TUBULAR ISCHEMIA AND TOXICITY

**TO035 ENDOTHELIAL PROGENITOR CELL-DERIVED EXTRAVASCULAR ECCELLS INHIBIT KIDNEY ISCHEMIA-REPERFUSION INJURY THROUGH THE TRANSFER OF mRNAs CODING FOR COMPLEMENT INHIBITORS TO INJURED TUBULAR EPITHELIAL AND ENDOTHELIAL CELLS**

Vincenzo Cantaluppi1, Davide Medica1, Federico Figliolini1, Stefano Gatti1, Stefania Bruno1, Alessandro D. Quercia1, Sergio Dellepiane1, Luigi Biancone1, Ciro Tetta1 and Giovanni Camussi1

1Nephrology, Dialysis and Renal Transplantation Unit University of Torino Torino Italy, 2Ospedale Maggiore Policlínico Milano Italy, 3Fresenius Medical Care Bad Homburg Germany

Introduction and Aims: Endothelial progenitor cells (EPCs) are bone marrow-derived precursors known to reverse acute kidney injury (AKI) by paracrine mechanisms including the release of extracellular vesicles (EVs), small particles playing a role in intercellular communication through the transfer of proteins and RNAs. Activation of the complement cascade in tubular epithelial and endothelial cells mediates kidney ischemia-reperfusion injury (IRI). The aim of this study was to evaluate whether the protective role of EPC-derived EVs in kidney IRI may be associated with complement inhibition.

Methods: EPCs were isolated from peripheral blood and EVs characterized for size, protein and RNA content. We evaluated the effects of EVs in a rat model of kidney IRI and in vitro in human tubular epithelial and endothelial cells cultured in hypoxia.

Results: EPC-derived EVs sized 60-130 nm and carried different subsets of mRNAs and microRNAs able to modulate cell proliferation and apoptosis. By RT-PCR, we found within EVs mRNAs coding for the complement inhibitors factor H, DAF (CD55) and CD59. After i.v. infusion, EVs localized within peritubular capillaries and tubular cells exerting morphologic and functional protection from AKI by reducing tubular cell apoptosis and leukocyte infiltration. EV administration reduced C5b9 deposition and enhanced the expression of factor H, DAF and CD59 in the ischemic kidney. The nonprotective effect of EVs was reduced after their treatment with RNase able to decrease mRNA expression of all complement inhibitors. In vitro, EVs reduced hypoxia-induced apoptosis of tubular epithelial and endothelial cells by decreasing the deleterious effect of C5b9 activation and by up-regulating the expression of factor H, DAF and CD59, thus confirming the in vivo data. The role of factor H, DAF and CD59 mRNA transfer to injured renal cells was inferred by experiments using RNase-treated EVs or EVs released from EPCs engineered to knock-down all complement inhibitors by specific siRNA.

Conclusions: EPC-derived EVs protect the kidney from ischemic AKI by delivering mRNAs coding for factor H, DAF and CD59 to injured tubular epithelial and endothelial cells. These results confirmed previous data on the relevance of complement inhibition after kidney IRI and suggest the potential use of EPC-derived EVs as therapeutic option to avoid delayed graft function after kidney transplantation.

**TO036 CONDITIONAL DELETION OF MACROPHAGES PROTECTS AGAINST ARISTOLOCIC ACID NEPHROPATHY IN MICE**

Li Zhou1,2, Xiaojie Dai1,2, Mei Feng1, Xiaoru Huang2, Ping Fu1 and Hui Yao Lan2

1Division of Nephrology, Department of Medicine West China Hospital of Sichuan University Chengdu Sichuan Province China, 2CUHK Shenzhen Research Institute, Shenzhen, Department of Medicine and Therapeutics, and Li Ka Shing Institute of Health Sciences CUHK Shenzhen China

Introduction and Aims: Aristoclastic Acid Nephropathy (AAN) is characterized by progressive tubular epithelial cell (TEC) loss and renal scarring, which has a similar manifestation with Balkan endemic nephropathy (BEN). Aristoclastic Acid (AA) has been shown to be a key mediator of Aristoclastic Acid Nephropathy (AAN). TGF-b/Smad3 has been proved to be a key signaling pathway in AAN. Interestingly, our recent studies in a rat macrophage cell line (NR8383) also showed that addition of AA significantly upregulated pro-inflammatory cytokines (IL-1b, TNF-a, MCP-1). Interestingly, progressive tubular necrosis and interstitial fibrosis were also prevented, resulting in 40-50% reduction in collagen I expression and a-SMA myofibroblasts accumulation. Further in vitro studies in a rat macrophage cell line (NR8383) also showed that addition of AA significantly upregulated pro-inflammatory cytokines (IL-1b, MCP-1 and TNF-a) and induced macrophage-dependent fibrotic response including collagen I and a-SMA expression, which was NF-κB dependent since addition of NF-κB inhibitor (Amonium pyrrolidinedithiocarbamate, PDTC) blocked AA-induced inflammation and fibrosis on macrophages.

Conclusions: Macrophages play important role in the development of of AAN. Results from this study suggest that targeting macrophages may represent a novel therapy for AAN.

**TO037 CD40 GENE SILENCING PREVENTS WARM RENAL ISCHEMIA-REPERFUSION INJURY**

Laura de Ramon1, Elia Ripoll1, Leonella Luzardo2, Ana Merino1, Nuria Bolaños1, Nuria Lioberras1, Josep M. Cruçado1, Josep M. Grinyó1 and Juan Torras1

1Nephrology Institut de la Llopatge Barcelona Spain, 2Nephrology Hospital de Clinics Montevideo Uruguay

Introduction and Aims: Ischemia-reperfusion injury has been associated with the incidence of both acute and chronic rejection. Together with the alloimmunome responses it is one of the most important causes of graft loss. Here, we test whether an anti-CD40 siRNA reduces kidney inflammation in a model of rat warm renal ischemia.

Methods: In the present study male Wistar rats were divided in 5 groups. SCR, group treated with scrambled siRNA(n=11); CD40-15, group treated with 15ug of siRNA (n=6); CD40-50, group treated with 50ug of siRNA(n=8); CD40-150, group treated with 150ug of siRNA(n=7) and CD40-500 group treated with 500ug of siRNA(n=6).

The siRNA anti CD40 was administered 1hour before the ischemia. Ischemia was induced by clamping both renal pedicles for 40minutes, followed by reperfusion. Animals were followed up during 48hours.

Results: Compared to scrambled controls, serum urea and creatinine levels were lower in treated groups. The histopathological analysis illustrates a renoprotective effect in those groups treated with higher doses of siRNA. Note that the highest dose of siRNA was the most effective reducing kidney interstitial infiltrate and tubular lesions.

Analysis of kidney gene expression showed that there was no activation of innate immunity (TLR3) due to the siRNA molecule itself, and that the siRNA reduced CD40 and also, proinflammatory cytokines such as IL4, IL2 and NFKB in treatment groups. Interstitial monocyte infiltrate (CD68+) showed a reduction in those kidneys with higher doses of siRNA treatment. An additional study with ICR mice using only the 50ug dose showed similar functional and structural protection.

Conclusions: Systemic administration of a siRNA anti CD40 in models of ischemia-reperfusion injury was highly effective diminishing the molecular and cellular inflammatory response, improving serum urea and creatinine levels, and reducing tubular and interstitial lesions. Thus, the blockade of costimulatory signal CD40 becomes a potential therapeutic tool to modulate ischemia-reperfusion injury.

**TO038 THE ROLE OF MICRORNAS IN RENAL ISCHEMIA-REPERFUSION INJURY**

Tamás Kauczás1, Csaba Révész1, Maria Godó1, Zsuzsanna Rácz1, Robert Tarszabó1 and Péter Hamár1

1Department of Pathophysiology Semmelweis University Budapest Hungary

Introduction and Aims: Ischemia induced acute tubular necrosis (ATN) is one of the main causes of acute kidney injury (AKI). Reperfusion paradoxically increases the injury due to apoptotic processes. The modulation of pro-apoptotic genes is currently under investigation. MicroRNAs are posttranscriptional regulators of gene-expression. Our aim was to investigate the miRNA expression profile and to elucidate their role in ischemia-reperfusion (IR) induced ATN.

Methods: After unilateral renal ischemia of C57BL/6 mice, renal function (urea) and ischemia-reperfusion (IR) induced ATN.

Analysis of kidney gene expression showed that there was no activation of innate immunity (TLR3) due to the siRNA molecule itself, and that the siRNA reduced CD40 and also, proinflammatory cytokines such as IL4, IL2 and NFKB in treatment groups. Interstitial monocyte infiltrate (CD68+) showed a reduction in those kidneys with higher doses of siRNA treatment. An additional study with ICR mice using only the 50ug dose showed similar functional and structural protection.

Conclusions: Systemic administration of a siRNA anti CD40 in models of ischemia-reperfusion injury was highly effective diminishing the molecular and cellular inflammatory response, improving serum urea and creatinine levels, and reducing tubular and interstitial lesions. Thus, the blockade of costimulatory signal CD40 becomes a potential therapeutic tool to modulate ischemia-reperfusion injury.

© The Author 2013. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com
Results: Three miRNAs (miR-21, miR-17-5p and miR-106a) were significantly elevated in IR injury after 24 hours of reperfusion (3.0, 1.5 and 1.4 fold, respectively, p<0.05). Real-time PCR analysis demonstrated, that these miRNAs started to elevate after 24 hours of reperfusion, in the maintenance phase, further increasing at 48 hours (miR-21: 2.3 fold, p<0.01; miR-17: 2.2 fold, p<0.01; miR-106a, 1.9 fold, p<0.01). After sublethal ischemia miRNA levels normalized together with kidney damage markers in the recovery phase.

Conclusions: We identified three miRNAs with altered expression in the maintenance phase of IR injury. Validated targets of the identified miRNAs have mostly pro-apoptotic effects. Therefore our results suggest that these miRNAs may be involved in the regeneration processes and could represent possible therapeutic tools in the treatment of ATN. Support: OTKA: K81972, NF69278; ETT: 011-07/2009.

Introduction and Aims: The protective role of a novel pathway, the Sigma-1 receptor (S1R)-Akt-endothelial nitrogen monoxide synthase (eNOS) axis has been recently described in heart ischemia/reperfusion (IR) injury. In renal IR we previously showed that S1R agonists are protective, however the exact mechanism is still unknown. Here in renal IR we studied the effect of S1R agonist fluvoxamine (FLU) and antagonist NE-100 on the S1R-Akt-eNOS signaling pathway.

Methods: Male Wistar rats were treated i.p. with FLU (20 mg/bwkg; FLU), FLU and NE-100 (20 mg/bwkg and 1 mg/bwkg; FN) or vehicle (VEH). 30 minutes after the treatment animals were harvested (T30') or subjected to renal ischemia for 50 minutes followed by 2 (T2) or 24 (T24) hours of reperfusion. Sham-operated, untreated animals served as controls (C) (n=10/group). The renal S1R-Akt-eNOS proteins were analyzed by Western blot and immunofluorescence microscopy.

Results: 30 min after FLU treatment renal Akt and eNOS expression were elevated compared to C. After IR both proteins continually increased with time (C vs. T2 vs. T24). While at T2 there was no difference among the groups, at T24 renal Akt and eNOS protein levels were higher in the VEH group compared to FLU. NE-100 diminished all effects of FLU. S1R expression remained unchanged in the different groups. S1R-Akt-eNOS were co-localized in renal tubular cells. In C and after FLU treatment a nucleus-associated staining was observed, while in VEH and FN groups S1R-Akt-eNOS showed a more cytoplasmic localization.

Conclusions: The S1R-Akt-eNOS axis could be a novel pathway in the pathophysiology of renal IR injury. The S1R agonist FLU might exert its renoprotective effect by altering these proteins. This work was supported by LP2011-008/2012 Lendulet Research Grant; NIH grant R01 DK56843 and a. It was also supported by grants of OTKA PD83431, ETT 06-066/2009 and TÁMOP 4.2.4.A/1-11-1-2012-0001.