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EXPERIMENTAL ACUTE KIDNEY INJURY

SP066 WNT10A OVEREXPRESSION IN KIDNEY FIBROBLASTS INDUCES KIDNEY FIBROSIS IN ACUTE INTERSTITIAL NEPHRITIS

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Introduction and Aims: Acute interstitial nephritis (AIN) is a common cause of acute kidney injury (AKI). In severe cases, AIN may progress to chronic kidney disease or end-stage renal disease. We previously reported that WNT10A is a novel angio/stromagenic factor in wound healing and organ fibrosis. In this study, we investigated the role of WNT10A in fibrotic progression of AIN.
Methods: Kidney biopsy specimens from 25 AKI patients (all men, ≥60 years) treated in our hospital between 2007 and 2013 were examined for WNT proteins, α-SMA, and fibronectin expression by immunohistochemistry. The relationship between each WNT proteins expression level and estimated glomerular filtration rate (eGFR) was evaluated by the Mann-Whitney U test. COS1 cells (kidney fibroblasts from African green monkey) were transfected with a WNT10A expression plasmid or a siRNA targeting peroxiredoxin 5 (PRDX5). The effects of WNT10A overexpression and PRDX5 knockdown on proliferation and hydrogen peroxide induced cytotoxicity were measured by WST-8 assay.
Results: The 10 patients exhibiting WNT10A expression in biopsy tissue had significantly lower eGFR values (median, 11.12 mL/min per 1.73 m^2; range, 7.16−28.15 mL/min per 1.73 m^2) than the 15 patients exhibiting no detectable WNT10A expression (34.70, 8.37−134.58; $p = 0.0033$). There was no significant relationship between eGFR and the expression level of any other WNT protein examined (WNT-1, -3, and -4). Overexpression of WNT10A in COS1 cells enhanced proliferation, fibronectin expression, PRDX5 expression, and resistance to hydrogen peroxide, while PRDX5 downregulation sensitized COS1 cells to hydrogen peroxide.
Conclusions: WNT10A expression may promote fibrotic progression and kidney dysfunction in AIN. Blockade of WNT10A expression may be a feasible therapeutic strategy against kidney fibrosis.

SP067 ADAMTS13-VON WILLEBRAND FACTOR (VWF) AXIS IS INVOLVED IN THE PATHOPHYSIOLOGY IN RENAL ISCHEMIA REPERFUSION INJURY

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Introduction and Aims: Renal ischemia-reperfusion injury (IRI) is one of the major causes of acute kidney injury (AKI), and inflammatory response is known to be a key mechanism in renal IRI. Recently, the ADAMTS13-Von Willebrand factor (vWF) axis has been suggested to play a causal role in the pathophysiology of IRI in different organs; however, its action in renal IRI is uncertain yet. Here, we investigated whether ultralarge vWF (UlvWF)-multimer was involved in the development of AKI and a potential role of ADAMTS13, a protease cleaving vWF multimer in renal IRI.
Methods: We performed renal IRI in ADAMTS13 knockout (KO) mice or wild type (WT) mice. After 24hrs, tissue ULvWF-multimer was detected by Western blot analysis, and functional and histological damage were assessed.
Results: In the immunohisotochemistry, vWF was detected in both medulla and cortex of injured kidney and significantly increased in the kidney of ADAMTS13 KO than in WT mice. Western blot analysis also showed increased expression of ULvWF-muiltimer in ischemic kidney, and the expression was significantly higher in ADAMTS13 KO mice than WT mice. The higher level of vWF-multimer in ADAMTS13 KO mice was correlated with more functional deterioration and severe tubular injury, suggesting an important role for VWF in the development of renal IRI. In addition, the number of Gr-1 (+) neutrophils increased in the kidney of ADAMTS13 KO mice compared to that of WT mice, whereas F4/80 macrophages were not.
Conclusions: Our data shows that ADAMTS13-Von Willebrand factor (vWF)axis could be involved in the pathophysiology in renal IRI. This is first report showing the involvement of UlvWF-multimer in renal IRI indicating that rhADAMTS13 could be of therapeutic value to limit renal ischemia/reperfusion injury.

SP068 EFFECTS OF CILASTATIN ON GENTAMICIN-INDUCED RENAL DAMAGE. IN VITRO AND IN VIVO EVIDENCE

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Introduction and Aims: Gentamicin is an aminoglycoside antibiotic that is widely used in human clinical practice to treat life-threatening Gram-negative infections. Unfortunately it causes nephrotoxicity in 10-20% of therapeutic courses, side effect that seriously limits its use. Thus, a therapeutic approach to protect or recover renal damage would have very important clinical consequences. We have found that cilastatin, a renal dehydrodipeptidase I inhibitor has protective effects on tubular cells and animals from cisplatin-induced renal damage. In this study, we have investigated the potential use of cilastatin as nephroprotector on gentamicin-induced renal injury in vitro and in vivo.
Methods: Primary proximal tubular epithelial cells (PTECs) obtained from pigs renal cortex were treated with increasing concentrations of gentamicin in the presence or absence of cilastatin (200 μg/ml) for 24 hours. Apoptosis was assessed by oligonucleosomes formation, apoptotic cellular morphology and cell detachment quantification by flow cytometry. On the other hand, male Wistar rats were divided into 4 groups: control rats, cilastatin-control rats, gentamicin-injected rats (80 mg/kg, daily i.p.), cilastatin-treated gentamicin-injected rats (150 mg/kg, daily, i.p.). Nephrotoxicity was assessed 9 days after the first dose of gentamicin treatment, by measuring serum creatinine, BUN, creatinine clearance, proteinuria and renal morphology. Renal apoptosis was measured by determination of TUNEL-positive cells and apoptotic mediator's levels of extrinsic (death receptor) and the intrinsic (mitochondrial) pathways of apoptosis. Gentamicin uptake into PTECs and kidneys was measured by TDX specific assay.
Results: Direct microscopy observation showed as gentamicin induced dose-dependent apoptosis on cultured PTECs with DNA fragmentation, and cell detachment. Cilastatin administration reduced these apoptotic events for every gentamicin concentration and decreased severe morphological changes induced by this antibiotic. The in vivo analysis displayed similar results. Gentamicin-treated rats showed significant elevations in BUN, creatinine and proteinuria, with severe morphological changes such as vacuolization and hyaline cast in the tubular lumen. The treatment with cilastatin resulted in amelioration of renal functions as shown by reduction of urea, creatinine and proteinuria and improved histological damage in gentamicin-treated animals. Cilastatin also reduced renal caspase 3-activation, bax, bax/Bcl-2 ratio, Fas ligand and TUNEL-positive apoptotic cells, previously increased by gentamicin. On the other hand, cilastatin partially attenuated gentamicin uptake by PTECs and kidney tissue.
Conclusions: This study provides evidence that cilastatin reduces in vitro and in vivo gentamicin nephrotoxicity by ameliorating apoptosis and might represent a novel strategy in the prevention of gentamicin-induced acute renal injury. The mechanism of the beneficial effect could be attributed, at least in part, to a decrease in drug accumulation by the cells.

SP069 DNASE 1 TREATMENT REDUCES RENAL I/R INJURY IN MICE

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Introduction and Aims: Acute kidney injury (AKI) is an important clinical problem among hospitalized patients in the ICU. It is associated with high mortality and increased risk for progression towards end stage renal disease (ESRD). AKI is often triggered by ischemia reperfusion (I/R), which leads to a complex interplay between coagulation, tubular epithelial and endothelial cell injury and sterile inflammation, resulting in renal tissue injury. Renal I/R injury is characterized by a massive influx and activation of neutrophils early after reperfusion, which play a crucial role in the patho physiology of post ischemic renal failure.It is known that upon activation, neutrophils can release nuclear material known as neutrophil extracellular traps (NETs). Initially described as a part of antimicrobial defense, recent studies indicate that NETs are also involved in the pathogenesis of non-infectious diseases. Deoxyribonuclease 1 (DNase 1) has been shown to dismantle the NET scaffold and administration of DNase 1 has a protective effect in vivo in murine models of e.g. myocardial I/R injury and acute lung injury. The presence of NETs in I/R injured renal tissue and the effect of DNAse 1 therapy on renal I/R injury is not known to date. In this study we investigated the presence of NETs in renal tissue after I/R and assessed the effect of DNAse 1 therapy on renal I/R injury and renal function in mice.

Methods: We subjected two groups of C57Bl/6J mice to renal I/R injury by clamping both renal arteries for 25 minutes followed by a reperfusion phase of 1 day. 24 hours and 1 hour prior to the operation and shortly after the operation we treated one group with 200 uL 20 mg/kg BW DNAse 1,and the other group with 200 uL 0.9 % NaCl. Mice were sacrificed after 24 hours.
Upon sacrifice, kidneys were isolated and snap frozen into liquid nitrogen or formalin-fixed.
Blood was collected by heart puncture.
As marker for NET formation in tissue we performed a double staining for citrullinated histone H3 (H3Cit) and granulocyte marker Ly6G. As marker for NETs in plasma we performed an ELISA for nucleosomes.
Results: Renal I/R tissue shows presence of NETs in both mouse groups.
Mice treated with DNAse 1 show a trend towards fewer NETs in tissue and significant lower levels of nucleosomes in plasma (P<0,05) compared to NaCl treated mice. Furthermore, mice treated with DNAse 1 have significantly reduced creatinine (P<0,05) and urea plasma levels (P<0,05). Moreover they show less expression of nGAL mRNA (P<0,05) and MPO (P<0,05) in renal tissue.
Conclusions: Our data indicate the presence of NETs formation in murine renal tissue after renal I/R.Furthermore, we show that DNAse 1 treatment protects against acute inflammation, renal I/R injury and loss of renal function.

SP070 ENDOTHELIAL DYSFUNCTION AND RENAL FIBROSIS IN SEPSIS-INDUCED ACUTE KIDNEY INJURY:POSSIBLE ROLE OF LPS BINDING PROTEIN

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Introduction and Aims: The pathophysiology of sepsis-induced acute kidney injury (AKI) is characterized by a complex activation of the host immune system and renal resident cells by pathogen derived pro-inflammatory products. The occurrence of renal fibrosis in this setting has been poorly investigated and is usually associated with later development of chronic kidney disease. Aim of the present study is to investigate the possible association between EC dysfunction and acute development of tissue fibrosis in a swine model of LPS-induced AKI. Moreover we studied the possible effects of coupled plasma filtration adsorption (CPFA) in this setting.
Methods: After 3 h from LPS infusion, 8 pigs were treated with CPFA for 6 h; 8 control pigs receive no treatment. Renal biopsies were performed before (T0) and 9 hours (T9) after LPS infusion. LPS Binding Protein (LBP) levels were quantified in sera by ELISA. Endothelial cells (ECs) were stimulated for 24h with LPS 4ug/ml and cultured in presence of 1% different swine sera for 12h and were analyzed by FACS.
Results: In a swine model of LPS-induced AKI, we found that acute tubulo-interstitial fibrosis occurred by 9h from LPS injection (Masson's Trichrome: 51.54±15.33, vs T0 p=0.04). Acute fibrosis was associated with dysfunctional α-SMApos ECs (T9:18.10±1.58 vs T0, p=0.003) characterized by active proliferation (Ki67pos) (T9: 12.5±0.7 vs T0, p=0.001), without apoptosis (Caspase 3neg). As in vivo, LPS activation led to EC dysfunction in vitro with significant Vimentin and N-cadherin expression and increased synthesis of mRNA for collagen I (normalized mRNA expression: 2.10±0.5 vs basal 0.31±0.09, p=0.03). Therapeutic intervention by CPFA significantly prevented acute fibrosis in septic pigs (Masson's Trichrome: 25.28±3.51 vs T9 LPS, p=0.04), by preserving EC phenotype both in peritubular capillaries and renal arteries (α-SMApos ECs 5.58±0.52 vs T9 LPS, p=0.0002). We found that the removal of LBP from septic plasma (0.21±0.03 ug/ml vs septic plasma:9.6±0.69, p=0.0001) was critical to abrogate the effects of LPS on EC dysfunction in vitro, by blocking LPS-induced collagen I production (normalized mRNA expression, ctr sera:1.03±0.28 vs septic sera: 4.17±0.72 , p=0.003).
Conclusions: Our data indicated the EC dysfunction might be pivotal in the acute development of tubulo-interstitial fibrosis in LPS-induced AKI. Selective removal of the LPS adaptor proteins LBP might represent a future therapeutic option to prevent EC dysfunction and tissue fibrosis in sepsis-induced AKI.

SP071 THE EFFECT OF ERYTHROPOIETIN ON THE EXPRESSION OF CYTOCHROME C AND FAS/FASL IN AN EXPERIMENTAL ACUTE RENAL ISCHEMIA/REPERFUSION MODEL

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Introduction and Aims: The role of cytochrome c (cyt c) and Fas/FasL in apoptosis are well established, however their participation in apoptotic signaling pathways in acute kidney ischemia-reperfusion (I/R) injury are not completely understood. Erythropoietin (EPO), through its antiapoptotic action, was found to be renoprotective in experimental models of I/R- induced acute kidney injury (AKI). The aim of this study was to investigate the effect of EPO on the expression of cyt c and Fas/FasL in an experimental model of I/R AKI at different time points after reperfusion.
Methods: Male Wistar rats randomly divided into two groups: the I/R group (n=12) and the EPO-I/R group (500 U/Kg,i.p. 20 min prior to ischemia, n=15) were subjected to bilateral renal ischemia 45min. Each group was allocated in three subgroups according to the timing the animals were sacrificed at 6, 24 and 48 hrs after reperfusion. Rats subjected to identical surgical procedure without occlusion of renal pedicles were used as sham-operated group (n=6). Renal injury was assessed by measurement of serum biochemical markers (urea and creatinine) and histological grading. Expression of cyt c and Fas/FasL mRNA was evaluated by RT-PCR and protein expression of cyt c by immunohistochemistry.
Results: EPO-I/R group had significantly lower serum urea and creatinine levels compared to I/R group at 6, 24 and 48 hours of reperfusion (p<0.05). Histological evaluation revealed significantly less tubular damage in the EPO-treated group compared to I/R group (p<0.001).Fas/FasL and cyt c mRNA were detected in normal kidneys. Fas/FasL mRNA expression was not altered by I/R and EPO administration, although differences in absolute numbers were observed. Cyt c mRNA was significantly increased in I/R group compared to sham group at all time points (p<0.05). EPO administration caused a significant reduction of cyt c mRNA expression compared to I/R group at 48hrs (p<0.05). A pronounced upregulation of cyt c protein expression was observed in I/R group, localized mainly in renal cortical tubules, compared to sham group. EPO administration markedly reduced cyt c staining compared to I/R group. In both groups immunoreactivity became gradually weaker from 6 to 48hrs.
Conclusions: EPO pretreatment reduced I/R-induced tubular injury and ameliorated renal function. Acute renal I/R injury and renoprotective action of EPO were not observed to correlate directly with changes in gene expression of Fas and FasL. Early induction (6hr) of cyt c expression following I/R might contribute to severe tissue damage observed at later time points. EPO reduced Cyt c immunostaining throughout reperfusion and gene expression at later stages suggesting a potential antiapoptotic action in I/R-induced renal injury through the endogenous pathway of apoptosis.

SP072 THE EXPRESSION OF NEURONAL NOS IN AN ACUTE RENAL ISCHEMIA/REPERFUSION INJURY EXPERIMENTAL MODEL: THE EFFECT OF ERYTHROPOIETIN

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Introduction and Aims: Neuronal NOS (nNOS) is involved in normal renal function regulation, although the individual mechanisms are not fully clarified. nNOS expression is affected under ischemic conditions but its role in acute renal ischemia/reperfusion (I/R) injury remains unknown. Furthermore, it has not been studied whether nNOS is implicated in erythropoietin (EPO) renoprotective action. The aim of this study was to investigate the effect of EPO on the expression of nNOS in an experimental model of acute renal I/R at different time points after reperfusion.
Methods: Male Wistar rats randomly divided into two groups: I/R group (n=12) and EPO I/R group (500 U/Kg, i.p, 20 min prior to ischemia, n=15) were subjected to bilateral renal ischemia 45min. Each group was allocated in three subgroups according to the timing the animals were sacrificed at 6, 24 and 48 hrs after reperfusion. Rats subjected to identical surgical procedure without occlusion of renal pedicles were used as sham-operated group (n=6). Renal injury was assessed by measurement of serum biochemical markers (urea and creatinine) and histological grading. Expression of nNOS was evaluated by RT-PCR and immunohistochemistry.
Results: EPO I/R group had significantly lower serum biochemical markers compared to I/R at 6, 24 and 48 hrs of reperfusion (p<0.05). Histological evaluation revealed significantly less tubular damage in the EPO I/R group compared to I/R (p<0.001). Acute renal I/R decreased nNOS mRNA at all time points of reperfusion compared to sham-operated group (p<0.01). A slight increase was observed in the later stages without restoring to normal levels even at 48hrs. EPO pretreatment delayed nNOS mRNA reduction as the onset of the reduction was detected at 24hrs (p<0.001). Comparison of nNOS mRNA expression between groups revealed significantly higher mRNA expression in the EPO I/R group at 6hrs (p=0.02). nNOS protein expression was observed in normal kidneys, mainly in macula densa regions and diffusely in renal tubules. Following I/R an obvious decrease in nNOS staining was detected in the early stages of reperfusion, which seemed to be gradually restored at 48hrs. EPO administration resulted in higher nNOS protein expression at 6 and 24hrs compared to I/R group. Immunoreactivity was similar in both groups at 48hrs of reperfusion regardless of the administration of EPO.
Conclusions: EPO pretreatment reduced I/R-induced tubular injury and ameliorated renal function. nNOS is expressed in normal kidneys. Severely damaged renal regions exhibited a weak expression of nNOS indicating that nNOS reduction could be involved in renal injury. A gradual increase in nNOS expression in the later stages of reperfusion might be correlated with the recovery of renal function. As EPO pretreatment resulted in higher levels of nNOS expression compared to I/R group in

the early stages of reperfusion, it could be suggested that restoration of nNOS levels is a possible contributory mechanism of EPO renoprotective effect in acute renal I/R injury.

SP073 OAT1/3 RESTORATION PROTECTS AGAINST RENAL DAMAGE AFTER ISCHEMIC AKI

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Introduction and Aims: Expression of proximal tubular organic anion transporters OAT1 and OAT3 is reduced by prostaglandin E2 (PGE2) after renal ischemia and reperfusion (I/R) injury. We hypothesized that impaired expression of Oat1/3 is decisively involved in the deterioration of renal function after I/R injury. Therefore, we administered probenecid, which blocks proximal tubular indomethacin uptake, to abolish the indomethacin mediated restoration of OAT1/3 regulation and its beneficial effect on renal functional and morphological damage.
Methods: Ischemic AKI was induced in rats by bilateral clamping of renal arteries for 45 min with 24h follow up. Low-dose indomethacin (1mg/kg) was given i.p. at the end of ischemia. Probenecid (50mg/kg) was administered i.p. 20 min later. PAH-net secretion, inulin- and PAH clearance as well as PGE2 clearance were determined. OAT expression, MCP1, caspase3 activity, iNOS expression and NO generation were measured. Pathohistomophologic damage of proximal tubules was scored and ED1 infiltration quantified.
Results: Indomethacin restored the expression of OAT1/3, PAH net secretion and PGE2 clearance. Additionally, indomethacin improved kidney function as measured by GFR and morphology, whereas in opposite it reduced renal cortical apoptosis and nitric oxide production. Notably, indomethacin did not affect parameters of inflammation in kidney tissue. On the other hand, probenecid blocked the indomethacin induced restoration of Oat1/3 and moreover abrogated all beneficial effects regarding renal function and morphology.
Conclusions: Our study indicates that the beneficial effect of low-dose indomethacin in iAKI is not due to its anti-inflammatory potency, but in contrast to its restoration of OAT1/3 expression and function. Inhibition of proximal tubular indomethacin uptake abrogates the beneficial effect of indomethacin by resetting the PGE2 mediated Oat1/3 impairment, thus re-establishing renal damage. This provides evidence for a mechanistic effect of OAT1/3 in a new model of the induction of renal damage following ischemic AKI.

SP074 THE ANTIDEPRESSANT FLUVOXAMINE IS PROTECTIVE AGAINST RENAL ISCHEMIA REPERFUSION INJURY

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Introduction and Aims: Previously we showed that pretreatment with the Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) improved postischemic survival and resulted in milder deterioration of renal function and kidney damage. Here we studied the effect of FLU on the S1R-Akt-NOS signaling pathway and on intrarenal vasoregulation.
Methods: Male Wistar rats were subjected to unilateral renal ischemia followed by 24 hours of reperfusion (T24). 30 min prior to the ischemia (I/R) groups were treated i.p. either with vehiculum (VEH); FLU; FLU+S1R antagonist NE-100 (FN); FLU +non-selective NOS blocker L-NAME; FLU+selective endothelial (e)NOS blocker L-NIO or FLU+selective neuronal (n)NOS blocker 7-NI. Controls were sham-operated animals. Renal S1R, pAkt, peNOS and nNOS protein levels were measured at different time-points. Alteration of intrarenal capillary diameters was determined in vivo using multiphoton microscopy. In vitro experiments were performed on HK2 human proximal tubular epithelial cells treated with 10μM FLU.
Results: In controls FLU had an acute vasodilatative effect which was suspended by L-NAME and 7-NI and even reversed by L-NIO (FLU+L-NAME Δd=0.23 μm; FLU +7-NI Δd=0.86 μm; FLU+L-NIO Δd=-0.57 μm vs. FLU Δd=2.18 μm). S1R, pAkt and peNOS protein levels were elevated 30min after FLU treatment, while nNOS expression was minimal. At T24 I/R induced renal vasoconstriction was ameliorated by FLU (C: 9.86 ± 1.23 μm; VEH: 8.29 ± 1.29 μm; FLU: 10.64 ± 2.53 μm; FN: 7.88 ± 1.67 μm). This increase was neutralized by all NOS blockers. S1R; pAkt; peNOS and nNOS levels were more elevated in the FLU group compared to VEH and FN. S1R expression of HK2 cells was elevated after 30 min, 2 hours and 12 hours of FLU treatment. FLU induced a slight increase in eNOS phosphorylation after 30 min, that became more robust after 2 and 12 hours.
Conclusions: The S1R agonist FLU pretreatment directly acts on proxmimal tubular cells through the activation of the S1R - NOS system in a time and NOS isoform specific manner. Thereby FLU - used in the long-term treatment of depression without notable side-effects - improves postischemic renal perfusion and is renoprotective in I/R. Based on this data one can hope to find a new therapeutic target in the treatment of renal I/R damage through the modulation of the S1R.
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SP075 REVERSAL OF RADIOCONTRAST MEDIUM TOXICITY IN HUMAN RENAL PROXIMAL TUBULAR CELLS BY *VITIS* SPP. EXTRACT

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Introduction and Aims: Radiocontrast media (RCM) are commonly used in medical practice but their use may lead to contrast-induced nephropathy. The toxicity of RCM may be due to several factors, with effects on renal haemodynamics and direct cell toxicity believed to be the main ones. Direct cell toxicity has been shown by the decrease in cell viability and an increase in cell apoptosis in several cell models. We have observed that HK-2 cells (an HRPTC line) pre-incubated with a *Vitis* spp. (white grape) juice extract (denoted as WGJe)dramatically reduced the decrease in cell viability induced by the RCM sodium diatrizoate (NaD). A possible mechanism for this apparent protective effect was also investigated.
Methods: HK-2 cells were incubated with NaD (75mg Iodine/ml) in the presence/absence of WGJe (250 μg of a spray-dried extract was added per ml of cell culture medium). After 2.5h, the culture medium was removed and replaced with fresh medium. After a further 30h cell viability was measured using the MTT (Thiazolyl blue tetrazolium bromide) chemical reduction assay. HK-2 cells were also incubated with NaD (75mgI/ml) for 30 and 60 minutes in the presence/absence of WGJe, after which the cells were harvested and whole cell lysates prepared and subjected to SDS-PAGE/Western blotting.
Results: HK-2 cell viability, as determined by the chemical reduction of MTT, was reduced by nearly 75%, but in the presence of WGJe the decrease observed was only 15%. Analysis of the cell lysates by Western blotting showed that NaD caused a decrease in the phosphorylation (activation) of the pro-survival/anti-apoptotic kinase Akt at Ser473, but in the presence of WGJe, the phosphorylation status of this kinase was maintained.
Conclusions: *Vitis* spp. extract can protect renal cells against cell death induced by RCM. Though the exact mechanism at present may not be fully known, maintaining the pro-survival kinase Akt in an active form may contribute to this.

SP076 MECHANISMS OF THE RENAL PROTECTION BY CYCLOSPORINE AFTER AN ISCHEMIA REPERFUSION : ROLE OF HSP70 AND CYCLOPHILINE D.

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Introduction and Aims: The aim of this study is to show that the preconditioning with CsA provides a protection against renal ischemia reperfusion (IR) by two pathways allowing together the delay of mPTP opening: the HSP70 pathways and by the direct inhibition of cyclophiline D (cypD).
Methods: We performed a right unilateral nephrectomy with a 30-minutes contralateral clamping of the left renal artery in C57BL6 male mice. First, we measured in 3 groups (a sham group, a control group and a pre-conditionning group with 10 mg/kg of CsA (preCsA) injected 10 min before ischemia) the calcium overload (CRC) in order to test mPTP, the oxydative phosphorylation to test respiratory chain, the renal expression of HSP70 and GSK 3-b by western blotting at 20 min of reperfusion. Secondly, we performed the same ischemia in CyclophilineD deficient mice to evaluate the role of CypD in mPTP. Finally, we used the inhibitor of HSP70 (Quercetin, 100mg/kg, 2h before ischemia) in mice preconditioned with CsA to evaluate the possible role of HSP70 in mPTP.
Results: At 20 min of reperfusion, we showed a delay of mPTP opening in the preCsA group compared to control group. We showed also a delay of mPTP opening in the cypD-KO group. However, we found no protection in the oxidative phosphorylation that is dramatically decreased at 20 min of reperfusion in all groups, as previously reported. Renal HSP70 and GSK3 phosphorylated protein (P-GSK3b) levels were higher in the preCsA group than in the control group, suggesting an enhancement of these 2 proteins by CsA. The pretreatment with Quercetin decreased HSP70 and P-GSK3b levels compared to preCsA group and eliminated the preconditionning protection with an early opening of mPTP.
Conclusions: Our study suggested that preCsA promotes two pathways of protection, the HSP70 pathways, mediated by P-GSK3B and by inhibition of cyclophilline D. These 2 pathways allow delaying the mPTP opening and protects against IR, suggesting that mPTP is the key event in the renal lesion of IR.

SP077 DIRECT EFFECT OF LPS ON GLOMERULAR RENIN ANGIOTENSIN SYSTEM (RAS) IN EXTRACORPOREAL RENAL PERFUSION

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Introduction and Aims: Recent experimental observations suggest that, at least in the early phases of septic acute kidney injury (AKI), significant changes involving the glomerular hemodynamics and results in the loss of glomerular filtration rate (GFR) occur. A variety of substances are released in the systemic circulation as a consequence of endotoxemia, leading to accented mutate in local renin angiotensin system. Cytokines increase the synthesis of nitric oxide, urging to marked reduction in vascular resistance, compensatory responses including increased of angiotensin II (Ang II). Increased sympathetic tone and renin-angiotensin system (RAS) activity induce renal hypoperfusion and ischemic damage certainly contributes to sepsis-related acute kidney injuries. In our laboratory demonstrated the inhibition of enzymatic activity of renin by the direct effect of LPS in immortalized human mesangial cell (HMC). If reproducible in vivo, inhibition of the RAS site by LPS have clinical importance given the role usually played by Ang II on glomerular cells (epithelial, endothelial and mesangial) and autoregulation of glomerular hemodynamics
Methods: FITC-LPS was infused into the aorta above the renal arteries of male Wistar rats weighing approximatey 250g. 1-2 hours after systemic injection, kidneys were removed and cortical glomerular cells were isolated through sieving technique and its stepwise gradient centrifugation in Ficoll solution and the supernatant incubated in DNAse , collagenase and Fetal Bovine Serum to confirm the presence of LPS-FITC in glomerular tissue homogenate, by flow cytometry. Before, the glomerular fixed material in the lamina technique of spin cell was analyzed to verify the absence of circulating blood cells. Histological evaluation by light microscopy performed in the control group underwent extracorporeal perfusion was carried out to demonstrate that tubules and glomeruli had maintained its integrity.To exclude systemic effects of LPS on the kidneys did the extracorporeal perfusion for 1-2 hours with preservation Euro-Collins (EC) solution and solution EC + LPS of E. coli concentrations of 0.10 mg/ml, 0.100 mg/ml and 1mg/ml , and the kidney subsequently stored at -80°C for determination of Ang I /II and renin activity. Homogenate of renal cortex and medulla are isolated and incubated with proteinase inhibitors and decapeptides excess for determination of Ang I /II and to measure renin activity in high performance liquid chromatography (HPLC). Furthermore, fragments of kidney tissue will be fixed and analyzed by immunohistochemistry (IHC) for RAS.
Results: The evaluation of the blades showed absence of blood circulating cells in glomerular homogenate, n=8. The fluorescence in flow cytometry showed a higher mean fluorescence intensity of glomerular cells in the renal cortex from animals treated with FITC-LPS,n=4, compared with animals control groups, n=4.Histological evaluation by light microscopy performed in the control group underwent extracorporeal perfusion showed that tubules and glomeruli had maintained its integrity. From measuring HPLC, we ruled out Ang I and II degradation, as levels of their metabolic fragments were also significantly decreased by LPS. ACE activity was slightly increased following LPS administratin. On the other hand, renin activity was significantly inhibited, as Ang I concentration elevation following exogenous angiotensinogen administration was blunted by LPS(70%)
Conclusions: So far we proved that circulating LPS-FITC is retained in renal glomerular tissue. We seek to determine that LPS, by direct action, can inhibit the RAS and be held responsible for changes in glomerular microcirculation and initial decline glomerular filtration rate in severe sepsis.

SP078 PROTECTIVE EFFECT OF SILDENAFIL CITRATE IN ISCHEMIC ACUTE KIDNEY INJURY DEPENDS ON RENAL DAMAGE EXTENSION

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Introduction and Aims: The renal ischemia-reperfusion injury pathophysiologycal processes include generation of reactive oxygen and reactive nitrogen species (ROS and RNS) besides the induction of protection mechanisms, such as the expression of heme oxygenase-1 (HO-1) enzyme. HO-1 is a protective enzyme against diverse insults in assorted tissues. Sildenafil citrate (SIL), a phosphodiesterase type-5 inhibitor, catalyzes the breakdown of cGMP.This study evaluated the effect of SIL in protecting kidney function in a time dependent ischemic AKI animal model.
Methods: Adult, male, Wistar rats weighing between 260 - 310 g were divided into following groups: SHAM, Ischemia 30 min (renal pedicles clamping for 30 min), Ischemia 30 + SIL (SIL 0.25 mg/kg 60 min before renal ischemia), Ischemia 45 min (renal pedicles clamping for 45 min), Ischemia 45 + SIL. Renal function (serum creatinine-sCr, inulin clearance-iClear, sodium fractional excretion-FENa), renal blood flow (RBF), oxidative injury (urinary peroxides - PU, thiobarbituric acid reactive substances - TBARS, nitric oxide - NO and thiols in renal tissue), RNA extraction and quantitative PCR of HO-1 and kidney histological analysis (fractional interstitial area - FIA and tubuleinterstitial injury) were evaluated.

SP078 Renal function, oxidative index. $\alpha p<0.05$ vs SHAM, β vs Isc30 and δ vs Isc45.

	SHAM (n=7)	Isc30 (n=6)	Isc30+SIL (n=6)	Isc45 (n=5)	Isc45+SIL (n=5)
sCr(mg/dl)	0.3± 0.1	2.1±0.3α	0.8±0.2αβδ	2.9±0.2α	2.4±0.2α
iClear(ml/min)	0.63±0.04	0.32 ±0.04α	0.50±0.04αβ	0.16 ±0.02αβ	0.31±0.03αδ
FENa(%)	0.29±0.03	2.85±0.50	0.68±0.11	9.17 ±1.38αβ	4.16.12αδ
RBF(ml/min)	10.4±0.8	4.0±0.3α	7.5±1.0β	2.3±0.1α	5.4±0.5αδ
UP (nmol/g Cr)	1.7±0.1	5.9±0.2α	2.3±0.3βδ	9.7±1.8αβ	4.0±0.6δ
Thiols(nmol/mg prot)	170±16	117±11α	111±11α	129±17α	68±12αδ
TBARS(nmol/g Cr)	57±7	114±16α	48±2β	117±33α	45±7βδ
NO(μmol/g Cr)	30.2±2.2	76.6±6.6α	33.5±3.5β	76.2±14.5α	46.9±5.3δ

Results: SIL treatment in 30 and 45 min ischemia models ameliorated renal function and RBF, decreased the oxidation metabolites, NO levels and the expression of HO-1. Histology studies showed that SIL reduced FIA and tubuleinterstitial injury at both ischemia models.
Conclusions: The study concludes that the functional and histological damage extension induced by the time of ischemia determines SIL protective effect in AKI, which mechanisms involve HO-1 and NO.

SP079 KIDNEY OXYGENATION DURING ACUTE SALINE VOLUME LOADING IN UNANESTHETIZED RATS

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Introduction and Aims: Administration of radiocontrast agents in a clinical setting is accompanied by the risk of development of acute kidney injury (AKI). Radiocontrast administration is associated with renal medullary tissue hypoxia, which is thought, in turn, to contribute to the pathogenesis of AKI. Individuals at risk of AKI are acutely volume loaded prior to radiocontrast administration. This reduces their risk of AKI. We hypothesized that acute volume loading increases outer medullary oxygen tension (PO2), which might explain its efficacy in the clinical setting as a prophylactic treatment to prevent radiocontrast-induced AKI.
Methods: Experiments were conducted using 10 - 12 week old male Sprague-Dawley rats (n= 18). A carbon paste electrode (270 μm diameter) attached to the telemetry transmitter was inserted 4 mm below the kidney surface, into the outer medulla, under isoflurane anesthesia. This allowed continuous measurement of outer medullary PO2. The left carotid artery and the right jugular vein were catheterized to allow measurement of mean arterial pressure and infusion of saline and radioactive markers respectively. After a 1 week recovery period, freely moving rats received isotonic saline acutely, at a rate of 1 ml/kg/min over 40 min, either in a metabolic cage where clearance studies were conducted or in their home cage where outer medullary tissue PO2 was measured. Glomerular filtration rate (GFR) was measured by the clearance of [3H]-inulin and effective renal plasma flow (eRPF) was measured by the clearance of [14C]-paraminohippurate.
Results: Compared to a 165 min control period, urine flow (n=18) and sodium excretion (n=7) had increased by 176±40% and 194±40% respectively during the period 15 to 180 min after volume loading was completed. However, outer medullary tissue PO2 did not change significantly (n=5, 2±3% change). GFR (n=9) was increased by 67±31% and total sodium reabsorption (n=4) tended to increase (P=0.075, 107 ±36%) Neither mean arterial pressure, heart rate, hematocrit, eRPF nor effective renal blood flow changed significantly in response to acute saline loading.
Conclusions: The absence of a detectable increase in outer medullary tissue PO2 in response to acute saline loading could be explained by the absence of increased renal oxygen delivery (as indicated by unchanged total renal blood flow and hematocrit) and increased, rather than decreased, renal oxygen consumption (as indicated by increased total sodium reabsorption). Effects of saline loading on responses of outer medullary PO2 to radiocontrast administration remain to be determined.

SP080 EFFECT OF TADALAFIL IN A RAT MODEL OF RENAL ISCHEMIA-REPERFUSION INJURY

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Introduction and Aims: The pathophysiology of ischemic acute kidney injury is very complex and still not completely understood. Experimental models of renal ischemia-reperfusion (IR) injury in uninephrectomised rodents are widely used to study the effect of therapeutic and preventing strategies. Previous studies demonstrated that Tadalafil, a selective phosphodiesterase type 5 inhibitor, attenuates renal IR injury

by decreasing leukocytes infiltration (1) or by reducing urinary injury markers (2). The aim of our study was to evaluate the effect of Tadalafil on kidney function and histological lesions after IR injury in uninephrectomised rats.
Methods: In uninephrectomised male Sprague Dawley rats, we induced IR injury by clamping the left kidney pedicle for 45 minutes followed by reperfusion for 24 hours. Renal function was determined by plasma and urine creatinine and urea measurements. Kidney histological lesions were evaluated by hematoxilin eosin staining and histopathological analysis. Tadalafil or its vehicle (0.5 % methylcellulose and 0.05 % Tween 80 in distilled water) were administrated by gavage (10 mg/kg p.o.) at 24 hours and 15 minutes prior to ischemia. Sham-operated animals underwent the same surgical procedure and vehicle treatement without clamping of kidney pedicle.
Results: Our results showed that, 24 hours after reperfusion, renal IR injury in nephrectomised rats induced a significant increase of controlateral kidney weight (IR: 1.8 ± 0.08 g vs sham:1.4 ± 0.06 g, P<0.01) as well as an increase of plasma levels for creatinine (IR: 272.6 ± 24.02 µM vs sham: 43.3 ± 1.4 µM, P<0.001) and urea (IR: 26.53 ± 2.40 mM vs sham: 4.58 ± 0.11 mM, P<0.001) compared to sham-operated animals. In parallel, we also observed a significant decrease of creatinine excretion in urine (IR: 4903 ± 857 vs sham: 13455 ± 1820 µmol/mg of proteins, P<0.01) and urea excretion (IR: 308.8 ± 56.9 vs sham: 1138.0 ± 138.9 mmol/mg of proteins, P<0.001) in IR rats compared to sham, showing an impaired kidney function. Tadalafil treatment had no effect on both creatinine and urea plasma levels and excretion in urine, as well as on kidney weight compared to IR vehicle treated animals. Histological analysis revealed adequate and successful lesion induction in IR animals compared to sham, including tubular degeneration and necrosis, tubular dilatation and cast formation. In contrast with previously published literature, our result showed that Tadalafil had no effects on tubular lesions.
Conclusions: Previous studies demonstrated that Tadalafil attenuates renal IR induced acute kidney injury. However, these data were obtained in the early phase of reperfusion (60-240 minutes). In the same experimental conditions, our study shows that Tadalafil has no beneficial effect at 24 hours of reperfusion, suggesting the important role of therapeutic window for drug treatments.
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2) Sohotnik R et al, Am J Physiol Renal Physiol, 304:F1099-104, 2013.

SP081 THE STRATEGY OF RENIN-ANGIOTENSIN SYSTEM BLOCKERS REDUCE THE INDOXYL SULFATE-MEDIATED RENAL DAMAGE ON THE DURATION OF ACUTE KIDNEY INJURY

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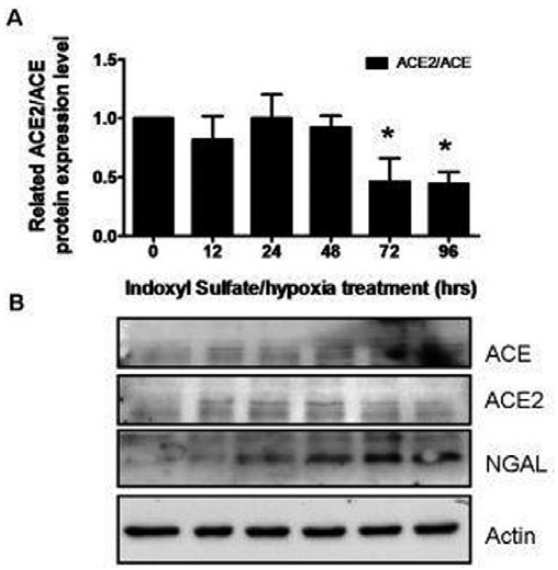
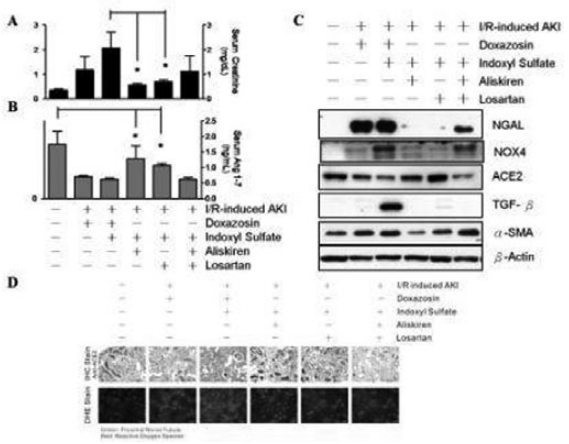
Introduction and Aims: Renal recovery from acute kidney injury (AKI) is often not achieved because of accompany with new injuries during the repair phase. Indoxyl sulfate (IS), a potential vascular toxin retains in AKI patients could significantly activate most of the intra-renal renin-angiotensin system (RAS) components. However, the use of RAS blockers such as direct rennin inhibitor (DRI) and angiotensin II receptor blockers (ARB) in the perioperative period remains controversial. The inappropriate activation of the RAS contributes to imbalance of ACE/AngII/AT1 axis over ACE2/Ang1-7/MAS axis and promotes oxidative stress, fibrosis, inflammation, EMT and dedifferentation after renal injury. Here we examined renal protective effects of direct renin inhibitor (DRI) and angiotensin II receptor blockers (ARB) in the IS-mediated AKI.
Methods: Human proximal tubular epithelial (HK2) cells were exposed to 1 mM IS and hypoxia condition in the absence or presence of DRI (20 nM Aliskiren) or ARB (200 nM Losartan) for 72 hours. The mice with IS-mediated AKI, induced by unilateral renal ischemia/reperfusion injury and IS (100 mg/kg/day, from day 1 to 3), were randomly divided into 5 groups: the Sham group, the Model group, the Aliskiren group (25 mg/kg/day), the Losartan group (10 mg/kg/day) and the Combination-Treatment group.
Results: In HK2 cells, most RAS components except ACE2 were up-regulated when expose to IS and hypoxia condition including angiotensinogen, ACE, angiotensin receptor type 1 and MAS receptor. In contrast to ACE, we found that ACE2 represent a bidirectional way which is increased during the early stage but decreased near-baseline levels at the later stage (Figure 1). The reduction of ACE2 in vitro and in vivo was accompanied with up-regulating NGAL which is reversed by Aliskiren or Losartan. These rescued effects by RAS blockers were inhibited by A-779 which is MAS receptor antagonist. IS-mediated AKI mice exhibited a lower serum Ang 1-7 ratio and renal ACE2 protein expression, higher serum BUN and creatinine, increased renal NOX4, TGF-beta1, alpha-SMA and phospho-p44/42 protein expression compared to administration with Aliskiren or Losartan groups (Figure 2). Furthermore, the rescued effect of RAAS blockers was less marked in combination-treatment groups compared with Aliskiren or Losartan only groups.
Conclusions: Individual RAS blocker including Aliskiren or Losartan could enhance ACE2/Ang1-7/MAS axis by up-regulating ACE2 protein expression, thereby inhibiting oxidative stress, inflammation and EMT in the kidney, as well as inhibiting ERK signaling pathway in vivo. Dual RAS blockade treatment yields no additional effect in renal protection but may impair the ACE2/Ang1-7/MAS signaling. Results from cell and animal studies do not support the use of combination therapy with DRI and ARB.



SP081 Figure 1:



SP081 Figure 2:

SP082 RENIN ANGIOTENSIN SYSTEM INDUCES RENAL INFLAMMATION VIA RENAL TLR2 ACTIVATION IN EXPERIMENTAL UNILATERAL URETERAL OBSTRUCTION

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Introduction and Aims: Although Toll-like receptor 2(TLR2) may play an important role, inhibition of TLR2 has not shown consistent results of amelioration in renal inflammation of obstructed kidney. There have been some reports that renin angiotensin system (RAS) may affect the activation of TLR signaling. However, there was few study for the relationship between RAS and renal TLR2 activation in experimental unilateral ureteral obstruction(UUO). We investigated the effect of RAS on the activation of renal TLR2 in UUO.
Methods: Male wild type and TLR2 knokout(KO) mice backgrounded C57BL/6 were divided into the 8 groups; 1)Sham, 2)Angiotensin II(Ang II)+ Sham, 3)AngII+TLR2 KO, 4)Aliskiren+Sham, 5)Aliskiren+TLR2 KO, 6)UUO only 7)TLR2 KO UUO, and 8) Aliskiren + TLR2 KO UUO. Ang II and aliskiren were administrated via an osmotic minipump(AngII;1,000ng/kg/min for 12 days, Aliskiren; 25 mg/kg/day for 8days). We performed realtime RT PCR and immunohistochemistry for molecular study and H&E stain and Masson trichrome (MT) stain for histologic examination of kidneys.

Results: Ang II increased the renal mRNA expression of TLR2 in wild type mice ($p < 0.05$). Ang II-infused TLR2 KO mice kidney showed significantly lower mRNA expressions of osteopontin(OPN) and TGF-β compared to those of Ang II-infused wild type mice. In TLR2 KO UUO kidneys, there were no differences of MCP-1, OPN and TGF-β mRNA expressions and renal histology compared with UUO kidneys of wild type mice($p < 0.05$, $p < 0.05$, respectively). The renal renin mRNA expression in TLR2 KO UUO was significantly higher than that of UUO kidneys of wild type mice ($p < 0.05$). Renin inhibition by aliskiren decreased the mRNA expressions of MCP-1, OPN and TGF-β, all of which were upregulated in TLR2 KO UUO kidneys (all, $p < 0.05$). Aliskiren also significantly reduced renal tissue injury score, MT-stained area, and immunostained area of TGF-β in TLR2 KO UUO kidneys ($p < 0.05$).
Conclusions: Although TLR2 inhibition did not attenuate renal inflammation, inhibition of RAS attenuates renal inflammation in TLR2 KO UUO kidneys. It is speculated that RAS may modulate renal TLR2 activation in experimental unilateral ureteral obstruction.

SP083 NAFAMOSTAT MESYLATE ATTENUATES ISCHEMIA-REPERFUSION INDUCED RENAL INJURY VIA INHIBITION OF APOPTOSIS

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Introduction and Aims: It has been reported that nafamostat mesylate (NM) inhibited inflammatory injury via inhibition of complementary activation in ischemic heart, liver and intestine. However, it has been little known that NM inhibits the apoptosis in ischemia-reperfusion (IR) injured kidney. We investigated whether NM attenuates IR renal injury and involves apoptosis inhibition.
Methods: We used HK-2 cell and male C57BL/6 mice. C57Bl/6 mice were divided into four groups; Sham, Nafamostat mesylate(NM,2mg/kg)+Sham, IR injury(IR injury; reperfusion 27 minutes after clamping of both renal artery and vein), and NM+IR injury. Kidneys were harvested 24hr after IR injury. BUN and serum creatinine(s-Cr) were measured 24 hrs after IR injury. We performed real time RT-PCR and immunohistochemistry for molecular study and H&E stain and Masson trichrome (MT) stain for histologic examination. For *in vitro* study, HK-2 cell were divided as into three groups; Control, IR-HK-2(HK-2 **cells**were incubated for 6 hours with **mineral** paraffin **oil for ischemic injury**) and **IR-HK-2+NM(2nM)** groups**. Cell survi**val and the magnitude of **apoptosis** were **evaluated**.
Results: BUN**, s**erum creatinine level and renal tissue injury score in NM+IR injured mice were significantly lower than those of control IR mice (all, $p<0.01$). NM treatment significantly improved cell survival in ischemic HK-2 cells ($p<0.01$). Renal Bax protein and mRNA expression were significantly increased in IR injured kidneys and ischemic HK-2 cells. NM treatment significantly decreased renal Bax expression ($p<0.05$). Renal Bcl-2 protein and mRNA expression were significantly decreased in IR kidney and ischemic HK-2 cells compared to those of sham and control groups. NM treatment increased renal Bcl-2 expression in IR injured kidneys and ischemic HK-2 cells ($p<0.05$). TUNEL positive cells were significantly lower in NM+IR injured kidneys, comparing to control IR injured mice ($p<0.05$).
Conclusions: In conclusion, NM attenuates ischemia-reperfusion renal injury via inhibition of apoptosis.

SP084 MELITTIN INDUCED ACUTE KIDNEY INJURY THROUGH TNF-A/NF-KB/MITOCHONDRIA DEPENDENT APOPTOTIC PATHWAY

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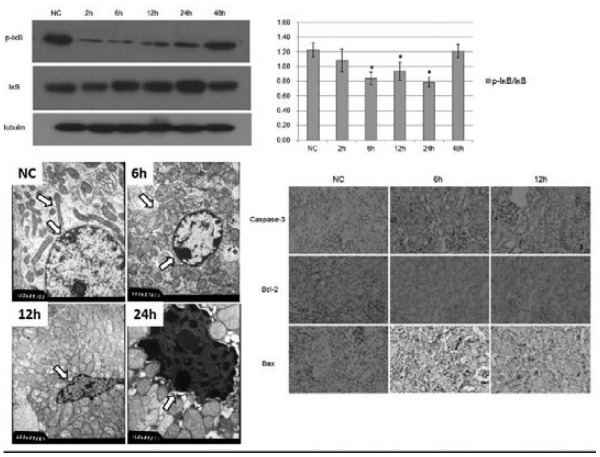
Introduction and Aims: Mechanism of acute kidney injury (AKI) following multiple bee stings is not clear. Melittin, a main component of bee venom, can induce apoptosis in many cell types. We aimed to investigate the mechanism of melittin induced apoptosis in renal tubular epithelial cells through TNF-α/NF-κB signaling pathway.
Methods: AKI model was established by injecting melittin (4.0 μg/g) through the caudal veins of BALB/c mice (n=60). AKI was defined as creatinine (Cr) increased over 2 fold of that in control group. Blood and kidney samples were collected at 0h, 2h, 6h, 12h, 24h and 48h. Cr, urea nitrogen and hemoglobin were measured by Abbott i-STAT blood-gas analyzer. Creatine kinase was measured by immunochemiluminometric assays. Serum TNF-α was measured by ELISA method. Apoptosis of renal tubule cells was detected by TUNEL staining and transmission electron microscope. Expression of caspase-3, caspase-8, caspase-9, Bcl-2, Bax, and cytochrome c was detected by RT-PCR and/or immunohistochemical method. Expression of IκB and p-IκB was detected by Western Blot.
Results: AKI was diagnosed at 6h after injection of melittin (Cr: 1.1±0.3 mg/dl at 6h vs. 0.4±0.1 mg/dl at 0h, $p<0.05$). Serum TNF-α level increased significantly since 2h after injection. Apoptosis was detected since 2h, and was most notable at 12h. Western Blot showed that p-IκB/IκB ratio was significantly decreased in melittin group during 6h to 24h, indicating that NF-κB signal pathway was inhibited. RT-PCR showed that the Bcl-2/Bax ratio was significantly lower in melittin group than control group, indicating that the mitochondrial dependent apoptosis pathway was activated.
Conclusions: We successfully established melittin induced AKI in mice. Melittin could induce mitochondrial dependent apoptosis in renal tubular epithelial cells through activating TNF-α and inhibiting NF-κB signaling pathway.



SP084

SP085 ENHANCED NITRIC OXIDE PRODUCTION AMELIORATES ACUTE KIDNEY INJURY IN EXPERIMENTAL ARISTOLOCHIC ACID NEPHROPATHY

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Introduction and Aims: Aristolochic acid nephropathy (AAN), a progressive tubulointerstitial injury of toxic origin, is characterized by early and transient acute tubular necrosis, followed by fibrosis and tubular atrophy leading to loss of renal function. A reduced NO production has been demonstrated, which may disturb the regulation of renal function. The present study tested the hypothesis that L-Arginine supplementation may restore renal function and reduce renal injury after aristolochic acid (AA) intoxication.
Methods: C57BL/6J male mice were randomly subjected to ip injection of either sterile saline solution (control) or AAI (2,5mg/Kg) for 5 days. To determine if the renal AA-induced injuries were related to NO reduction, L-Arginine (L-Arg), a substrate for NO synthase, was supplemented in drinking water.
Results: Mice intoxicated with AAI displayed polyuria, significantly increased plasma creatinine level, proteinuria and FENa+ ($P<0.05$). As compared to controls, histological analyses showed severe proximal tubular cell necrosis, renal inflammation and increased oxidative stress ($P<0.05$). These lesions were associated with a significant reduction of NO bioavailability, measured by the urinary nitrite/nitrate (NOx) excretion. L-Arg supplementation in AA-treated mice significantly improved kidney function, as attested by significant reductions in plasma creatinine level, urine volume, proteinuria and FENa+ (see Table). Moreover, L-Arg treatment resulted in a significant reduction of tubular cell necrosis score, as well as in reduced renal inflammation and oxidative stress along with a normalized NO bioavailability (see Table). These results suggest that a preservation of the NO concentration leads to a kidney protection in AAN.

SP085 Renal functional parameters and NOx excretion in experimental groups

	Control (n=6)	AA (n=8)	AA+L-Arg (n=8)
Plasma creatinine level(mg/dl)	0.16±0.01	0.58±0.03*	0.40±0.03&
Urine volume(ml/24h)	0.54±0.09	1.70±0.34*	1.01±0.13
Urinary protein level(mg/mg CRE)	1.58±0.14	22.10±2.03*	15.11±1.6&
Fractional excretion of sodium - FE Na (%)	0.30±0.02	1.43±0.26*	0.82±0.06&
Urinary NOx level (μmol/mg CRE)	2.53±0.15	1.49±0.20*	2.71±0.20&

Statistical analysis : ANOVA test. *P<0.05: AA vs Control, &P<0.05 : AA+L-Arg vs AA.

Conclusions: In the present mouse model of acute AAN, NO seems to act as a strong mediator of renal function. Increased NO bioavailability by L-Arg supplementation was demonstrated beneficial in improving renal injury in AAN.

SP086 CELL CYCLE ARREST IS ASSOCIATED WITH ACQUIRED RESISTANCE TO RECHALLENGE INJURY IN RATS RECOVERED FROM RENAL DAMAGE WITH URANYL ACETATE

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Introduction and Aims: Rats that recovered from renal damage with uranyl acetate (UA), which mainly injures S3 segment of proximal tubule (PT), can develop resistance to subsequent UA insult with less morphological and functional damages. We investigated cell cycle status and progression in this acquired resistance model.
Methods: Normal rats and rats 14 days after recovering from renal damage, which was induced by 1 mg/kg of UA, were injected with 38 mg/kg of lead acetate (a proliferative stimulus) or 4 mg/kg of UA. Isolated tubular cells after the second stimuli were separated into PT and distal tubule (DT) cells by Percoll density-gradient centrifugation. The cell cycle status was analyzed by flow cytometry. G0 and G1 phase cells were separated by Hoechst33342/Pyronin Y method or immunohistochemistry for Cdt1 (a G1 phase marker).
Results: Cell cycle status in PT cells acquiring resistance was comparable to that in normal PT cells. When compared to the control group, delayed G0-G1 transition and less increased S phase cell number were found in response to lead acetate in the resistance group, indicating repressing S phase progression. PT cells stayed at G1 phase for longer time and increased G2/M phase cell number without increased S phase cell number were found in response to UA in resistance group, comparing to control group.
Conclusions: G1 arrest was induced in PT cells recovered from renal damage with UA in response to proliferative stimulus or injurious stimulus with UA. Induction of inhibiting proliferation pathway may closely link to inhibiting cell death pathway in PT cells with the acquired resistance.

SP087 THE PURIFIED MICRONIZED FLAVONOID FRACTION EFFECT IN THE SEPSIS-ASSOCIATED ACUTE KIDNEY INJURY

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Introduction and Aims: Sepsis is a severe inflammatory response accompanied by a depression in immunological function which causes multiple organ injury. Sepsis-associated acute kidney injury (AKI) pathophysiology induces renal vasoconstriction, ischemia and acute tubular apoptosis. The concept of renal vasoconstriction and kidney ischemia as a key pathogenic factor is certainly valid for sepsis models. Sepsis-associated AKI may result from global renal hypoperfusion, which is locally mediated by the upregulation of pro-inflammatory cytokines (TNF-α and IL-6), with subsequent tubular cell apoptosis also in distan organs. The purified micronized flavonoid fraction is being mentioned to improve venous tone and reduce capillary hyperpermeability by protecting the microcirculation from inflammatory processes. The aim of this study is to evaluate the effect of the purified micronized flavonoid fraction (Diosmin) on the creatinine clearance, urinary peroxides and the kidney histology in sepsis associated AKI.
Methods: Adult male Wistar rats, weighing 250-300g were used. Renal function (creatinine clearance, Clcr), urinary peroxides (UP, FOX-2), kidney, lung and intestinal tract levels of TNFα and IL-6 (ELISA) and kidney histology were evaluated. Sepsis was induced by cecal ligature and puncture (CLP). Groups: Sham (without CLP); Sepsis (CLP); Sepsis+Diosmin (Diosmin 3mg/Kg 30 minutes before CLP).
Results: A similar quantitative inflammatory response in distant organs and in the kidneys was observed in CLP animals. CLP induced a decrease in mean arterial pressure, body temperature and creatinine clearance, accompanied by a prominent increase in UP. These paramethers were significantly changed in the pretreated Diosmin group. In the kidney histology, loss of brush border was evident in sepsis group and it was observed a severe dilation of the tubular lumen after CLP.
Conclusions: Sepsis is an injury repair stereotyped response across remote organs. Distant organ inflammation can be considered a significantly mediator of sepsis-associated AKI as shown in this study. These data provide a new insight about Diosmin attenuating injury effect in the sepsis AKI.

SP087 Renal function, inflammatory response. α p<0.05 vs SHAM, β vs Sepsis.

	SHAM	Sepsis	Sepsis+Diosmin
Cr Cl (ml/min)	0.60±0.01	0.20±0.06α	0.35±0.03β
UP (nmol/g Cr)	3.5±0.7	13.9±5.1α	7.2±3.1β
TBARS (nmol/g Cr)	4.4±2.2	13.0±3.4α	7.5±2.8β
IL−6 intestine	11.9±1.6	14.2±3.1	11.7±4.5
IL−6 kidney	13.3±2.0	19.4±5.8	21.5±9.1
IL−6 lung	00±00	3.9±3.2α	0.7±0.8
TNF−α intestine	4.2±0.9	4.3±2.5	1.3±0.9β
TNF−α kidney	9.2±2.7	9.7±1.4	7.9±1.3
TNF−α lung	0.2±0.3	0.2±0.1	00±00

SP088 THERAPEUTIC S100A9 BLOCKADE IN EXPERIMENTAL GLOMERULONEPHRITIS

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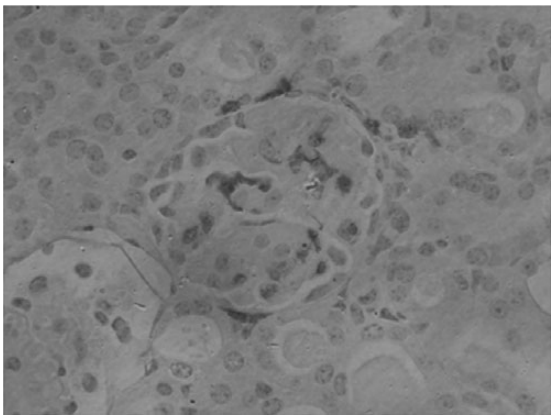
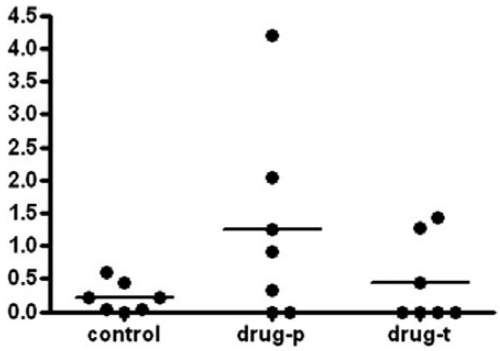
Introduction and Aims: The S100A9 protein has been identified as one target molecule of paquinimod, a quinoline-3-carboximide compound, disrupting binding this protein to the proinflammatory receptor toll-like receptor 4. The S100A9 in association with S100A8 forms the heterodimer S100A8/S100A9, named calprotectin. Calprotectin is found abundantly in both neutrophils and monocytes and has been demonstrated to be upregulated in patients with vasculitis ANCA positive (Pepper et al 2013, Kidney International). Moreover, our group demonstrated that S100A9 knock-out mice have significantly less glomerular disease than wild type counterparts using the nephrotoxic nephritis (NTN) model of immune mediated glomerulonephritis (Pepper et al submitted). The aim of this study was to assess the efficacy of paquinimod in prevention and treatment of murine NTN model using histological, urinary and serum markers
Methods: 21 C57BL/6 mice had NTN induced. Animals were pre-immunized with sheep IgG at day -5, followed by intravenous injection of sheep nephrotoxic serum at day 0. Paquinimod (25mg/kg), dissolved in drinking water, was started at day 0 (prevention group) or day 2 (treatment group). Mice were sacrificed 8 days later. 24 hours prior the sacrifice, mice were placed in individual metabolic cages and urine was collected. Proteinuria was quantified using the sulphosalicylic acid assay. Creatinine,



SP088 Figure 1: Immunohistochemistry of renal calprotectin expression



SP088 Figure 2: Calprotectin score in control and treated groups

urea, ALT and albumin were measured in the serum. Kidneys were removed, fixed in formalin and embedded in paraffin for periodic acid-Schiff staining and immunohistochemical staining for infiltrating macrophages and calprotectin expression. Histological analysis included enumerating the percentages of crescents and degree of glomerular thrombosis on the PAS stained sections.
Results: By day 8, 4 mice had died; 2 from the control group, 1 from the prevention group and 1 from the treatment group. In relation to proteinuria, Creatinine, Urea, ALT or albumin we could not find any statiscally significant differences between the 3 groups. Analyzing the renal histology in the PAS staining, we could not find any significant differences in the percentages of crescents or the thrombosis score, but we could see higher levels in the prevention group.We found an increase of the calprotectin expression in the prevention group that was statistically significant comparing to control group (p: 0.05) and slight increase in the treatment group (not statiscally different). In the macrophages staining we could not find any significant differences in the expression of MAC2 antibody between the three groups, but again a slight increase in the groups that we administered paquinimod.
Conclusions: Our results show that using a high dosing of paquinimod we did not successfully prevent or treat mice with nephrotoxic nephritis as assessed by levels of glomerular thrombosis, serum creatinine and urea, number of crescents and degree of proteinuria. Interestingly, an increase in glomerular macrophages and calprotectin expression was found in the paquinimod treated cohorts, in keeping with previous reports of temporary augmentation of inflammation during early paquinimod treatment. One explanation maybe the use of LPS in this model in association with a S100A9 inhibition, which may have resulted in an increase of S100A8 expression, and loss of S100A9 regulation of S100A8 and a subsequent higher influx of inflammatory renal cells.

SP089 EVALUATION OF THE ANTIOXIDANT AND RENOPROTECTIVE EFFECTS OF ELLAGIC ACID ON ISCHEMIA / REPERFUSION INDUCED NEPHROPATHY IN RATS

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Introduction and Aims: Renal ischemia-reperfusion injury (IRI) is one of the important cause of acute kidney injury (AKI). Reactive oxygen species and inflammatory cytokines play major role in the pathogenesis of IRI. Ellagic acid (EA), a phenolic compound, have shown to exert antioxidants, anti-inflammatory, anticarcinogenic, antihyperlipidemic effects. We aim to evaluate, the effect of Ellagic Acid on Renal Ischemia / Reperfusion induced nephropathy in rats.
Methods: Twenty-eight male Sprague-Dawley rats were divided into four groups; control, control + EA, IR, and IR + EA. EA (85 mg/kg, perorally) was administered 60 min prior to the ischemia. Rats were unilaterally nephrectomized and subjected to 45 min of renal pedicle occlusion followed by 60 min of reperfusion. Both groups were subsequently studied by renal function tests, oxidant and antioxidant parameters, and kidney histology.
Results: Serum/kidney TAC, NO and Paraoxonase levels were significantly higher, while serum urea and creatinine, serum/kidney MDA and TOS were significantly lower in IR + EA group compared to IR group (p<0.05). Renal function tests, oxidant and antioxidant parameters of the groups are presented in Table 1. Histopathologic examination revealed that less deterioration in glomerular structure, atrophic structure, and diffuse hydropic degeneration in the proximal and distal tubules in IR+EA group compared to IR group (score 2 vs. score 3, respectively, Figure 1, 2).
Conclusions: Ellagic acid contributed to the amelioration of renal ischemia-reperfusion injury by reducing oxidative stres and preventing histological injuries.

SP089 Table 1. Results of renal function tests, oxidant and antioxidant parameters

Parameters	Control (n=7)	Control + EA (n=7)	IR(n=7)	IR + EA(n=7)
Serum				
Urea (mg/dl)	43,5±7,41	40,85±5,87	108,71±5,87	68,14±15,44
Creatinine (mg/dl)	0,60±0,06	0,50±0,06 a	1,04±0,12	0,78±0,11
MDA (mmol/ml)	34,42±12,28	15,85±3,07 a	107,14±40,92	58,28±11,22b
TOS (µmol /L)	60,86±49,77	22,03±5,94 a	331,06±99,34	194,08±41,57b
TAC (mmol /L)	0,76±0,32	1,48±0,35 a	3,25±4,76	11,87±2,72b
NO (µM)	4,57±2,07	60,42±10,73 a	29,85±18,09	140,14±138,36b
Paraoxonase (U/L)	8,76±4,56	16,98±4,46 a	36,01±17,42	60,68±11,93b
Kidney Tissue				
MDA (mmol/ml)	117,57±13,18	60,87±14,33 a	185,14±7,08	83,71±10,98b
TOS (µmol /L)	213,56±59,60	113,57±16,63 a	398,63±109,76	233,06±54,36b
TAC (mmol /L)	8,40±6,86	35,41±14,39 a	6,17±4,44	34,04±22,11b
NO (µM)	35,57±11,90	60,42±10,73 a	89,00±47,03	203,57±100,03b
Paraoxonase (U/L)	27,25±8,88	38,82±8,58 a	44,62±19,03	74,47±9,87b

Note: MDA: malondialdehit; TOS: total oxidant status; TAC: total antioxidant capacity; NO: nitric oxidea P<0.05 vs control group, b P<0.05 vs IR group

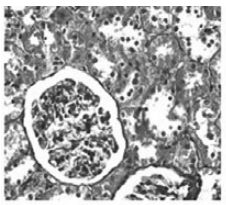

Figure 1. Histopathologic evaluation of the IR+EA group

SP089 Figure 1:

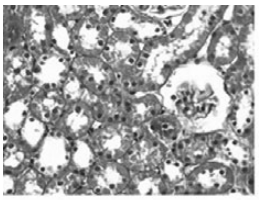

Figure 2. Histopathologic evaluation of the IR group

SP089 Figure 2:

SP090 POLYMYXIN B TOXICITY IN LLC−PK1 CELLS IS MEDIATED BY THE HEME OXYGENASE 1 ENZYME

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Introduction and Aims: In the acute kidney injury, the mechanisms of defense act as "protector genes", as the protein heat shock 32 (HSP 32) known as heme oxygenase 1 (HO 1). The polymyxin B (PMB) is a nephrotoxic antimicrobial. The aim of this study was to distinguish the role of the HO 1 enzyme in the PMB toxicity in LLC−PK1 cells.
Methods: The cells were submitted to the following treatments: Control (CTL− 0µM); PMB (375µM); PMB + Hemin (25µM of hemin, one hour before PMB); PMB + ZnPP (10µM of ZnPP, one hour before PMB). All groups were evaluated in 72 hours. The following parameters were analysed: cellular viability, apoptosis, lactate dehydrogenase (LDH), lipid peroxidation (MDA), genic expression of HO 1 (RT−PCR)and nitric oxide (NO, Griess).
Results: PMB decreased the cellular viability and increased LDH and apoptosis (p<0.05) Hemin improved viability and reduced apoptosis (p<0.05). No changes were observed in MDA and HO-1 genic expression. Additionally, PMB itself or with Hemin or ZnPP increased nitric oxide (NO) (p<0.05).
Conclusions: The PMB was confirmed as a cytotoxic drug by increasing apoptosis, cell damage and reducing viability. Its association with Hemin or ZnPP improved cellular viability by reducing apoptosis, confirming the heme system role associated with NO in this model of toxicity.

SP090 PMB toxicity and HO-1. α p<0.05 vs Control; β vs PMB.

Groups/n	Control(8)	PMB(9)	PMB+Hemin(7)	PMB+ZnPP(7)
Viability(%)	81±5	40±2α	55±1αβ	53±3αβ
Apoptosis(%)	8±2	36±3α	22±1αβ	24±2αβ
LDH(%)	1.7±0.1	22.6±1.7α	21.3±0.7α	22.9±1.2α
NO(µM/mg protein)	0.6±0.1	1.4±0.3	2.9±0.3αβ	2.0±0.1α

SP091 CONDITIONED MEDIUM FROM MESENCHYMAL STEM CELLS PROTECTS HUMAN PROXIMAL TUBULAR CELLS LESIONS BY LPS

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Introduction and Aims: Recent studies emphasize the contribution of mesenchymal stem cells (MSCs) in the regeneration related to acute kidney injury (AKI). MSCs mitigate the damage and / or accelerate the repair and participate in the immunomodulation processes, probably by paracrine pathways. This protection

involves the conditioned medium (CM) that contains soluble factors or microvesicles (MVs). We studied the paracrine effect of MSCs in vitro models of AKI induced by LPS in culture.
Methods: Human proximal tubular cells (HK2) cells were cultured and treated for 24-96 h with LPS (100mg/ml), with or without CM from MSCs. The CM was also previously treated with LPS (100mg/ml) or Gentamicin (GENTA) (2 mM) for 48 h before the experiments and it was employed in frozen form (CMc). Cell proliferation was evaluated by MTT assay.
Results: We observed an increase in cell proliferation in the CM+LPS group compared with the group treated only with LPS (0.12 ± 0.02 DO vs 0.10 ± 0.02 DO, $p < 0.05$), and the major proliferation happened with the CMc+LPS (0.16 ± 0.04 DO, $p < 0.05$). When comparing the groups that received CM stimulated previously it was observed higher proliferation in CM (with GENTA)+LPS (0.16 ± 0.05 DO, $p < 0.05$) than in MC (with LPS)+LPS (0.09 ± 0.02 DO, $p < 0.05$). The latter result was very similar to the LPS group. Comparing only the different CM with HK2 cells in culture, it was measured greater proliferation in MC (with LPS) (0.27 ± 0.07 DO, $p < 0.05$) than with CM and CMc+GENTA (0.21 ± 0.03 DO vs. 0.20 ± 0.06 DO, $p < 0.05$). MC (with GENTA) group showed the lowest proliferation compared with others (0.17 ± 0.02 DO, $p < 0.05$).
Conclusions: These preliminary results suggested that CM from MSCs exerts a protective effect by stimulating cell proliferation during the treatment with LPS. Also, it is possible to "conditioned" the CM and stimulate the higher production of the potential factors produced by MSCs in culture. Moreover, results may indicate that it is possible to minimize AKI induced by LPS without introducing stem cells but only with their CMs, avoiding potential harmful effects of cell therapy. It is clear that more studies are needed to enable the use of CMs stimulated and frozen.

SP092 INVESTIGATING RENAL HANDLING OF HEPCIDIN: IS THERE A ROLE FOR THE MEGALIN RECEPTOR?

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Introduction and Aims: Hepcidin is an iron regulating hormone. Recent studies suggest that urine hepcidin may protect against acute kidney injury after coronary artery bypass surgery by attenuating tubular injury. However, renal handling of hepcidin is unknown.
Methods: A single dose of 10 µg human hepcidin-25 (hhep25) was injected intraperitoneally in C57Bl/6 mice and urine, plasma and tissue were collected after 1h or 24h.
Results: Hhep25 was measured in plasma at 1h after injection, but was undetectable 24h after injection. Presence of hhep25 in plasma led to a compensatory decrease in endogenous hepcidin-1 concentration in plasma (from 5.6±1.4 to 1.4±0.5 nM, $p<0.05$) and a 10-fold reduction in hepatic hepcidin-1 mRNA expression level ($p<0.05$) compared to control, showing that hhep25 is active in mice. In urine, hhep25 was present 1h after injection and the amount of excreted hhep25 increased over 24h, demonstrating glomerular filtration. Immunohistochemistry revealed clear hhep25 staining in the proximal tubules at 1h after injection, which was not present at 24h after injection. Detection of the hhep25 isoforms hhep22 and hhep20 in urine, but not in plasma, shows that hhep25 degradation takes place in the kidney. Administration of gelofusin 5 min prior to hhep25 injection resulted in increased urinary excretion of hhep25 (from 2.4±1.6 to 10.4±5.9 pmol, $p<0.05$) and its isoforms (hhep22: from 4.8 ±3.1 to 16.7±7.1pmol, $p<0.01$; hhep20: from 1.0±0.3 to 2.1±0.7 pmol, $p<0.05$), suggesting proximal tubular reabsorption via megalin.
Conclusions: Hhep25 is excreted in urine via glomerular filtration, after which it can be degraded and/or reabsorbed via megalin.

SP093 BIOMARKERS OF TRANSITION FROM CYCLOSPORINE-INDUCED RENAL DYSFUNCTION TO NEPHROTOXICITY

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Introduction and Aims: Calcineurin inhibitors, in particular Cyclosporin A (CsA), remain the cornerstone of immunosuppressive regimens in many transplantation centres worldwide, regardless of drug-induced nephrotoxicity. The pathogenesis of CsA-induced nephropathy remains to be fully elucidated, but it seems to be affected by the duration of drug exposure. This experimental animal study aimed to clarify the pathways involved in short and long-term CsA-induced nephrotoxicity and the putative biomarkers of transition from cyclosporine-induced renal dysfunction to nephrotoxicity.
Methods: The study comprised 24 male Wistar rats, divided in two models: Short- and Long-term treatments (3 and 9 weeks, respectively). Each model included two rat groups (n=6 each), receiving orally: Control group - vehicle; CsA group - 5 mg/Kg BW/day. Renal function was assessed on serum, urine and kidney tissue samples, through creatinine, BUN, TBARs and NGAL measures, including clearances. Renal tissue was also used to evaluate the gene expression (qRT-PCR) profile of proliferation/fibrosis markers (TGF-β1, PCNA, Ki67, mTOR, TP53 and NF-κβ), as wells as to determine the protein expression by immunohistochemistry of CTGF, KIM-1, mTOR, PCNA, NF-κβ and TGF-β1. Hematoxilin & eosin, periodic acid of schiff and masson's trichrome staining were used to evaluate glomerular, tubular and vascular kidney lesions. Statistical analysis: ANOVA and Bonferroni post hoc test; $p<0.05$ was considered significant.
Results: In short-term treatment, creatinine and BUN levels increased and clearances decreased, accompanied by GFR reduction, but without kidney lesions. Short-term CsA exposure induced PCNA, TGF-β1, NF-κβ and TP53 kidney mRNA up-regulation. In long-term exposure, renal dysfunction (BUN and creatinine clearances) was accompanied by glomerular and tubulointerstitial lesions, associated with remarkable mTOR and Mki67 up-regulation, but without urine and serum NGAL correlation. Moreover, immunohistochemical analysis showed that lesions and expression profile of some proteins were positively associated.
Conclusions: CsA-induced nephrotoxicity is aggravated over time and the results indicate that distinct mechanisms and biomarkers are involved in regulating short or long-term toxicity. Functional impairment starts earlier but it is aggravated with time, while renal lesions only appeared after the long-term exposure, accompanied with significant mRNA up-regulation of Mki67 and mTOR. These findings reinforce the rationale for the early substitution of CsA by less nephrotoxic agents, with the mTORinhibitors as a validated choice, in order to prevent chronic CsA-induced nephrotoxicity.

SP094 CAFFEIC ACID PHENETHYL ESTER PROTECTS AGAINST AMPHOTERICIN B INDUCED NEPHROTOXICITY IN RAT MODEL

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[1]Süleyman Demirel University School of Medicine, Isparta, Turkey, [2]Mevlana University School of Medicine, Konya, Turkey, [3]Isparta State Hospital, Isparta, Turkey, [4]Süleyman Demirel University School of Medicine, Isparta, Turkey, [5]Süleyman Demirel University School of Medicine Department of Internal Medicine, Division of Nephrology, Isparta, Turkey

Introduction and Aims: The present study was conducted to investigate whether caffeic acid phenethyl ester (CAPE), an active component of propolis extract, has a protective effect on amphotericin B induced nephrotoxicity in rat models.
Methods: Male Wistar-Albino rats were randomly divided into four groups: (I) control group (n = 10); (II) CAPE group (n = 9) received 10 µmol/kg CAPE intraperitoneally (i.p.); (III) amphotericin B group (n = 7) received one dose of 50 mg/kg amphotericin B; and (IV) amphotericin B plus CAPE group (n = 7) received 10 µmol/kg CAPE i.p. and one dose of 50 mg/kg amphotericin B. CAPE started one day before the administration of amphotericin B and continued for 7 days. The left kidney was evaluated histopathologically for nephrotoxicity. Levels of malondialdehyde (MDA), nitric oxide (NO), enzyme activities including catalase (CAT) and superoxide dismutase (SOD) were measured in the right kidney.
Results: Histopatological damage was prominent in the amphotericin B group compared to controls, and the severity of damage was lowered by CAPE administration. The activity of SOD, MDA, and NO levels increased and catalase activity decreased in the amphotericin B group compared to the control group ($P=0.0001$, $P=0.003$, $P=0.0001$, $P=0.0001$ respectively). Amphotericin B plus CAPE treatment caused a significant decrease in MDA, NO levels, and SOD activity ($P=0.04$, $P=0.002$, $P=0.0001$ respectively) and caused an increase in CAT activity compared with amphotericin B treatment alone ($P=0.005$).
Conclusions: Amphotericin B toxicity remains high despite developments in drug formulations. The main strategies are based on prevention. The role of oxidative stress in amphotericin B toxicity is clear. Therefore, administration of CAPE seems to be an alternative agent for the management of amphotericin B toxicity. Further clinical studies are necessary for confirmation of these positive effects in clinical settings.

SP095 SIRT2 REGULATES LIPOPOLYSACCHARIDE-INDUCE RENAL INFLAMMATION

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Introduction and Aims: SIRT2 an NAD-dependent histone deacetylase, plays important roles in genomic instability, carcinogenesis and genomic instability. However, its role in renal inflammatory injury has not yet been demonstrated.
Methods: In this study, we explored the expression pattern of CXCL-2 and CCL-2 in kidney tissues from *sirt2*$^{-/-}$ and *sirt2*$^{+/+}$ mice and in MPT cell lines after treatment with lipopolysaccharide.

Results: CXCL-2 and CCL-2 was significantly down-regulated at both the mRNA and protein levels in kidney of *sirt2*$^{-/-}$ mice than *sirt2*$^{+/+}$ mice after lipopolysaccharide. CXCL-2 and CCL-2 expression was decreased in MPT cells treated with the SIRT2-siRNA compared with the blank control. Overexpression of SIRT2 using adenovirus significantly increased the expression of CXCL-2 and CCL-2 in lipopolysaccharide-treated MPT cells. Moreover, down-regulation of SIRT2 in the kidney increased the acetylation of p65 of nuclear factor kappa- B.
Conclusions: Decrease of SIRT2 was associated with increase of renal proinflammatory cytokine CXCL-2 and CCL-2 and regulation of SIRT2 might be an important target for renal inflammatory injury.

SP096 INFUSION OF RECOMBINANT IL-6 IN HEALTHY HUMANS ELEVATES PLASMA NGAL CONCENTRATIONS WITHOUT A REDUCTION IN RENAL FUNCTION

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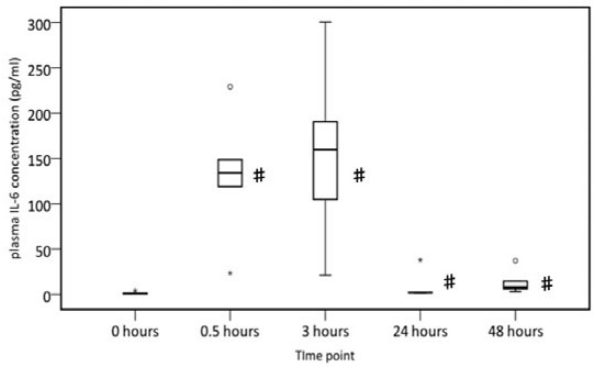
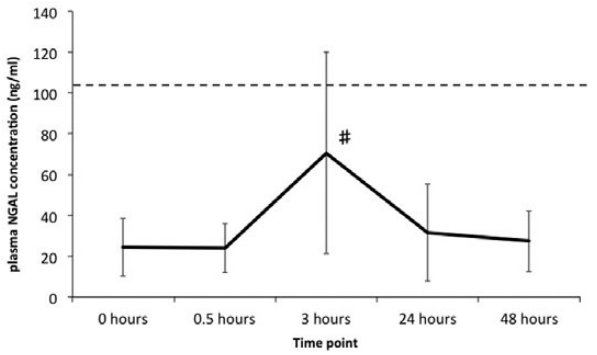
Introduction and Aims: Interleukin-6 (IL-6) is believed to be an important cytokine in acute kidney injury (AKI) and may be both a marker and mediator of AKI. Plasma concentrations of IL-6 correlate with poor outcomes. We determined whether IL-6 per se was able to influence an AKI biomarker (NGAL) and kidney function in healthy humans.
Methods: A three-hour rhIL-6 infusion was administered to six males at a rate of 5 μg/h. Plasma IL-6, NGAL, creatinine, and cystatin C concentrations were measured at 0h, 0.5h, 3h and post-infusion at 24h and 48h. Tympanic temperature was recorded at 0h and 3h.



SP096 Figure 1: Effect of rhiL-6 infusion upon plasma IL-6 concentrations. Data are medians (thick lines) and ranges. Open circles and asterixes are outliers. There was a significant effect of time (*P*=0.007). #, difference compared to 0 hours sample point.



SP096 Figure 2: Effect of rhiL-6 infusion upon plasma NGAL concentrations. Data are means and standard deviations. Dotted line indicates upper limit of normal range for plasma NGAL (106 ng/ml). There was a significant effect of time (*P*=0.025). #, difference compared to 0 h sample point.

Results: IL-6 concentrations were 0.7 {0.6, 1.2} {interquartile range} pg/ml at 0h and peaked at 3h to 159.7 {114.6, 186.7} pg/ml (figure 1). By 48 hours this fell to 8.2 {6.8, 13.0} pg/ml (main effect of time, p = 0.007). Plasma NGAL concentrations were 24.3 (13.2) ng/ml at 0h and peaked at the end of the infusion at 3h to 70.6 (49.3) ng/ml (figure 2). At 48 hours, this fell to 31.7 (16.5) ng/ml (main effect of time, p = 0.025). Plasma creatinine and cystatin C concentrations were unchanged. Tympanic temperature rose from 36.9 (0.2) 0C to 37.5 (0.6) 0C at 0h and 3h, respectively (p = 0.046). Plasma NGAL was positively correlated with tympanic temperature (r = 0.945, p = 0.004).
Conclusions: Although we achieved IL-6 concentrations found in clinical models of AKI, elevations in plasma NGAL were below the range typically associated with AKI and no changes to renal function were evident. This suggests IL-6 per se is not responsible for AKI or kidney dysfunction but other physiological aberrations are needed.

SP097 N-ACETYLCYSTEINE EFFECTIVELY AMELIORATE HEAT STROKE INDUCED ACUTE KIDNEY INJURY

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Introduction and Aims: The heat-related illness has become more prevalent and contributed to increased morbidity and mortality in the world with global warming. Heat stroke (HS) is the most severe and potentially fetal heat-related illness. However, specific and effective therapeutic strategies are not yet available to date. Heat stress is known to generate ROS which play a central role in disease process leading to multiple organs damage including acute kidney injury (AKI). We assessed the efficacy of N-acetylcysteine (NAC), a thiol-containing free radical scavenger and antioxidant, therapy for HS induced AKI.
Methods: Adult male Sprague-Dawley rats, weight between 325 ± 15 g (age 9~10 weeks) exposed to a Ta of 40°C under anesthesia in pre-warming chamber (with relative humidity of 55%) for 60 mins were performed to induce experimental HS. The rats are randomly allocated into four groups. Two experimental HS groups of rats pre-treated with either saline, or NAC and another two control groups of rats treated identically kept at room temperature of 24.0°C used as normothermic controls. Oral NAC, 10mg/mL, in drinking water was used. All physiological and biochemical variables are measured during the observation. Disease severity was verified by with serum and urine metabolic profiles and with renal histopathology. The expression of cytokines and oxidative stress markers, cell apoptosis, and the associated mechanisms were also determined.
Results: HS rat treated with NAC displayed a better survival rate and the hemodynamics were more stable including body temperature, mean arterial pressure and heart rates. NAC also significantly ameliorate reduce severity of AKI based on biochemical (levels of blood urea nitrogen, creatinine) and histopathlogical evidence. The NGAL-positive cells and TUNEL-positive apoptotic cells in the kidney were also significantly reduced in theNAC-treated HS rat. Oxidative stresses markers (Advanced Oxidation Protein Products, AOPP and Malondialdehyde, MDA) in the serum were significantly reduced in NAC -treated HS rat. Cytokines studies ndicated that NAC significantly modulate serum proinflammatory, and anti-inflammatory cytokines. The effect on other organs revealed similar pattern as kidney.
Conclusions: Our studies have demonstrated NAC administration significantly attenuated organ damage and enhanced survival in HS rats. These protective effects may be associated with its anti-inflammatory capacity and anti-oxidant activity. Our study suggests that NAC could be potentially therapeutic for clinical HS induced AKI in the future.

SP098 EXERCISE AND CALORIE RESTRICTION ATTENUATES CISPLATIN-INDUCED ACUTE KIDNEY INJURY

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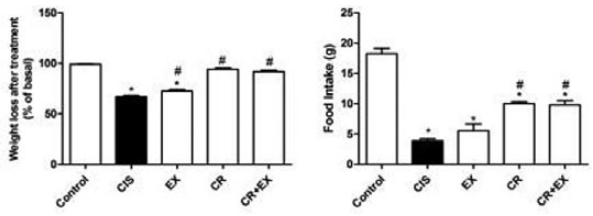
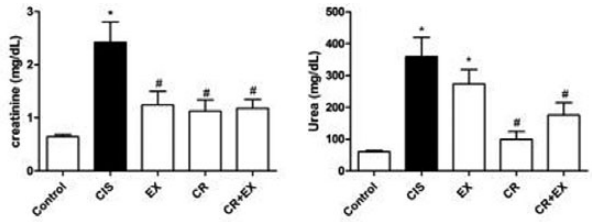
Introduction and Aims: Cisplatin treatment has been adopted in some chemotherapies; however, this drug can induce acute kidney injury due its ability to negatively affect renal function. Inflammation and apoptosis is the general cause of cisplatin-inducing acute kidney injury. Several works showed that exercise and calorie restriction are two good tools to modulate inflammatory response, diminishing apoptosis and expression levels of pro-inflammatory cytokines. Its well estabilished that those interventions can revert the inflammation in cardiovascular disease, obesity, diabetes and many others.
Methods: To examine the role of exercise and calorie restriction in cisplatin nephrotoxicity C57/Bl6 mice were separated in 5 different groups: Cisplatin treatment group that was submmited to 20mg/kg of cisplatin; Exercise group that was submmited



SP098 Figure 1:



SP098 Figure 2:

to swimming training and treated with 20mg/kg of cisplatin; Calorie Restriction group, that was submmited to 30% of food restriction and treated with 20mg/kg of cisplatin; Exercise + Calorie Restriction group that was submmited to swimming training plus 30% of food restriction and was treated with 20mg/kg of cisplatin: and Control group that received saline 0.9%.
Results: Cisplatin treatment causes body weight loss and diminishes food intake, the calorie restriction and exercise + calorie restriction groups were able to reverse this side effect. Moreover cisplatin administration leads to renal dysfunction augmenting creatinine and urea levels, all three treatments were capable to maintain creatinine levels at basal state and only the exercise group was not able to prevent the higher levels of urea. Additionally those treatments can attenuates the acute tubular necrosis and mRNA expression of pro-inflammatory cytokines levels.
Conclusions: Our data suggest that exercise and calorie restriction are non-pharmacological interventions to prevent the nephrotoxicity caused by cisplatin administration.

SP099 EFFECT OF XANTHINE OXIDASE INHIBITORS (XOI) IN RATS SUBMITTED TO LPS

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Introduction and Aims: The systemic inflammatory response of sepsis is marked with the production of reactive oxygen species, follow to instability components pro- and antioxidant. The antioxidant system is composed among others by glutathione (intracellular) and uric acid (intravascular). The latter is one of the most important circulating antioxidant. The higher consumption of antioxidants is observed in severe sepsis with multiple organ failure. Thus, it was studied the role of the xanthine oxidase inhibitors XOi (allopurinol and febuxostat) in the LPS model of sepsis in rats made hyperuricemic by oxonic acid (OxAc) administration.
Methods: Mean arterial pressure (MAP) was measured by tail method and uric acid (UrAc) by uricase method. The LPS was administered i.p. (10 mg/Kg) every 24 hours until the 3rd day. XOi were given by gavage each 24 hours for 3 days. Allopurinol (ALLO,2 mg/ kg) and febuxostat (Febux,1 mg / kg) were used in an equivalent dose as in clinical use . To increase UrAc it was administered OxAc by gavage (750mg/Kg/day) during 5 days. UrAc measurements were done at baseline and at 6Th day. The animals were divided into 9 groups (n = 6): 1-Control, 2-ALLO treated animals, 3-Febux treated, 4-LPS group, 5-LPS+ALLO, 6-LPS+Febux, 7-OxAc treated animals, 8-OxAc +LPS+ALLO and 9-OxAc+LPS+Febux.Data were evaluated by Student's t test and survival curves (Chi square) considering the value of $p \geq 0.05$. The data were reported as mean and standard deviation.
Results: ALLO administration with LPS treatment caused a significant increases in the mortality , inducing death in 28 of 34 animals (82%) , while the same did not occurred with LPS+ Febux (11/17; 65%) or LPS alone (10 /16; 63%) , $p<0.05$. Administration of OxAc after 5 days increased the UrAc in the plasma from 2.98 ± 0.17 to 3.76 ± 0.59 mg/dl (p= 0,015) and also induced MAP to rise from 125 ± 8 to 149 ± 29 mmHg ($p<0.02$). Surprisingly no mortality was observed in the group OxAc+LPS+Febux ($p<0.02$). There was a significant lower number of deaths when we associated OxAc +LPS+ALLO in sepsis (12 /18; 67%) compared with LPS+ALLO (28/ 34; 82%). It was also interesting that AcOx +LPS+ALLO (67%) vs. LPS+ALLO (82%), p= 0.08, did not differed in the mortality, despite the tendency of AcOx to protect agains ALLO in this protocol.
Conclusions: In this study the administration of ALLO in LPS treated animals, decreased significantly the chance of survival. This effect was not observed with Febux. On the other hand elevation of plasma UrAc by the OxAc protected the animals from the LPS. Thus, high plasma UrAc, by its potential antioxidant capacity, decreases the effects of LPS and the use of OxAc, increasing the blood uric acid, elevate the chance of survival. More studies are needed to elucidate if the administration of the xanthine oxidase inhibitors can worsen the sepsis and to evaluate different effects among XOi (ALLO vs. Febux) in this experimental model.

SP100 PROTECTIVE EFFECT OF MITOCHONDRIA-TARGETED ANTIOXIDANTS IN AN ACUTE BACTERIAL INFECTION

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Introduction and Aims: The main approach to treat acute pyelonephritis is antibiotic therapy. However, the pathology is accompanied by inflammation and oxidative stress phenomena that can also be a target for intervention when direct antibacterial measures are impossible or inefficient. In our study, in vitro and in vivo models of experimental pyelonephritis were used to define the role of mitochondria in this pathology and to find a way to alleviate the kidney damage.
Methods: We used an in vivo experimental model of APN where bacteria are introduced in the bladder of rat. The inoculum (5 mL per kg, 1 × 108 cfu/mL of rat fecal bacterial composition) was injected slowly to avoid any leakage into the bladder. On the second day after the injection, blood samples were taken, and kidneys were excised for the mitochondria isolation, determination of MDA in the tissue, Western blotting, and histopathological examination. Inflammation Modeling in Vitro. Renal cells and leukocytes (neutrophils) were cocultivated for 24 or 48 h with bacterial lysate or LPS. Cell death (both necrotic and apoptotic) rate was evaluated using Annexin-V FITC Kit (Invitrogen).
Results: In APN, we found an excessive ROS generation strongly depending on the activities of neutrophils, monocytes, and mesangial cells migrating to the primary inflammatory loci. This traffic results in elevation of the levels and activities of myeloperoxidase that persists after transformation to chronic type. After activation initiated by interaction with bacterial products, leukocytes secrete ROS, NO, and TNFα. The suppression of leukocytic NAD(P)H oxidation yields a dramatic decrease of ROS within renal cells, which may serve as an argument to support the idea that leukocytes are specifically responsible for excessive ROS production in the pyelonephritic kidney. A number of agents involved in different signaling mechanisms were tested for their ability to alleviate oxidative stress in renal cells. We found that the antioxidant Trolox reduced oxidative stress-related phenomena, apparently by diminishing transfer of ROS from leukocytes to kidney cells. Protective effects of mitochondria-targeted antioxidants (SkQ1, SkQR1) and of an inhibitor of GSK-3β (Li +) may be explained by the targeted depletion of mitochondrial ROS and the prevention of the mitochondrial permeability transition, respectively. Oxidative stress observed in pyelonephritic kidney was accompanied by a reduced level of mitochondrial B-cell lymphoma 2 (Bcl-2). Importantly, renal cell death and animal mortality were both alleviated by mitochondria-targeted antioxidant 10 (6′-plastoquinonyl) decylrhodamine 19 (SkQR1).
Conclusions: We emphasize that, in a majority of pathological steps, prodeath and prosurvival signaling is related to mitochondrial function. Mitochondria-targeted antioxidants appear to be effective antipyelonephritic drugs with high potency to prevent renal dysfunction in the cases where direct antibacterial measures are restricted (in children, pregnant women, individuals with primary immunodeficiency, etc.) or inefficient (in cases of antibioticresistant bacterial strains).

SP101 THE PROTECTIVE EFFECT OF THE PURIFIED MICRONIZED FLAVONOID FRACTION IN THE SEPSIS-ASSOCIATED ACUTE KIDNEY INJURY (AKI)

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Introduction and Aims: Sepsis is characterized by a severe inflammatory response accompanied by depression in immunological function which causes multiple organ injury. Sepsis-associated AKI pathophysiology changes from renal vasoconstriction, ischemia and acute tubular apoptosis. The concept of renal vasoconstriction and kidney ischemia as a key pathogenic factor is certainly valid for sepsis models. Sepsis-associated AKI may result from global renal hypoperfusion, which is locally mediated by the upregulation of pro-inflammatory cytokines (TNF-α and IL-6), with

subsequent tubular cell apoptosis also in distan organs. The purified micronized flavonoid fraction is being mentioned to improve venous tone and reduce capillary hyperpermeability by protecting the microcirculation from inflammatory processes. The aim of this study is to evaluate the effect of the purified micronized flavonoid fraction (Diosmin) on the creatinine clearance, urinary peroxides and the kidney histology in sepsis associated AKI.

Methods: Adult male Wistar rats, weighing 250-300g were used. Renal function (creatinine clearance, Clcr), urinary peroxides (UP, FOX-2), kidney, lung and intestinal tract of levels of TNFα and IL-6 (ELISA) and kidney histology were evaluated. Sepsis was induced by cecal ligature and puncture (CLP). Groups: Sham (without CLP); Sepsis (CLP); Sepsis+Diosmin (Diosmin 3mg/Kg 30 minutes before CLP).

Results: A similar quantitative inflammatory response in distant organs and in the kidneys was observed in CLP animals. CLP induced a decrease in mean arterial pressure, body temperature and creatinine clearance, accompanied by a prominent increase in UP. These paramethers were significantly changed in the pretreated Diosmin group. In the kidney histology, loss of brush border was evident in sepsis group and it was observed a severe dilation of the tubular lumen after CLP.

Conclusions: Sepsis is an injury repair stereotyped response across remote organs. Distant organ inflammation can be considered a significantly mediator of sepsis-associated AKI. These data provide a new insight about Diosmin attenuating effect in the sepsis AKI.