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

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

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

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

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

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

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

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

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

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

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

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

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

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

