OS003 IPC REDUCES IRI IN THE RAT KIDNEY BY REPRESSING ITS UNIQUE MICRORNA EXPRESSION PROFILE

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Background: MicroRNAs are important post-transcriptional regulators of gene expression, implicated in many physiological and pathophysiological processes, including kidney disease. Ischaemia reperfusion injury (IRI) is an inevitable consequence of transplantation and results in delayed graft function and primary non-function. The aim of this research was to characterise the role of microRNAs in kidney IRI and their response to the therapeutic strategy of Ischaemic Preconditioning (IPC).
Methods: An *in vivo* model of IRI and IPC was utilised, in which adult male lewis rats underwent surgery and were divided into 3 groups: sham; IRI (45 min bilateral renal pedicle cross-clamping); and IPC+IRI (3 cycles of 2 min ischaemia and 5 min reperfusion, prior to 45 min of IRI). Kidney tissue was retrieved at 48 h and blood samples taken at 0 h and 48 h. Histological, biochemical and mRNA AKI marker analysis was undertaken. MicroRNAs were profiled using Next Generation Sequencing (NGS) and hybridisation arrays, and changes in selected microRNAs confirmed by RT-qPCR.
Results: IRI was characterised by: marked histological damage including acute tubular necrosis and endothelial cell loss; increased serum creatinine; and increased NGAL and KIM-1 expression. In contrast IPC reduced the histology scores, serum creatinine and NGAL and KIM-1 expression. NGS and Microarray analyses identified 18 differentially expressed microRNAs in IRI, which were confirmed by RT-qPCR. This microRNA expression profile was attenuated by IPC, with particular changes noted in 4 microRNAs ((miR-21, -221, and -222, up-regulated in IRI and down-regulated by IPC) and (miR-375-3p, down-regulated in IRI and up-regulated by IPC)).
Conclusion: These data have identified a unique microRNA signature of IRI in the rat kidney, and have shown that pulsatile IPC improved injury by attenuating this microRNA signature. MicroRNAs thus show significant promise as biomarkers of injury and potential therapeutic targets in this context.

OS004 ADMINISTRATION OF MESENCHYMAL STROMAL CELLS BEFORE RENAL ISCHEMIA/REPERFUSION ATTENUATES KIDNEY INJURY AND MODULATES RENAL LIPID METABOLISM IN RATS

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Background: Mesenchymal stromal cells (MSC) have been demonstrated to attenuate renal ischemia/reperfusion (I/R) damage in rodents. The mechanisms of such nephroprotection remain unclear.
Materials and Methods: Male *Lewis* rats aged of 8–10 weeks received tail i.v injection of $1.5x10^6$ MSC in 1 mL saline (MSCD-7, $n = 11$) or saline alone (SD-7, $n = 6$) 7 days before renal I/R. Left renal ischemia (by clamping the renal pedicle) lasted 45 min. Right nephrectomy was simultaneously performed. Blood sample was collected from inferior vena cava 48 h post reperfusion. Renal function was assessed by measuring serum creatinine (SCr) levels. Expressions of inflammatory and apoptotic markers by real-time (RT)-qPCR were comparatively quantified. High-throughput RNA sequencing was applied to MSCD-7 vs. SD-7 non-ischemic right kidneys. Relevant pathways were detected using an Over-Representation Analysis with WebGestalt, and confirmed by RT-qPCR.
Results: Scr levels reached 1.4 ± 0.7 vs. 2.4 ± 0.8 mg/dL in MSCD-7 vs. SD-7 group ($p < 0.05$). MSC infusion significantly reduced mRNA expression of *Casp3, Hsp 70, Kim-1, Mcp-1* and *Il-6* and increased mRNA expression of *Bcl* compared to saline. Among 25 908 genes, 748 were identified as significantly differentially expressed (False Discovery Rate (FDR), <0.05) between MSCD-7 and SD-7 non-ischemic kidneys. Among the most affected metabolic pathways, renal lipid metabolism was significantly altered, with down-regulation of fatty acid biosynthesis and an up-regulation of PPARα pathway in MSCD-7 vs. SD-7 groups. By immunoblotting, PPARα and phosphorylated-PPARα were significantly increased in MSCD-7 vs. SD-7 kidneys, in both non-ischemic and ischemic conditions. Moreover, levels of malondialdehyde-derived lipid peroxidation products were decreased in MSCD-7 ischemic kidneys in comparison to SD-7 ischemic kidneys.
Conclusion: MSC infusion at day 7 prior injury critically impacts renal lipid metabolism, which may condition kidney parenchyma against I/R.

OS005 COLLECTIN-11 PROMOTES RENAL TUBULOINTERSTITIAL FIBROSIS FOLLOWING RENAL ISCHEMIA REPERFUSION

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Collectin-11 (CL-11) is a newly identified collectins of the innate immune system and recently has been suggested to play a pathogenic role in acute kidney injury induced by renal ischemia reperfusion (IR). However, the impact of CL-11 on the late phase of renal IR injury is unknown. In the present study, we investigated whether CL-11 is involved in the pathogenesis of renal tubulointerstitial fibrosis following renal IR and the underlying mechanisms.

We employed a murine model of renal IR injury and CL-11-/- mice to determine the roles of CL-11 in renal inflammation and tubulointerstitial fibrosis. To investigate cellular mechanisms that CL-11 contributes to renal inflammation and the development of tubulointerstitial fibrosis we performed a series of *in vitro* experiments using freshly prepared peritoneal neutrophils or mono/macrophages and primarily cultured renal fibroblasts.

We show that CL-11 deficiency protected mice from the development of tubulointerstitial fibrosis following renal IR. Compared to the wild littermates, CL-11-/- mice had significantly reduced renal fibrosis, as evidenced by reduced renal function impairment, tubular injury, renal leukocyte infiltration (i.e. CD45, neutrophils, macrophages), collagen deposition in the kidney as well as intrarenal gene expression of proinflammatory (TNF-alpha, IL-1beta, IL-6) and profibrotic (TGF-beta) molecules. *In vitro* study showed that CL-11 had potent effects in promoting leukocyte migration and stimulating renal fibroblast proliferation.

Therefore, our findings demonstrate a pathogenic role for CL-11, particularly locally produced, in renal tubulointerstitial fibrosis following renal IR and suggest a novel mechanism for CL-11 in promoting leukocyte chemotaxis and stimulating fibroblast proliferation in renal fibrosis. CL-11 may represent a novel therapeutic target in both the early and late phases of kidney IR injury.

Translational Kidney Ischemia-reperfusion and preservation

OS006 DIRECT COMPARISON OF HYPOTHERMIC AND NORMOTHERMIC MACHINE PERFUSION IN A PORCINE *EX-VIVO* KIDNEY MODEL

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Background: Hypothermic machine perfusion (HMP) is a well established method for deceased donor organ preservation, assessment and preconditioning. Translational studies have shown that normothermic perfusion (NMP) offers similar and perhaps greater advantages. However, data on a direct comparison of the two methods are scarce. Therefore, the aim of this study was to compare the two methods in an *ex-vivo* model using porcine kidneys.
Methods: 16 kidneys from 8 donor pigs retrieved at an abattoir after 25 min of warm ischaemia time were stored on ice for 24 h. They were then perfused hypothermically (4°C, $n = 7$) or normothermically (37°C, $n = 7$) for 4 h using an RM3 pulsatile perfusion machine or left on ice ($n = 2$). Kidneys were reperfused with whole blood for 2 h at 37°C. Physiological parameters e.g. perfusate flow rate, urinary output and oxygen consumption were compared. Levels of IL-1β and NGAL in perfusate samples were measured by ELISA and mRNA expression of TNFα, IL-1β, NGAL and EDN-1 were determined by RT-PCR. Statistical analysis was performed using ANOVA.
Results: Kidneys after HMP showed significantly higher urinary output (5.7 ± 2.26 ml/min vs. 2.15 ± 1.24 ml/min, $p = 0.0048$) as well as oxygen consumption ($p = 0.0032$) and perfusate flow rates ($p = 0.036$) at reperfusion than kidneys after NMP. At mRNA level, expressions of proinflammatory markers were higher for the HMP group, which reached significance for the expression of EDN-1 ($p = 0.03$). IL-1β levels in perfusate samples were similar between the two groups.
Conclusion: In direct comparison to normothermic machine perfusion, hypothermic machine perfusion of porcine kidneys resulted in improved physiological parameters and led to significantly increased urinary output rates despite showing a higher upregulation of inflammatory markers at mRNA level. Further investigations of the physiological and immunological parameters of both preservation methods are needed to optimise outcomes.