Nephrology Dialysis Transplantation 29 (Supplement 3): iii63–iii64, 2014
doi:10.1093/ndt/gfu138

EXPERIMENTAL KIDNEY INJURY MECHANISMS

TO051 MESENCHYMAL STEM CELLS (MSC) IMPROVE THE ARCHITECTURE AND FIBROTIC PROCESSES IN BOTH STENOTIC AND CONTRALATERAL KIDNEYS

Elizabeth B. Oliveira-Salles[1], Edgar Maquigussa[1], Patricia Semedo[1], Luciana G. Pereira[1], Vanessa M. Ferreira[1], Niels O. Camara[1], Cassia T. Bergamaschi[1], Ruy R. Campos[1] and Mirian A. Boim[1]
[1]Federal Univesrty of São Paulo, São Paulo, Brazil

Introduction and Aims: Chronic stenosis of renal artery leads to chronic renal hypoxia, renovascular hypertension, renal vascular rarefaction, fibrosis and renal failure. Previous data from our Laboratory showed beneficial effects of mesenchymal stem cells (MSC) in the reconstruction of renal parenchyma of the stenotic kidney, improving the vascular rarefaction and fibrosis. Here we evaluated the effect of MSC on the contralateral kidney. It was used the 2 Kidney-1 clip (2K-1C) model in rats.
Methods: Three weeks after left renal artery occlusion, fluorescently tagged mesenchymal stem cells (MSC) (2x105 cells/animal) were injected weekly into the tail vein of the 2K-1C rats. Flow cytometry showed labeled MSC in the cortex and medulla of both ischemic (clipped) and contralateral kidneys (unclipped).
Results: MSC prevented further increase in the MAP and significantly reduced proteinuria in 2K-1C rats. Renal function parameters were unchanged. Contralateral kidney presented altered structure, fibrosis and vascular rarefaction. Similar to stenotic kidney, MSC improved the morphology and decreased the fibrotic areas in the cortex and medulla of contralateral kidney. In contrast, the efficiency of MSC improving vascular rarefaction, as observed in stenotic kidney, was not observed in the contralateral kidney.
Conclusions: In conclusion, MSC therapy in the 2K-1C model prevented the progressive increase of MAP, improved renal morphology and reduced fibrosis in both stenotic and contralateral kidneys but it was more efficient improving vascular rarefaction in stenotic than in contralateral kidney. Although this therapy may be a promising strategy to treat renovascular hypertension and its renal consequences, further studies are necessary to improve the efficiency of MCS in order to ameliorate the morphology of the contralateral kidney.

TO052 REGENERATIVE POTENTIAL OF EXTRACELLULAR VESICLES DERIVED FROM ENDOTHELIAL PROGENITOR CELLS IN SEPSIS-ASSOCIATED ACUTE KIDNEY INJURY

Vincenzo Cantaluppi[1], Davide Medica[1], Federico Figliolini[1], Alessandro D Quercia[1], Sergio Dellepiane[1], Grazia Maria Virzi'[2], Claudio Ronco[3], Ciro Tetta[4], Luigi Biancone[1] and Giovanni Camussi[1]
[1]Nephrology, Dialysis and Kidney Transplantation Unit, Torino, Italy, [2]Nephrology, Dialysis and Kidney Transplantation Unit, Vicenza, Italy, [3]Nephrology, Dialysis and Kidney Transplantation Unit, "San Bortolo" Hospital, International Renal Research Institute Vicenza (IRRIV), Vicenza, Italy, [4]Emeala Medical Board, Fresenius Medical Care, Bad Homburg, Germany

Introduction and Aims: Sepsis is the main cause of acute kidney injury (AKI) in hospitalized patients. Sepsis-associated AKI is not merely related to tissue hypoperfusion, but also to a direct detrimental effect of pro-apoptotic circulating plasma factors able to induce endothelial (EC) and tubular epithelial (TEC) cell injury. Endothelial Progenitor Cells (EPC) are bone marrow-derived precursors able to accelerate tissue regeneration through the release of paracrine mediators such as extracellular vesicles (EV). EV are nanoparticles involved in cell-to-cell communication through transfer of proteins, lipids, mRNA and microRNA. The aim of this study was to evaluate the protective role of EPC-derived EV on EC and TEC injury induced by plasma of septic patients.
Methods: We enrolled in the study 20 critically ill patients with septic shock and AKI (Failure group RIFLE criteria) requiring renal replacement therapy. Peripheral blood EPC were evaluated by FACS (CD34+/CD133+/flk-1+ cells) and isolated on fibronectin-coated plates. EV were isolated from EPC supernatants by ultracentrifugation and characterized for protein, mRNA and microRNA content. EV isolated from EPC of healthy subjects were isolated and added in culture to human kidney-derived EC and TEC in presence of septic plasma, evaluating apoptosis and functional alterations.
Results: In comparison to healthy subjects, patients with sepsis-associated AKI presented an increased number of circulating CD34+/CD133+/flk-1+ EPC. However, EPC of septic patients isolated on fibronectin-coated plates showed a decrease of proliferation and of EV release. EV isolated from EPC of healthy donors were internalized in both EC and TEC through a L-selectin-mediated mechanism, protecting from septic plasma-induced injury. Indeed, on EC, septic plasma induced leukocyte adhesion, decreased angiogenesis through eNOS reduction and induced apoptosis through activation of the complement cascade (increase of C5b9). EPC EV reduced all these detrimental effects through the transfer of specific pro-angiogenic microRNA (miR-126, miR-296) and mRNA coding for inhibitors of apoptosis (Bcl-XL) and of complement activation such as CD55 (DAF), CD59 and Factor H. On TEC, septic plasma induced death receptor (Fas, TNF-R) apoptosis and mitochondrial dysfunction through decrease of PGC-1alpha. In addition, septic plasma induced loss of cell polarity and function as assessed by trans-epithelial electrical resistance (TEER) and decreased expression of the tight junction protein ZO-1, the endocytic receptor megalin and other TEC-specific solute carriers. All these septic plasma-induced injurious effects on TEC were reduced by EPC EV stimulation. However, the EV-associated protection on both EC and TEC was significantly abrogated by their pre-treatment with RNase, the enzyme able to destroy mRNA and microRNA within EV.
Conclusions: EPC isolated from peripheral blood of patients with sepsis-associated AKI showed functional alterations and decreased proliferation and EV release. EPC-derived EV may be exploited as innovative therapeutic approach in sepsis-associated AKI for their ability to inhibit apoptosis and functional alterations of EC and TEC.

TO053 EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) INHIBITION WITH ERLOTINIB ATTENUATES CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

Yukihiro Wada[1], Masayuki Iyoda[1], Kei Matsumoto[1], Yuki Shindo-Hirai[1], Yoshihiro Kuno[1], Taihei Suzuki[1], Yasutaka Yamamoto[1], Tomohiro Saito[1], Ken Iseri[1] and Takanori Shibata[1]
[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan

Introduction and Aims: EGFR expression is upregulated in various renal diseases. However, the effects of blocking the EGFR in acute kidney injury (AKI) are controversial. In the current study, we investigated the renoprotective effect of erlotinib, a selective tyrosine kinase inhibitor that can block EGFR activity, in cisplatin (CP)-induced AKI
Methods: CP nephrotoxicity (CP-N) was induced in 6-week-old male Sprague-Dawley (SD) rats (n=28) by intraperitoneal injection of CP (7 mg/kg) on day 0. Groups of animals were given either erlotinib (CP+E, 20 mg/kg, n=14) or vehicle (CP+V, n=14) daily by oral gavage from day -1 to day 3. Five SD rats were used as normal control (NC). All rats were sacrificed on day 4. Renal morphological investigations were performed at sacrifice. *In vitro*, we used human renal proximal tubular cells (HK-2). Cells were pretreated with erlotinib or medium alone, and phosphorylation of MEK1 and Akt induced by CP were analyzed by Bio-Plex® Suspension Array System.
Results: Compared to the NC rats, the CP+V rats exhibited marked AKI characterized by deterioration of renal function, severe tubulointerstitial (TI) damage, and increase in renal cortical mRNA expressions for proinflammatory cytokines, profibrogenic genes, and pro-heparin-binding EGF-like growth factor. Compared to vehicle, erlotinib treatment significantly prevented body weight loss ($p < 0.05$) and increased urine volume ($p < 0.05$) in CP-N rats. Erlotinib treatment also resulted in a reduction in serum creatinine level (1.6 ± 0.3 vs. 0.8 ± 0.2 mg/dL, $p<0.01$) and urinary N-acetyl-β-D-glucosaminidase (NAG) activity ($p < 0.05$). TI injury (the number of casts/HPF: 2.0 ± 0.7 vs. 0.7 ± 0.1, $p < 0.01$), and the number of PCNA+ ($p < 0.01$), TUNEL+ ($p < 0.01$), and caspase-3+ cells ($p < 0.05$) were also significantly reduced in the CP+E rats. Furthermore, the CP+E rats had a significant reduction of renal cortical mRNA expression for profibrogenic genes (TGF-β ($p < 0.05$), collagen type I ($p < 0.05$), and type III ($p < 0.01$)) and Bax/Bcl-2 ratio ($p < 0.05$) compared to the CP-V rats. However, erlotinib treatment did not affect either macrophage infiltration in tubulointerstitium or mRNA expressions for proinflammatory cytokines in CP-N. *In vitro*, pretreatment of erlotinib significantly reduced the CP-induced phosphorylation of MEK1 ($p < 0.01$) and Akt ($p < 0.01$) in HK-2.
Conclusions: Erlotinib has renoprotective property that is likely in part through degradation of tubular cell apoptosis and proliferation by inhibiting the downstream signaling pathway of EGFR including MAPK and Akt. Our results suggest that erlotinib may be useful for preventing AKI in patients receiving CP chemotherapy.

TO054 RESVERATROL INHIBITS THE INTRACELLULAR CALCIUM INCREASE AND RAS/ENDOTHELIN SYSTEMS ACTIVATION INDUCED BY SOLUBLE URIC ACID IN MESANGIAL CELLS

Guilherme Albertoni[1], Luciana Aparecida Reis[1] and Nestor Schor[1]
[1]*Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil*

Introduction and Aims: Hyperuricemia is associated with increase in the renal disease risk. We and others previously observed that intracellular calcium increases and renin-angiotensin system (RAS) and Endothelin are involved in UA effects. RESV is a polyphenolic flavonoid with potent antioxidant activity, has reno-cardioprotective effects and it is gaining attention due to health benefits. The objective of this study was to evaluate the effect of RESV on the intracellular calcium increase and RAS/Endothelin activation induced by UA in immortalized human mesangial cells (ihMCs) in culture and rat primary cells.
Methods: The cells were pre incubated with RESV (12,5 μM) for 1 h and than treated with UA (10 mg/dL) for 6 and 12hr. Angiotensinogen (AGT) and Pre-pro ET (ppET-1) expression was evaluated by the PCR technique. Angiotensin II (AII) and endothelin -1 (ET-1) protein synthesis were assessed by ELISA technique. Additionally, the level of [Ca++]i was quantified by fluorescence with Fluo-4 AM by flow cytometry and expressed as fluorescence intensity (FI). It was also examined the effects of pretreatment of RESV in rats by immunohistochemical analyses quantitated in the renal tissue that expressed AII, ET-1 and renin markers.
Results: UA significantly increased AGT expression and AII synthesis in comparison the control after 6 and 12h (Table). When ihMCs were pre incubated with RESV, there were a significant decrease in AGT expression and AII synthesis in comparison the UA in both periods analyzed. Incubation of ihMCs with UA increased ppET-1 expression and ET-1 synthesis in comparison to control after 6h and 12h (Table). When ihMCs were pre incubated with RESV, it was observed a significantly decrease in ppET-1 mRNA and ET-1 protein synthesis in comparison the UA. There was an increase of 80% in [Ca++]i when ihMC were stimulated with UA (8 ± 0.2 vs. 4.5 ± 0.1 FI) in comparison to the fluorescence at baseline ($p<0.01$). The pre incubation with RESV significantly inhibited the increase in [Ca++]i induced by UA of 75% (5 ± 0.2 vs. 8 ± 0.2 FI, $p<0.05$). At tissular level, UA significantly increased AII , ET-1 and renin production of 1.400% (50 ± 2 vs. 3 ± 1% in the control group), 800% (90 ± 12 vs. 10 ± 2% in the control group) and 1.200% (80 ± 2 vs. 6 ± 1% in the control group), respectively ($p<0.05$). When the animals were pre treated with RESV, it was observed a significantly decrease in AII , ET-1 and renin production of 70% (15 ± 4 vs. 50 ± 2), 92% (7 ± 1 vs. 90 ± 12) and 85% (12 ± 0.5 vs. 80 ± 2) compared with UA alone group, respectively ($p<0.05$).
Conclusions: These findings suggest that resveratrol can minimize the impact of UA on the angiotensin, endothelin and [Ca2+]i increases in mesangial cells, suggesting that, at least in part, resveratrol can prevent the effects of soluble UA in mesangial cells. These novel observations corroborate with previous data that UA is able to stimulate endothelial Ang II and ET-1 production in endothelial cells and thus, UA-induced mesangial stimulation can potentially induce glomerular damage observed after long-term hyperuricemia.

TO055 HEME OXIGENASE 1 AS A POTENTIAL ANTIOXIDANT AGENT FOR CONTRAST– INDUCED NEPHROPATHY IN DIABETIC RATS.

Cassiane Dezoti Fonseca[1], Mirian Watanabe[1], Mariana Hayashi Mendonca[1], Sheila Marques Fernandes[1] and Maria De Fatima Fernandes Vattimo[1]
[1]*University of Sao Paulo, Sao Paulo, Brazil*

Introduction and Aims: Contrast–Induced Nephropathy (CIN) is a toxic acute kidney injury (AKI) that results from intrarenal vasoconstriction, direct tubular toxicity with generation of reactive oxygen species (ROS). The decreased tissue oxygen tension in medula in preexisting renal dysfunction, as in Diabetes Mellitus (DM), is a risk factor for CIN. This study evaluated the effect of HO 1 in the restoration of oxidant injury of the contrast-induced AKI in diabetic rats.
Methods: Adult male Wistar rats (250-300g) were used. Physiological parameters; renal function (creatinine clearance, crCl); oxidative injury (urinary peroxides, UP, tiobarbituric acid reactive substances–TBARS and thiols in renal tissue) and kidney histological analysis were evaluated. Rats were submitted to left uninephrectomy (Nx) on the 1° day. The DM was induced by a single dose of intravenous streptozotocin (65mg/kg i.v.) on the 20° day. The iodine contrast (IC) meglumine ioxithalamate 6ml/kg and the hemin (HO 1 inducer;10mg/kg) were administrated intraperitoneal (i.p.) on the 85° day. Groups: Control (Nx+Citrate), Nx+DM, Nx+DM+IC; Nx+DM+IC+H (hemin was administrated 60 minutes before IC).
Results: Diabetic groups showed polyphagia, polydipsia, polyuria, increase in the blood glucose and reduction in body weight ($p<0.05$). IC reduced the crCl and thiols in renal tissue and a proeminent increase in UP and TBARS was observed. These paramethers were significantly changed by hemin. Kidney histology showed that the IC animals presented tubular cells vacuolization and edema with moderate injury.
Conclusions: The data highlight the HO 1 renoprotective effect in oxidative damage in the CIN associated with DM.

TO055 Renal function, oxidative index. αp<0.05 vs Control;β vs Nx+DM; δ vs Nx+DM+CI.

Groups/n	cr/Cl/100g (ml/min)	UrinaryPeroxides (nmol/g creatinine)	TBARS(nmol/g creatinine)	Thiols(nmol/mgtotalprotein)
Control(6)	0.5±0.8	9±2	0.2±0.7	12±4
Nx+DM(6)	0.3±0.5α	46±21α	2.2±0.6α	7±2
Nx+DM +CI(6)	0.2±0.3αβ	45±10α	4.2±1.0αβ	5±1α
Nx+DM +CI+H (6)	0.4±0.7αβδ	26±13αβδ	1.6±0.2αδ	14±3βδ