discounting creatinine decrements because of dialysis itself.

Secondary objectives:

1) Assess the effect of CT-1 on kidney function after transplant through: DGF rate, defined as the need of dialysis during the first week after transplantation. Glomerular filtration rate post-transplant. Incidence and severity of biopsy-proven acute rejection within 30d post-transplant. Serum creatinine levels post-transplant. Levels of serum and urine biomarkers. Anatomopathological assessment of transplanted kidneys before treatment administration and there after 2) Asses economic impact of CT-1 through the days of hospitalization after transplant 3) Asses safety of CT-1

MACHINE PERFUSION

P19-0073 ANALYSIS OF PRESERVATION SOLUTION (KPS AND UW) WITH HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY DURING COLD PRESERVATION: COMPARISON OF METABOLITC CONTENT DURING COLD ISCHEMIA WITH MACHINE PERFUSION AND STATIC STORAGE.

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Introduction: Preservation conditions of kidney graft before transplantation play a crucial role on graft outcome. Nuclear Magnetic Resonance (NMR) spectroscopy NMR is a non invasive method which allows a direct quantitation of compounds in preservation solutions in order to find pronostics biomarkers of a better recovery function. This preliminary study is a comparison of metabolic content of preservation solutions during cold ischemia with machine perfusion (MP) or static storage.

Methods: We worked on an auto-transplantation model on Large White pig mimicking deceaded after cardiac arrest donor. 60 min of warm ischemia were followed by 24 hours of preservation at 4° C in static condition or with MP. We used KPS or UW solutions (n = 3). Perfusates samples at T = 0 and 24 hours were analysed with a NMR spectrometer Bruker 500 MHz.

Results: NMR analysis of preservation solution showed an evolution of numerous metabolites. Among them, the glutathione, a redox cellular buffer, decreased over time when using MP. In the same time, we observed the inverse evolution regarding glutamate and glycine. In static preservation, glutathione concentration did not significatively change. We also observed a increase of lactate or TMAO whatever the preservation type. Lactate increase was significatively higher with MP and testified the establishment of anaerobic metabolism during cold ischemic time.KPS or UW, did not show difference whatever the preservation type.

Conclusion: Those preliminary NMR results allow a rapid comparison of numerous metabolites in one experiment during preservation. It could give informations of kidney state during preservation, especially when using MP. We have to correlate those results with graft outcome in order to consider NMR as a pronostic tool.

P20-0052 HYPOTHERMIC MACHINE PERFUSION OF HUMAN LIVER GRAFTS: COMPARISON OF SINGLE VS DUAL VESSELS PERFUSION

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Background: Hypothermic machine perfusion (HMP) has shown superior results to conventional cold storage method in kidney preservation.

Preliminary data has suggested that hypothermic machine perfusion may be beneficial for human liver grafts. Whether perfusion of both hepatic artery and portal vein is required has not been addressed.

Methods: Sixteen human livers which were declined for clinical use were randomised to 7 hours cold storage followed by one hour of machine perfusion via the hepatic artery alone (group 1;n = 4), portal vein alone (group 2;n = 4), dual perfusion through hepatic artery and portal vein (group 3;n = 4), or no machine perfusion (cold storage group; group 4;n = 4)..Livers were perfused at 4 to 8 degree using Belzer MPS (KPS-1). ALT and AST was measured in the perfusate every 30 min. Histology and electron microscopy of 3 liver biopsy samples were compared for Ischemia reperfusion injury before and after HMP.

Results: There was no damage to the hepatocytes and sinusoidal endothelium as determined by light and electron microscopy in both single and dual perfused livers.

The transaminase levels in the perfusion solution were significantly elevated after machine perfusion but levels were not significantly different between single and dual perfusion.

BRAIN DEAD DONORS

P21-0018 MNTMPYP, A SELECTIVE SUPEROXIDE DISMUTASE MIMETIC, REDUCES OXIDATIVE STRESS IN BRAIN DEAD DONORS

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Brain dead-derived kidney grafts have inferior transplantation outcomes compared to living donated kidneys. A major factor leading to inferior kidney quality is oxidative stress resulting from the diminished organ perfusion and increased neutrophil influx during the brain death period.

We investigated whether MnTMPyP, a selective superoxide dismutase mimetic, can reduce oxidative stress and improve kidney donor quality in an in vivo brain death rat model.

Male F344 rats (275−300 g,n = 14) underwent slow induction of brain death and were kept brain dead for 4 hours We administered MnTMPyP (5 mg/kg ip) or a saline vehicle 30 min prior to brain death induction. Sham-operated animals (n = 14) received the same treatment.

Plasma malondialdehyde (MDA) levels in MntMPyP-treated brain dead rats were lower compared to saline-treated controls ($P < 0.017$), indicating decreased lipid oxidation. Remarkably, plasma MDA levels of MnTMPyP-treated brain dead rats were comparable to sham-operated rats. Highly increased numbers of infiltrating PMNs were found in brain dead rats which was not counteracted by MnTMPyP.

These results suggest that MnTMPyP effectively removes oxygen radicals formed during the brain death period, despite the high influx of oxidative radical producing PMNs.

To further explore the benefits of MnTMPyP on organ quality, we want to assess morphological damage in kidney tissue, serum creatinine, and mRNA levels of KIM-1, BAX/Bcl-2, E-selectin, HO-1, and IL-6.

P22-0049 ROLE OF BIOCHEMICAL- AND HISTOLOGICAL-BASED SCORES FOR THE PREDICTION OF GRAFT FAILURE AFTER LIVER TRANSPLANTATION

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Background and aims: Ischemia reperfusion injury (IRI) is a complex phenomenon often encountered in surgical practice. In transplantation, this type of damage may significantly affect the evolution of the implanted graft. The purpose of this study is to analyze the role of pre- and immediately post-operative scores in a cohort of liver transplant recipients with the intent to find the best prognostic index for graft survival.

Methods: A retrospective analysis of 88 patients transplanted from January 2004 to December 2010 in Rome ''La Sapienza'' center was performed. All the patients underwent a protocollar biopsy after reperfusion (1 hour after arterial declamping).

The following indexes were adopted for the study: Model For End-Stage Liver Disease (MELD)> 15, initial poor graft function (IPGF) according to Gonzalez et al. (1994), IPGF according to Nanashima et al.(2002), donor risk index (DRI)> 1.35, and Suzuki score> 6.

Results: at univariate logistic regression analysis IPGF according to Nanashima was the strongest predictor for graft failure (p value 0.003, odds ratio 3.29), followed by IPGF according to Gonzalez (p value 0.007, odds ratio 3.01) and Suzuki score > 6 (p value 0.04, odds ratio 2.80). DRI> 1.35 and MELD score> 15 were not significant (p value 0.17 and 0.45, respectively).

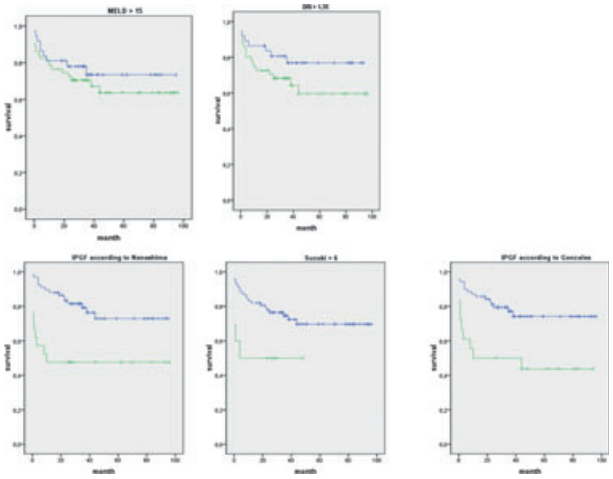
At multivariate analysis, only IPGF definition according to Nanashima resulted an independent risk factor for graft failure (p value 0.003, odds ratio 3.29), showing its superiority respect to the other indices.

Similar data were also showed by Kaplan-Meier analysis, in which graft survival curves were obtained for the 5 variables (Figure 1).

Conclusions: In the present study, parameters related to the pre-transplant donor and recipient clinical condition (namely, MELD and DRI) failed in their intent to predict graft failure. Post-reperfusion damage investigated by Suzuki score could represent an efficacious parameter for post-transplant patient and graft evaluation.

However, transaminases behaviour evaluated within the first days after transplant consented to obtain the best prognostic index. A possible combination of these biochemical and histological parameters can increase this prognostic ability: a larger cohort is required for better define this new scoring system.

Figure 1. Graft survival according to the 5 analyzed variables: months from liver transplantation.



DONORS AFTER CARDIOCIRCULATORY DEATH

P23-0022 EARLY URINARY BIOMARKERS OF WARM AND COLD ISCHAEMIC INJURY IN AN EXPERIMENTAL KIDNEY MODEL

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Introduction: Early urinary biomarkers may be useful in determining the severity of ischaemic injury in donation after circulatory death (DCD) kidneys. The aim of this study was to evaluate the efficacy of a collective series of urinary biomarkers in relation to the warm and cold ischaemic intervals.

Methods: Porcine kidneys were retrieved after 0, 10 and 25 min of warm ischaemia (WI) then preserved by static cold storage (CS) for periods of 2 and 18 hours. After preservation, kidneys were reperfused on an isolated organ perfusion system to assess renal function and injury. Levels of IL-6, TNFα, endothelin-1 (ET-1) and neutrophil gelatinase-associated lipocalin (NGAL) were measured in urine samples after 3 hours of reperfusion.

Results: There was no significant difference in renal functional parameters or urinary biomarkers between the WI times when kidneys were stored for 2 hours ($P > 0.05$). After 18 hours CS, kidneys with 10 and 25 min of WI demonstrated a significant decline in renal function compared to kidneys without WI ($P < 0.05$). Levels of ET-1 and NGAL were significantly higher in kidneys with 25 min WI (ET-1 25 m, 30.1 ± 21.2, vs. 0 m 2.25 ± 1.5 pg/ml;$P = 0.002$: NGAL, 25 m 77 ± 51 vs. 0 m 10 ± 0.1 pg/ml;$P = 0.005$). Levels of IL-6 and TNFα, were significantly higher in kidneys with 10 and 25 min of WI ($P = 0.001$, 0.001) respectively.

Conclusion: Early urinary biomarkers are a useful means to determine graft injury. ET-1 and NGAL are more accurate in predicting the severity of ischaemic injury compared to inflammatory markers.

P24-0058 EVALUATION OF CARDIAC GRAFTS FROM NON-HEART-BEATING DONORS: EARLY REPERFUSION HEMODYNAMIC PARAMETERS PREDICT CONTRACTILE RECOVERY IN ISOLATED RAT HEARTS

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Background: The gap between supply and demand for donor hearts limits cardiac transplantation. Non-heart-beating donors (NHBDs) are a potential source of additional donor organs; however, these organs undergo a period of warm ischemia, which may lead to irreversible injury and preclude transplantation. With NHBDs, evaluation of cardiac graft suitability for transplantation may not be possible until the time of procurement. We thus aimed to identify means to predict cardiac recovery prior to transplantation.

Methods: Hearts ($n = 31$) harvested from male Wistar rats were aerobically perfused with Krebs-Henseleit buffer in working-mode for 20 min, subjected to global, no-flow ischemia for 30, 50, 55 or 60 min at 32 °C and reperfused. During reperfusion, hearts were initially perfused in an unloaded-mode for 20 min and subsequently in loaded working-mode for 40 min. Hemodynamic parameters were assessed with the aid of an intraventricular micro-tip pressure catheter.

Results: After 60 min reperfusion, percent recovery of LV work (developed pressure-heart rate product) ranged from 5 to 90%. Several hemodynamic parameters measured during early, unloaded reperfusion correlated significantly with LV work after 60 min reperfusion ($P < 0.001$). Early reperfusion measures of heart rate (HR), developed pressure (DP) and end-diastolic pressure were used to generate a composite, weighted predictive parameter, which was evaluated against the LV work after 60 min of reperfusion. Effective discriminating ability for this novel parameter was observed for four 60 min reperfusion LV work cut-off values ($P < 0.01$).

Conclusions: Hemodynamic parameters measured during early reperfusion may be used as predictors for subsequent contractile recovery. This simple and rapid approach could be of use in the development of a clinical protocol to evaluate suitability of NHBD cardiac grafts prior to transplantation.

P25-0038 DUAL KIDNEY TRANSPLANTATION FROM UNCONTROLLED DECEASED DONORS AFTER CARDIAC ARREST (UDDCA)

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Kidney grafts from uDDCA has been used in France to counteract the organ shortage. Some kidneys from uDDCA remained unsuitable for solitary transplantation and may be considered for dual kidney graft (DKG) transplantation. The aim of the study was to evaluate graft survival and outcomes in single (SKG) and DKG.

Five patients received a DKG and 24 patients a SKG from uDDCA. All kidneys were placed on MPP (pulsatile perfusion machine, RM3) and all patients received the same immunosuppressive treatment. Estimated MDRD and inuline clearance were analysed until 36 months after transplantation and systematic biopsies were performed at M3 and M12 to evaluate the chronic interstitial fibrosis (IF) by colour image quantification.

Our experience with RM3 perfusion machine leads us to perform a SKG when the resistance index (RI) is lower than 0.4 after 6 hours of perfusion and a DKG when the RI is between 0.4 and 0.6.

Donor's and recipient's characteristic (mean age, gender), mean numbers of HLA mismatch were not significantly different. The mean duration time of MPP and the mean warm ischemic time were higher in the DKG than in the SKG group (1319 vs. 689 min, $P = 0.05$ and 105 vs. 121 min, $P = 0.017$). Patient and graft survivals were 100% in both groups. PNF (not observed in our cohort) and DGF rate were not statistically different between the 2 groups (100% vs. 78%). Acute rejection rate was not different between the groups. Graft outcomes and IF results are reported table 1.

Table 1 : Evolution of graft function and histology

	DKG	SKG	*P*
eGFR M3	37.0 (6.8)	38.6 (7.6)	0.70
eGFR M12	42.0 (6.9)	44.3 (4.1)	0.81
eGFR M24	45.0 (3.3)	45.9 (14.0)	0.96
eGFR M36	40.3 (15.5)	45.1 (14.0)	0.52
m GFR M12	43.6 (5.0)	44.4 (13.0)	0.94
m GFR M36	36.7 (16.0)	42.5 (12.0)	0.60
IF score M3	37.8 (9.2)	29.2 (7.6)	0.25
IF score M12	34.6 (12.8)	36.2 (13.4)	0.92

eGFR : estimated by MDRD formula, m GFR : measured by inulin clearance, IF : interstitial fibrosis. All data are expressed as mean (SD).

Results between SKG and DKG are not significantly different. Kidneys from uDDCA with high resistance index should not be discarded and could be used as DKG with acceptable results.

P26-0065 PROLONGED COLD ISCHAEMIA POTENTIATES THE MITOCHONDRIAL DAMAGE THAT OCCURS DURING WARM ISCHAEMIA IN RAT KIDNEYS

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Introduction: Kidneys donated after cardiac death (DCD) undergo a variable period of warm ischemia. They have reduced tolerance to prolonged cold storage and ischemia-reperfusion injury and have higher rates of delayed graft function (DGF). As mitochondria are responsible for both oxidative damage and cell survival during ischemia, we investigated mitochondrial damage after variable periods of warm and cold ischemia to elucidate their relative contributions to organ damage prior to transplantation.

Methods: Rat kidneys were exposed to 0, 30, 60 or 90 min of warm ischemic time (WIT) post-mortem followed by cold storage in University of Wisconsin solution for 6, 12 or 24 hours. Tissues were subsequently analysed for mitochondrial respiratory function, ATP/ADP, lactate, oxidative damage (protein carbonyls) and mitochondrial DNA (mtDNA) damage and histology.

Results: Increasing WIT significantly reduced mitochondrial respiration capacity, ATP/ADP, mtDNA integrity and increased markers of oxidative damage. Cold storage <6 hours did not potentiate the damage incurred during warm ischemia. Twelve hours cold storage resulted in a significant increase in mtDNA and oxidative damage in kidneys exposed to 60'–90' WIT, but not in those exposed to 0' or 30' WIT. Prolonged cold storage (24 hours) resulted in significant mtDNA and oxidative damage in all kidneys exposed to warm ischemia (30', 60' or 90').

Conclusion: Prolonged warm ischemia in the kidney results in significant functional impairment and damage to mitochondria. Furthermore, increasing cold ischemic time (>12 hours) potentiates the mitochondrial damage incurred during warm ischemia. This may contribute to the increased DGF rates in DCD kidneys.

P27-0005 HEMODYNAMIC STUDY OF THE PORTAL VENOUS SYSTEM IN A MODEL OF WARM LIVER ISCHEMIA/REPERFUSION INJURY IN RATS

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Background: Liver transplantation and resections require temporary interruption of the hepatic blood flow resulting in ischemia/reperfusion (IR) injury.

The hepatic microcirculatory impairment is related to the severity of the IR injury and may be reflected in the liver macro hemodynamic. There is an inverse correlation between hepatic blood flow and the postoperative transaminases. The aim of the study was to evaluate the hemodynamic of the portal venous system after warm liver IR injury.

Methods: Eighteen Wistar rats were divided into 3 groups: I) Control; II) sham: rats submitted to resection of right and caudate liver lobes; III) Ischemia (IR): rats subjected to 60 min of partial warm liver ischemia of left and median lobes, followed by resection of non-ischemic lobes at reperfusion. Four hours after reperfusion rats were anesthetized and submitted to mechanical ventilation. Carotid artery was cannulated for mean arterial pressure (MAP). Mean portal venous flow (MPF) was assessed by a perivascular flowprobe. A micropressure probe was introduced into portal vein to assess the mean portal venous pressure (MPP). At the end blood was collected for AST and ALT analysis.

Results: The mean weigh of rats was 229 ± 18 g. AST and ALT were increased in IR group (6.675 ± 1.687 and 5.793 ± 1.119 U/L) compared to sham (897 ± 303 and 815 ± 433 U/L) and control groups (99 ± 28 and 64 ± 27 U/L), $P < 0.05$. MAP was decreased in IR group (90 ± 17 mmHg) compared to control group (114 ± 9 mmHg), $P < 0.05$; but there is no difference when compared to sham group (106 ± 16 mmHg). MPP was increased in sham group (9.3 ± 1.8 mmHg) compared to control (6.2 ± 1.7 mmHg) and IR (4.5 ± 1.7 mmHg) groups, $P < 0.05$. MPF was decreased in IR group (5.0 ± 2.2 ml/min) compared to sham (12.3 ± 2.1 ml/min) and control (12.2 ± 1.9 ml/min) groups, $P < 0.05$.

Conclusions: This study showed that four hours after warm liver ischemia/reperfusion, the total hepatic blood flow is reduced, demonstrated by the decrease of 60% in mean portal flow and 21% in mean arterial pressure. These changes in the hepatic hemodynamics may be correlated with the severity of the liver ischemia/reperfusion injury. This model can be a tool to evaluate protective strategies against liver IR that impact hepatic macro hemodynamics. (FAPESP 2011/05214-3)

P28-0004 METABOLIC CHANGES IN A MODEL OF WARM LIVER ISCHEMIA/REPERFUSION INJURY IN RATS

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Background: Liver ischemia/reperfusion (IR) is characterized by tissue injury associated with metabolic derangements as occurs in Liver transplantation (LT) and hepatic surgery. We hypothesized that a model reproducing whole liver ischemia may allow studying with more accuracy the metabolic derangements that follows particular clinical scenarios like LT.

Methods: Eighteen Wistar rats were divided into 3 groups of 6 animals: I) Control: normal rats not subjected to liver resection or IR; II) sham: rats submitted to resection of right and caudate liver lobes; III) Ischemia (IR): rats subjected to 60 min of partial warm liver ischemia of the left lateral and median lobes, followed by resection of non-ischemic lobes at beginning of reperfusion. Four hours after reperfusion rats were anesthetized and submitted to mechanical ventilation. The carotid artery was cannulated and arterial blood was collected for analysis of lactate, potassium, glucose, hemoglobin and liver transaminases. Then animals were killed by exsanguination.

Results: The mean weigh of the rats was 229 ± 18 g. AST and ALT were increased in the IR group (6.675 ± 1.687 and 5.793 ± 1.119 U/L) compared to the sham (897 ± 304 and 815 ± 433 U/L) and control groups (99 ± 28 and 64 ± 27 U/L), $P < 0.05$. Glucose was decreased in the IR group (115 ± 30 mg/dl) compared to the sham (224 ± 101 mg/dL) and control (298 ± 115 mg/dl) groups, $P < 0.05$. Lactate was increased in the IR group (24.7 ± 10.2 mg/dl) compared to the sham group (14.8 ± 6.3 mg/dL), $P < 0.05$. There were no differences in potassium and hemoglobin between groups. However the potassium in the IR group tended to be higher than control and sham groups.

Conclusions: This experimental model of warm liver I/R showed that after 60 min of ischemia the hepatic metabolism is reduced. There was a decrease of 40% in the glucose and an increase of 60% in lactate serum levels. This impairment of these metabolic variables was associated with an increase in liver transaminases indicating a correlation with the I/R injury. This model may be useful to study the metabolic hepatic disorders that accompanies liver ischemia/reperfusion. (FAPESP 2011/05214-3)

ORGAN EX VIVO REPAIR

P29-0067 BIOMARKERS ASSESSING WARM ISCHAEMIC INJURY USING AN ISOLATED PORCINE KIDNEY HAEMOREPERFUSION MODEL

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Background: Prolonged warm ischaemia (WI) occurred in marginal kidney donors, together with reperfusion injury determines allograft survival, in which apoptosis and inflammation play crucial roles. There is no single valid biomarker, so far, to assess the degree of injury of kidney donors.

Methods: Porcine kidneys subjected to 7, 15, 25 and 40 min WI and 2 hours cold storage were further perfused with normothermic autologous blood for 6 hours using an isolated organ perfusion system. Caspase-3, caspase-7, apoptosis, inflammation, HSP70, and renal structural changes were examined.

Results: Caspase-3 activity was gradually increased by prolonged reperfusion, with a decrease trend against WI time (WIT). This was verified by raised 17 kD active caspase-3 in post-reperfusion kidneys, elevated 12 kD active caspase-3 and lowered caspase-3 precursor at 7 min WI. Active caspase-7 was doubled by reperfusion and its precursor was decreased at 7 min WI, with a declination against prolonged WIT. Apoptotic cells in tubular and interstitial areas were greatly increased by reperfusion at 7 min WI, but decreased against prolonged WIT. Myeloperoxidase+ cells were dramatically increased by reperfusion and presented as a bell shape against WIT. HSP70 was significantly increased at 7 min WI, but decreased at 40 min WI by reperfusion. In post-reperfusion kidneys, there were tubular dilation and cell shedding at 7 and 15 min WI, tubular vacuolation and cell debris in tubular lumens at longer WIT; and early nuclear pyknosis, tubular cell detachment and peritubular capillary dilatation at 40 min. Furthermore, caspase-3, caspase-7, apoptosis were correlated with renal function, but not MPO+ cells or HSP70.

Conclusions: Haemoreperfusion activates caspase-3 and caspase-7, promotes apoptosis of damaged cells in kidneys with limited WI, which might be beneficial to renal structural remodeling and functional recovery. Caspase-3, caspase-7 and apoptosis appear to be better biomarkers than MPO+ cells or HSP70 for assessing warm ischaemic injury of isolated kidneys.

P30-0051 BACMAM; A NOVEL GENE DELIVERY VECTOR FOR ISCHAEMIA

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Virus vectors offer a unique way to deliver protective genes to organs to ameliorate disease conditions. The *baculoviridae* family comprise a unique group of insect-specific DNA viruses. Over a decade ago, baculoviruses were modified to contain mammalian cell-active promoters upstream of the target gene, now referred to as BacMam vectors. Their non-replicative nature and high safety profile in mammalian cells, low cytotoxicity and ability to accommodate multiple genes or large DNA inserts make them an attractive alternative to mammalian virus vectors such as Adenovirus. This BacMam system has evolved rapidly in recent years and is currently also being used for recombinant expression of therapeutic proteins *in vitro*. Organ transplantation is a field that is becoming increasingly relevant for gene therapy applications. Ischaemia reperfusion injury (IRI) occurs during organ transplantation and is associated with hypoxia, free radical formation and organ failure. Previous studies by other workers suggested the possibility to ameliorate the effects of IRI by introducing anit-oxdidant, anti-apoptotic and cytoprotective genes using virus vectors. In this study BacMam was tested as a novel vector for delivery of mitochondrial manganese superoxide dismutase (*Mnsod*), *bcl-2* and heme-oxigenase 1 (*ho-1*) to ameliorate IRI in human kidney cells. An *in vitro* ischaemia model was developed and optimized in human kidney cells using Antimycin A, mitochondrial respiration inhibitor, in combination with a non-metabolizable 2-deoxyglucose and calcium inophore. BacMam viruses containing a **cytomegalovirus (CMV)** immediate-early promoter **upstream of** targets gene were constructed and the protective effect of the target gene on IRI was investigated *in vitro*.

P31-0062 ESTABLISHING A LIVER EX-VIVO NORMOTHERMIC PERFUSION MODEL: LESSONS LEARNED

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Introduction: Normothermic *ex-vivo* perfusion of the liver represents an emerging preservation modality in the transplantation field. While this technology has been applied with exceptional results in clinical lung transplantation, it is still evolving in animal liver models. Unique challenges presented by the liver are 1) Dual inflow, in which the arterial and portal venous system are subjected to completely different flows and pressures. 2) Lack of animal studies aimed to investigate the principles behind this preservation modality (ideal temperature of perfusion, type of perfusate that best maintains the organ closest to its physiological state, etc). Aim of this study is to describe the lessons learned in the establishment of our *ex-vivo* normothermic liver perfusion model as well our preliminary results with different types of perfusates.

Materials and methods: Between July 2011 and January 2012, 8 porcine DCD livers underwent normothermic *ex-vivo* perfusion after 1 hour of WIT. The first 5 livers were utilized to establish a stable and reproducible model able to provide with physiological flows and pressures in the hepatic artery, portal vein and hepatic veins. These 5 livers were crucial in elucidating the challenges related to the establishment of a normothermic perfusion machine as well as the importance of CVP and arterial perfusion in the function of the organ. Furthermore, we were able to modify the machine and obtain an easy to set up and adjust apparatus. The last 3 livers were utilized to test different perfusates: 1) whole blood, 2) Steen solution and 3) Steen with O2 carrier (red cells).

Results: Functional markers as well as histological impact of the three perfusates are reported in Figure 1 and Table 1.

Conclusions: This study provides us with valuable information regarding the establishment of a normothermic *ex-vivo* liver perfusion system and could simplify other researchers' attempt to set up a similar model. Furthermore it emphasizes the importance of research aimed to investigate the principles behind this preservation modality.

PRE/POST CONDITIONING/PRETREATMENT

P32-0006 EFFECTS OF ANAESTHETIC PRECONDITIONING 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. We hypothesized that pharmacological preconditioning with sevoflurane 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 animals: I) IR: rats subjected to 45 min of warm liver ischemia of the left and median lobes, followed by resection of the non-ischemic lobes at early reperfusion; and II) SEVO+IR: rats were exposed to sevoflurane 2.5% for 15 min, followed by washout during 5 min, before IR. The carotid artery was cannulated for mean arterial pressure (MAP) monitoring. The mean portal venous flow (MPF) was assessed by perivascular flowprobe. MAP and MPF were recorded at baseline, pre reperfusion and 4 hours post-reperfusion. Liver transaminases, creatinine, pH, bicarbonate (BIC) and base excess (BE), potassium (K), glucose and lactate were measured at 4 hours post-reperfusion.

Results: AST and ALT were decreased in SEVO+IR group (10.056 ± 5.830 and 8.586 ± 5.296 U/L) compared to IR group (16.890 ± 1.630 and 13.418 ± 1.088 U/L), $P < 0.05$. BIC, BE and K were increased in SEVO+IR group (12.42 ± 4.39, -14.72 ± 4.46 mmol/l and 6.3 ± 0.9 mEq/dl) compared to IR (6.70 ± 3.32, -20.48 ± 4.22 mmol/l and 4.7 ± 0.7 mEq/dl), $P < 0.05$. MAP at 4 hours post-reperfusion was decreased in SEVO+IR group (65 ± 24 mmHg) compared to IR (93 ± 14 mmHg), $P < 0.05$. There were no differences in MPF, creatinine, glucose and lactate. Glucose tended to be higher and lactate lower in SEVO+IR group (54.0 ± 22.7 and 42.8 ± 18.6 mg/dl) compared to IR (35.0 ± 18.4 and 66.8 ± 25.9 mg/dl).

Conclusions: In liver IR, sevoflurane preconditioning reduced hepatocellular injury demonstrated by lower levels of transaminases. Despite the lower mean arterial pressure presented in sevoflurane treated animals, no detrimental effect was observed in portal venous flow, hepatic metabolism and renal function. This study highlight the need for clarifying the mechanisms of sevoflurane preconditioning, and if there is additional hepatoprotection against cold IR injury. (FAPESP 2011/05214-3)

P33-0028 OZONE POSTCONDITIONING IN RENAL ISCHAEMIA-REPERFUSION MODEL. FUNCTIONAL AND MORPHOLOGICAL EVIDENCES

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Background: Ischaemia-reperfusion is one of the main causes of kidney complications. The most frequent lesion is acute tubular necrosis. Ozone oxidative preconditioning exerts a modulatory effect of redox state of renal cells in models of ischaemia-reperfusion, by stimulating endogenous antioxidant mechanisms. Similar results have been obtained in more recent studies using ischaemic postconditioning.

Objectives: To evaluate the effect of ozone oxidative postconditioning on renal function and morphology in an ischaemiareperfusion rat model.

Methods: We used forty female Wistar rats weighing between 150 g−200 g randomly divided into 4 groups (negative control, positive control, oxygen and ozone). The groups: positive control, oxygen and ozone were subjected to 60 min of ischaemia and 10 days of reperfusion. During reperfusion, the oxygen group was given 26 mg/kg body weight of oxygen, and the ozone group 0.5 mg/kg body weight of ozone, rectally. At the end of the experiment urine and blood samples were taken for renal function tests, left nephrectomy for studies of oxidative stress in homogenate and right nephrectomy for histological study was performed.

Results: The ozone group showed no significant differences for filtration fraction and proteinuria compared to the negative control group. The glomerular filtrate rate, renal plasma flow and creatinine showed a slight improvement in comparison with oxygen and Positive Control groups. In the biochemical tests in the group Ozone enzyme activity of superoxide dismutase and catalase showed the highest values and decreased lipid peroxidation. In the Oxigen group no significant differences with respect to Positive Control group were observed. Ozone group showed significantly lower overall histological damage than the Positive Control and Oxygen groups.

Conclusions: Ozone postconditioning had a protective effect on the preservation of renal function, increased antioxidant capacity and preserved renal morphology.

P34-0063 INTERMITTENT SELECTIVE CLAMPING ENHANCES LIVER TOLERANCE TO ISCHEMIA/REPERFUSION: A POWERFUL PROTECTIVE MODALITY IN THE FIELD OF LIVING-RELATED LIVER TRANSPLANTATION

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Background: Donor safety is undoubtedly the highest priority for living-related liver transplantation (LDLT). An effective protective technique would be very attractive to decrease the risk of surgery in the healthy donor. Here we used a rat model of partial hepatectomy (70%) to analyse the effect of intermittent (total or selective) clamping on the hepatocellular damages and liver regeneration. Methods: Sprague-Dawley rats were divided into 4 groups. In the intermittent total clamping group (ITC) left lateral and median lobes to be resected were exteriorized and entire hepatic pedicle was subjected twice to 10 min of ischemia followed by 5 min of reperfusion (I/R10-5) prior to hepatectomy. In the intermittent selective clamping group (ISC), lobes to be resected were exteriorized and subjected twice to I/R10-5 prior to hepatectomy. Sham and standard hepatectomy groups were used as controls. At different time points after hepatectomy, blood and tissues were collected for subsequent analysis.

Results: ISC significantly attenuated hepatocellular injury (LDH), oxidative stress (MDA production, catalase and SOD activities), mitochondrial damage (cytochrome C and caspase 9 activities) and apoptosis (caspase 3 and caspase12 activities). ISC also significantly protected against ER stress by reducing GRP78, GRP94, CHOP, XBP1, TRAF2 expression as well as the activation of three pathways of unfolded protein response (PERK, IRE-1 and ATF6) compared to ITC. The protective effect of ISC was also associated with an improvement in liver regeneration (BrdU and mitotic index), as well as ATP recovery. Conclusion: ISC triggers protective mechanisms against stress response associated with liver resection and improves liver regeneration. This procedure could be applied as a powerful protective modality in the field of LDLT.

P35-0012 THE EARLY PROTECTIVE EFFECT OF ISCHEMIA PRECONDITIONING AND GLUTAMINE PRETREATMENT IN RENAL ISCHEMIA-REPERFUSION INJURY OF RAT

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Heat shock protein (HSP) is one of the main stress proteins, which help to protect cells against stress. The hypothesis of this rat model study is that pretreatment with glutamine (Gln) and ischemia preconditioning (IPC) increase the expression of HSP, that is attenuates the renal ischemia-reperfusion (I/R) injury Sprague−Dawley (SD) rats were randomized into four groups [group I: Gln injection (+), IPC (+); group II: Gln injection (+), IPC (-); group III: saline injection (+), IPC (+); group IV: saline injection (+), IPC (-)]. In the Gln group, 3% glutamine (0.75 g/kg) was administered 12 hours before I/R injury. IPC underwent a 15 min period of ischemia followed by a 10 min reperfusion before warm renal ischemia induced by clamping the renal pedicle for 45 min. Renal HSP 70 expression was determined by Western blotting and kidney function was assessed by serum BUN/Cr. Renal cross sections were microscopically examined for tubular necrosis, exfoliation of tubular epithelial cell, cast formation, and monocyte infiltration. Gln pretreatment increased the intra-renal HSP expression ($P = 0.031$). But there was no statically significant association between IPC and HSP ($P = 0.088$). The Group I showed some ischemic tubulointerstitial abnormalities, which were clearly slighter than those in other groups ($P = 0.00$). After I/R injury, serum Bun/Cr were not different among the four groups. Gln pretreatment increased the intra-renal HSP expression ($P = 0.031$). But there was no statically significant association between IPC and HSP ($P = 0.088$). The Group I showed some ischemic tubulointerstitial abnormalities, which were clearly slighter than those in other groups ($P = 0.00$). After I/R injury, serum Bun/Cr were not different among the four groups.