Results: The majority of immunosuppressive drugs act by inhibiting activation of T cell. This prevents graft rejection, but also blocks induction of transplantation tolerance. We observed that immunosuppressive drugs have decreased the percentage of $CD4^+CD25^+$ and $CD4^+CD25^+FOXP3^+$ cells *in vitro*. The level of $CD4^+CD25^+FOXP3^+$ cells was decreased significantly in the presence of cyclosporine A.
Conclusions: The investigation and characterization of new features of regulatory T cells throw light on the mechanism of tolerance induction. Cyclosporine A in contrast to rapamycin might have an inhibitory effect to tolerance induction.

MP060 EXISTENCE OF THE AUTOREACTIVE T CELLS FOUND IN GOODPASTURE'S DISEASE IS ACCOUNTABLE BY PROPERTIES OF ENDOSOMAL PROCESSING

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Introduction and Aims: The predominant 3(IV)NC1-specific T cells in recent onset Goodpasture's disease have a strikingly consistent peptide specificity with all patients having T cells that recognise the $3(IV)NC_{171\text{-}91}$ and $3(IV)NC_{131\text{-}151}$ (called P14). We recently reported that the stimulatory self-peptides have in common exquisite in vitro susceptibility to rapid destruction by whole lysosomal extracts, and identified the destructive activity was mediated by the endosomal aspartic proteases Cathepsin D and E (abstract to RA and submitted). The observations suggested that the autoimmune response in Goodpasture's disease could be focussed on these peptides because they are constitutively presented at too low abundance to establish self-tolerance, as was recently suggested for a major T cell epitope in Multiple Sclerosis. Towards testing this hypothesis we here report an investigation of the influence of Cathepsin D activity upon in vivo 3(IV)NC1 presentation by intact human antigen presenting cells (APC).
Methods: To assess 3(IV)NC1 presentation by intact human APC we made murine T cell hybridomas specific for 3(IV)NC1 peptide/HLA DR15 complexes by immunising DR15-transgenic mice (provided by Dr. D. Altmann) with 3(IV)NC1 peptides. DR15-expressing mice were immunised with P14 and P14-specific T cells expanded in vitro. Lines were fused with a human myeloma T cell line (TcR negative) and shown to produce IL-2 when incubated with intact 3(IV)NC1 as well as P14-pulsed APC and to be restricted by HLA DR15. The murine T cell hybridomas were used to measure presentation of P14 on HLA DR15 by human B cells and macrophages that had been pulsed with P14 or intact 3(IV)NC1, and to evaluate the influence on presentation of manipulations that enhanced or diminished Cathepsin D processing.
Results: Human macrophages and B cells were able to process intact 3(IV)NC1 and present the P14 epitope on HLA DR15, but presentation sufficient to stimulate the T cells was considerably retarded (2-4 hours) compared with exogenous antigens (typically 30-60 minutes). P14 presentation was abrogated by prior incubation with Cathepsin D, but both accelerated and potentiated when APCs were treated with Pepstatin A, a peptide inhibitor of Cathepsin D and E.
Conclusions: Our results demonstrate that the previously in vitro-demonstrated rapid endosomal destruction of the P14 epitope by lysosomal extracts is also discernable in the kinetics of 3(IV)NC1 presentation by intact human APC, consistent with a role in shaping constitutive presentation of 3(IV)NC1 and the scope of self-tolerance.

MP061 ★ RENAL CELL CARCINOMA (RCC) AND IMMUNITY: MUTATIONAL SCREENING OF JAK3 GENE IN RCC PATIENTS AND IDENTIFICATION OF THREE NOVEL MUTATIONS

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Introduction and Aims: RCC is a relatively uncommon solid tumor, accounting for about 3% of all adult malignancies and it is an histological subtype that is responsive to immunotherapeutic approaches with any consistency. It has been hypothesized that immune-mediated mechanisms play important roles in limiting tumor growth and that T cells are the dominant effector cells that regulate tumor progression in situ. JAK3 protein and its interaction with γ chain of T-cell receptor plays a key role in T cell activation. The aim of this study was to investigate nucleotide variants in JAK3 gene which may induce susceptibility to RCC.
Methods: One hundred RCC Italian patients were enrolled and screened for changes in JAK3 gene. A normal control group of 100 healthy blood donors was also studied. Genomic DNA was extracted from peripheral blood samples. All 23 exons of JAK3 gene were PCR amplified and analysed with denaturing high-performing liquid chromatography (DHPLC). PCR products showing abnormal elution profile were re-amplified, purified and subjected to automatic sequence analysis.
Results: JAK3 mutational analysis allowed to identify 4 missense mutations in the coding region of JAK3 gene; three of these have not been previously described and are completely absent in control population. All mutations were present in heterozygous status in 7 different RCC patients. The first patient presented a nucleotide change (c.96 C>A), in the exon 1, resulting in an amino acidic substitution (p.Gln13Lys) in the FERM domain, important for cytokine receptor binding and catalytic activity modulation. The second patient showed a nucleotidic variant (c.2832 C>A), in exon 19, causing an amino acidic substitution (p.Arg925Ser) in the kinase domain, necessary to ensure protein activity. The third patient resulted compound heterozygote for 2 missense mutations: the first one (c.2088 G>A), in the exon 14, causes an amino acidic change (p.Ala677Thr) in the pseudokinase domain, important for kinase activity regulation; the second one (c.2223 G>A), in exon 15, consists of an amino acidic substitution (p.Val722Ile) in the same pseudokinase domain. This last mutation was detected in heterozygous status in other 5 RCC patients and in 5 control subjects. p.Val722Ile has been already reported in a patient with Severe Combined Immunodeficiency (SCID), a rare hereditary syndrome, characterized by lymphocyte deficiency. Moreover, 3 silent mutations and 17 nucleotide variants in no coding region of JAK3 gene were also identified.
Conclusions: In this study, we report for the first time JAK3 mutational analysis in RCC patients. Four missense mutations were identified in 7 unrelated RCC patients. Three of mutations (c.96C>A; c.2832C>A; c.2088 G>A), not previously described, are localized in important functional domains of JAK3 protein and may compromise protein activity and immune response of RCC patients.

Acute renal failure: experimental

MP062 HAEMATOPOIETIC LINEAGE-COMMITTED MARROW CELLS, BUT NOT CLONED CULTURED MESENCHYMAL STEM CELLS, CONTRIBUTE TO REGENERATION AFTER ACUTE TUBULAR NECROSIS

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Introduction and Aims: Our previous studies have demonstrated that endogenous bone marrow cells (BMCs) contribute to tubular regeneration after acute tubular necrosis. The aim of this study was to examine which fraction of BMCs [haematopoietic lineage marrow cells (HLMCs) or mesenchymal stem cells (MSCs)] are effective.
Methods: Female mice (6 week-old) were lethally irradiated and transplanted with female enhanced green fluorescent protein-positive (GFP^+) plastic-nonadherent marrow cells (as a source of HLMCs) plus cloned cultured male GFP[oline] MSCs, and four weeks later assigned into two groups: control mice with vehicle treatment, and mice treated with $HgCl_2$. Tritiated thymidine was given 1 hour before animal killing at interval over

2 weeks. Kidney sections were stained for a tubular epithelial marker, cell origin derived by GFP immunohistochemistry or Y chromosome *in situ* hybridisation, periodic acid-Schiff staining, and subjected to autoradiography. One thousand consecutive renal tubular epithelial cells per mouse in S-phase were scored as either female (indigenous), GFP^+ (HLMC-derived) or male (MSC-derived).

Results: The results showed that HLMCs and MSCs stably engrafted BM and spleen, but only HLMC-derived cells, not MSCs, were found in the renal tubules and able to undergo DNA synthesis after acute renal injury. A few MSCs were detected in the renal interstitium, but their importance needs to be further explored.

Conclusions: HLMCs, but not cloned cultured MSCs, can play a role not only in the normal wear-and-tear turnover of renal tubular cells, but also after tubular injury.

MP063 GENTAMICIN (G) PHARMACOLOGICAL PRECONDITIONING (PC) ON NITRIC OXIDE (NO) SYNTHESIS IN RAT RENAL ARTERY SMOOTH MUSCLE CELLS (rVSMC)

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Introduction and Aims: Gentamicin is an antibiotic largely used against Gram-negative bacteria but its use is limited by nephrotoxicity. In our previous studies G decreased NO in rVSMC primary culture. NO, a potent vasodilator, plays a role in the maintenance of glomerular hemodynamics and its decrease could be related to the G nephrotoxicity. Ischemic or pharmacological PC is defined as a situation when an animal, an organ or a cell, after submitted to an insult, gets resistance against a new insult from the same or different nature. The aim of the present study is to evaluate the effect of pharmacological PC with G on NO synthesis and on cell viability in rVSMC.

Methods: The cells were cultured from Wistar rats and treated during 5 days, as follows: Control (CTL): water as G vehicle; LPS: *E. coli* lipopolysaccharide (100μg/ml) as a positive control of NO synthesis; G: 2mM; G+LPS. PC was performed either with water (PC-CTL) or G 2mM (PC-G) during 24, 48 or 72 hours. PC-CTL+G: PC with water followed by G 2mM for 5 days; PC-G+G: PC with G followed by G 2mM for 5 days. At the end of the treatment, the supernatant was collected for NO determination (nmoles/mg protein) by the Griess and chemiluminescence methods; the results were normalized by the intracellular protein (Lowry method). The cell viability (%) was evaluated in all groups by the acridine orange method.

Results: Data were expressed as mean±SEM and analyzed by the one-way ANOVA; the significance is defined as $P<0.05$. N=5 for all groups.

By the Griess method, the NO (estimated by its metabolite, the nitrite) was increased in the LPS as compared to CTL(43.3±3.9 vs 12.0±0.7;$P<0.05$); in the G group NO was decreased when compared to CTL and to LPS(5.4±0.2;$P<0.05$); NO was reduced in G+LPS relatively to LPS(27.4±0.7;$P<0.05$). NO was significantly increased in all PC-G+G groups when compared to PC-CTL+G: 24 hours(15.8±0.5 vs 12.1±0.3); 48 hours(18.6±0.5 vs 14.1±0.6); 72 hours(17.1±0.3 vs 12.5±0.3).

By the chemiluminescence method we verified the same behavior of NO; the values for each group were higher, probably because of the better sensitivity of this method as compared to the Griess. NO was increased in the LPS(1034±4.7) and decreased in G(80.8±4.1) comparatively to CTL(145±4.7;$P<0.05$); in G+LPS(363±2.9) it was reduced when compared to LPS($P<0.05$). NO was significantly increased in PC-G+G vs PC-CTL+G after 24(148.6±6.4 vs 87.9±5.3), 48(227±9.6 vs 97.3±0.9) or 72hours(95.2±3.1 vs 87.1±1.1).

Regarding cellular viability, the groups G and G+LPS presented a significant reduction(79.5±3.0 and 89.5±2.5, respectively); when compared to CTL(98.7±1.0). In LPS it was similar to CTL(95.5±4.5). In the cells PC with G the viability was increased(PC-G+G vs PC-CTL+G) at 24(97±1 vs 88±3), 48(97.1±0.7 vs 89.1±0.5) or 72hours(97.1±2.3 vs 87.1±1.1).

Conclusions: In this study the G treatment during 5 days reduced NO and cell viability in rVSMC. When these cells were preconditioned with G during 24, 48 or 72 hours, there was an improvement in the NO production and in the cellular tolerance to G injury.

MP064 RENAL ISCHEMIA-REPERFUSION INJURY IN HUMAN TISSUE KALLIKREIN TRANSGENIC RATS

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Introduction and Aims: Renal ischemia-reperfusion injury (I-R) is an important cause of acute renal failure in which inflammation, particularly macrophage infiltration, can promote long term fibrotic injury. Tissue kallikrein cleaves prinicipally low molecular weight kininogen to generate kinin peptide hormones which have been variously ascribed both renoprotective (anti-fibrotic and blood pressure related) and renal damaging properties (pro-fibrotic and pro-inflammatory). We aimed to compare the outcome of I-R between transgenic adult male rats harbouring a stress inducible human tissue kallikrein-1 transgene (tKLK-1 TGR) and their wildtype littermates, and to examine whether any related changes were associated with changes in inflammation.

Methods: Rats (tKLK-1 TGR and wild type) were subjected to 45 minutes of left sided (I-R) with contralateral nephrectomy (n=6 per group) while sham-operated rats (n=3 per group) received right nephrectomy, only. Functional damage was assessed by plasma creatinine and blood urea nitrogen. The renal fibrotic response of the left ischemic kidney (I) was assessed as an indictator of injury severity and measured by Northern blotting analyses of TGF-b1 and collagen IV (a1) mRNA's and Western blotting analysis of fibronectin. As the kinin system is involved in the inflammatory response, differences in endothelial activation and neutrophil/monocyte infiltration were measured using vascular cell adhesion molecule-1 (VCAM-1) immunoblotting and tissue myeloperoxidase assay, respectively. Statistical comparisons were made using one-way ANOVA/Scheffes and statisitical significance set at $p<0.05$. The left kidney of sham rats was used as control (S).

Results: Two days after I-R, significant elevations in plasma creatinine and blood urea nitrogen were observed in both groups of rats. Increases were significantly higher in the t-KLK-1 TGR (plasma creatinine p=0.025, blood urea nitrogen p=0.05, versus wild type rats). I kidneys of all rats showed upregulation of TGF-b1 and collagen IV(a1) mRNAs and fibronectin protein expressions, these being significantly higher in the I kidneys of tKLK-1 TGR (p=0.05, p=0.003 and p=0.05, respectively), versus I kidneys of wild type rats. I-R also induced myeloperoxidase activation, significantly more markedly in the I kidney of tKLK-1 TGR (p=0.004 versus wildtype I kidneys).

Conclusions: Human tKLK-1 TGR have a worsened response to renal I-R injury showing a higher physiological and fibrotic damage at 2 days after I-R. A primary role for increased inflammation may be implicated in this response. This is in line with the role for kinins in promoting acute inflammatory injuries and studies showing that the tissue kallikrein inhibitor, aprotinin can prevent renal I-R injury.

MP065 ★ THE ROLE OF STEROIDS HORMONES REACHING THE KIDNEY THROUGHT THE ADRENAL RENAL VASCULAR CONNECTION IN ACUTE KIDNEY ISCHAEMIA

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Introduction and Aims: The presence of a direct vascular connection between the kidney and the adrenal gland (AVRC) was reported in the cat, rat, dog, rabbit and man.The vessels permit for unidirectional renal blood flow (RBF) – from the adrenal gland to the kidney.It is likely that the biologically active substances reach the kidney directly trough the ARVC. Therefore it has been assumed that the adrenal cortical and medullary hormones may flow by the adrenal-renal vascular connection. The aim of the present study was to examine the role of steroid hormones reaching kidney by ARVC in regulation of the RBF during reperfusion following acute renal ischemia.

Methods: The study included 36 male Sprague-Dawley rats weighing 250-300g.The animals were divided into two series. Each series was divided

into two groups. The right nephrectomy was done in each group. In series I and II the rats were subjected to renal ischemia for 60 minutes after prior renal denervation. In series I the effect of elimination of ARVC on RBF was examined after kidney ischemia. In series II the role of steroids reaching kidney by ARVC during the acute kidney ischemia was examined. Aminogluthetimide – the compound which inhibits the cholesterol conversion to pregnenolon, was used to inhibit steroid synthesis.
Results: Destruction of ARVC in series I resulted in a significant lowering of RBF during reperfusion following acute renal ischemia as compared to the group with the preserved ARVC. Inhibition of synthesis of steroids reaching the kidney through the ARVC exerted similar effects to the destruction of this connection. RBF was significantly lower as compared to the respective baselines, there was no difference in RBF.
Conclusions: 1. Adrenal steroids may play an important role in improving RBF during reperfusion of the kidney following an acute renal ischemia.

MP066 THE ROLE OF THE ADRENAL-RENAL VASCULAR CONNECTION IN ACUTE KIDNEY ISCHEMIA

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Introduction and Aims: The presence of a direct vascular connection between the kidney and the adrenal gland (AVRC) was reported in the cat, rat, dog, rabbit and man. The vessels permit for unidirectional renal blood flow (RBF) – from the adrenal gland to the kidney. The aim of the present study was to examine the role of the ARVC in regulation of the RBF during reperfusion following acute renal ischemia.
Methods: The study included 62 male Sprague-Dawley rats weighing 250-300g. The animals were divided into four series. Each series was divided into two groups. The right nephrectomy was done in each group. In series I, in which there was not kidney ischemia, the changes of RBF under resting conditions were examined. In series II, III, IV the rats were subjected to renal ischemia for 60 minutes after prior renal denervation. In series II the effect of elimination of ARVC on RBF was examined after kidney ischemia. In series III the effect of elimination of ARVC on kidney excretory functions during the reperfusion period after acute kidney ischemia was examined. Endogenous creatinine clearance plasma creatinine and sodium levels were measured. In series IV the role of the collateral circulation through the renal capsule in regulation of RBF during reperfusion after acute renal ischemia was examined.
Results: Elimination of ARVC did not influence resting RBF during 60 min of the observation period. Destruction of ARVC in series II resulted in a significant lowering of RBF during reperfusion following acute renal ischemia as compared to the group with the preserved ARVC. In both groups, RBF during the reperfusion period was significantly lower in comparison to the baseline. Renal ischemia resulted in elevation of creatinine level and lowering of creatinine clearance during the first following days the ischemia. Also in this series in both groups, RBF was significantly lower during the reperfusion period in comparison to the baseline. There was no difference in RBF between the group with lacking and that with the preserved vessels of the capsule.
Conclusions: 1. Presence of the ARVC improves the renal blood flow during reperfusion of the kidney following an acute renal ischemia.
2. Presence of the renal collateral circulation through the renal capsule does not play a significant role in determining the renal blood flow during reperfusion following an acute renal ischemia.

MP067 SIMVASTATIN AMELIORATES GENTAMICIN -INDUCED RENAL INJURY IN RATS

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Introduction and Aims: Nephrotoxicity is one of the most common side effects of aminoglycosides and gentamicin is used widely in clinical settings. Statins (SIMVASTATIN) can improve renal function in gentamicin induced nephrotoxicity via inhibition of isoprenylation, lipid peroxidation, angiotensin-1 production, and increasing endothelial Nitric Oxide (NO), increasing vasodilator PGs and increasing renal perfusion. In this study we evaluated the ameliorating effect of simvastatin in rats with gentamicin induced renal injury.
Methods: The experiments were performed in Sprague-Dawley rats, weighting 140-230gr. Rats were provided with rat chow and tap water after allowing 5 days for adaptation on the new enviroment. Rats were assigned in a random order to one of eleven groups (n=8 for each group) according to use of different doses of gentamicin (50 and 80 mg/kg/d) and simvastatin (2, 10, and 20mg/kg/d). All injection of gentamicin was done intraperitoneal for 8 days and gavages of simvastatin were given for 12 days (4 days before use of gentamicin). Blood samples were drawn at 13th day from the rat carotid and 24-hour urine collection were taken in first day and in the end of 12th day both kidney removed and processed for histopathological examination. In histopathological examination four categories of injury were distinguished: 0, no alteration, 1+ isolated cell necrosis, 2+ several necrotic cells in a tubular profile, 3+ complete or almost complete necrosis of a tubular profile.
Results: All groups receiving gentamicin and simvastatin compared with groups receiving gentamicin without simvastatin. In all groups simvastatin ameliorates gentamicin induced histopathological alteration ($p=0.001$), declines serum creatinine ($p<0.001$) and improves creatinine clearance ($p=0.001$) with doses of 2 and 10mg/kg/d.
Conclusions: We conclued that simvastatin improved renal function in gentamicin-induced nephrotoxicity with doses of 2 and 10 mg/kg/d but increasing dose to 20 mg/kg/d accompanied with decreasing renal function.

MP068 CHARACTERIZATION OF LABEL-RETAINING CELLS IN S3 SEGMENT OF NEPHRON IN RESPONSE TO URANYL ACETATE-INDUCED ACUTE RENAL FAILURE IN RATS

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Introduction and Aims: There are recent reports suggesting the existence of renal tubular progenitor cells in S3 segment of proximal tubule (PT). However, the source of regenerating tubular cells after acute tubular injury has not been understood very well. We recently documented that the initial regenerating PT cells raised from the distal area of S3 after PT injury by high dose uranyl acetate (UA) are dedifferentiated pre-existing PT cells which contributed to renewal of the entire S3 in rats. The initial regenerating PT cells also showed persistent label-retaining cells (LRCs) at least till day 42. We also reported that the target LRCs in the distal area of S3 had ability to produce daughter cells and were the main source of tubular regeneration in S3 after UA injury. In addition, 5 fluorouracil (FU)-treatment arrested cell cycle of the target LRCs at G0/G1, which again proliferated and differentiated after stopping 5FU. In the present study, to further characterize the target LRCs in the distal zone of S3, we chased them for longer period, examined whether they are the same population of LRCs labeled under normal condition reported by Maeshima et al.[J Am Soc Nephrol 14: 3138, 2003] and whether they are activated again by the second UA insult.
Methods: Acute renal failure with extensive PT injuries in the S3 segment were induced by i.v. injection of UA (4mg/kg) into rats. First, the target LRCs, which had been previously identified in the distal zone of S3, were pulse labeled at days 2, 2.5 and 3 after UA by ^{3}H-thymidine and followed for 12 weeks. Secondly, BrdU was injected intraperitoneally into rats daily for 1 week after the first UA. After 6-month chase period, rats injected with the second UA were received ^{3}H-thymidine 3 days after the second UA and killed at days 3 and 7. Finally, BrdU was injected intraperitoneally into normal rats daily for 1 week. After 2 week-chase period, rats were injected with UA and killed at days 3 and 7. The kidneys were processed for autoradiography or immunohistochemistry.
Results: LRCs labeled by ^{3}H-thymidine were restrictedly found in the distal 1/4 of S3 and remained there until 12 weeks, confirming slow cycling cell. After 6 month-chase period, significantly higher number of LRCs labeled by BrdU after the first UA was again labeled by ^{3}H-thymidine in the distal 1/4 of S3 than in the proximal 3/4 of S3 after the second UA, suggesting

the target LRCs were repeatedly involved in S3 repair. LRCs labeled by BrdU under normal condition did not show proliferating cell marker Ki67 positivity after UA insult.
Conclusions: LRCs in the distal zone of S3 are slow cycling cell, not activated under normal condition but activated by UA insult, and are capable to repair S3 even after the second UA insult. These data suggest that LRCs in the distal zone of S3 have characteristics of progenitor-like cell.

MP069 ROLE OF HYPOXIA IN CISPLATIN INDUCED RENAL INJURY

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Introduction and Aims: Cisplatin induced renal failure is a major side effect of cisplatin chemotherapy which limits therapeutic efficacy. Cisplatin can directly damage renal tubular cells, but also reduces renal blood flow leading to hypoxia of the outer medulla. Hypoxia inducible transcription factors HIF-1α and -2α control the expression of a variety of cytoprotective genes in response to hypoxia including EPO and Heme Oxygenase 1. These HIF target genes have already been shown to ameliorate cisplatin-induced renal failure. However, the role of hypoxia in cisplatin induced tubular cellular damage, possible direct effects of cisplatin on the HIF system and a potential protective effect mediated by HIF are discussed controversially. We therefore investigated the effects of cisplatin exposure on the HIF system in vitro and in vivo, assessed the effects of hypoxia on cisplatin induced cell injury and tested the impact of cisplatin on apoptosis in the presence or absence of a functioning HIF system.
Methods: Primary mouse tubular cells were prepared using collagenase digestion. HIF-1α protein was analysed by immunoblot. For transactivation assays cells were transiently transfected with a HIF-responsive reporter construct. Apoptosis was determined by a fluorimetric measurement of caspase-3 activity. HIF defective cells were MEF (lacking HIF-1α) and Hepa1 (lacking HIF-1β).
Results: Rats treated with 8mg/kg bw cisplatin i.p. developed renal failure, but both HIFα isoforms were not detectable in the kidney by immunohistochemistry. In a human proximal tubular cell line (HKC-8) different doses of cisplatin did not stabilize HIF-1α in normoxia in vitro. Conversely, the increase of HIF-1α protein levels under hypoxia (1% O2) or after treatment with the hypoxia mimetic Dipryridyl (DP) was blunted following pre-incubation with cisplatin. This inhibitory effect was dose dependent, but independent of ongoing cisplatin exposure. Moreover, cisplatin exposure did not increase reporter activity of a HIF-1α responsive reporter construct, but activation of this reporter with DP was significantly reduced after pretreatment with cisplatin. To investigate the potential protective effects of HIF-stabilization prior to the insult of cisplatin both primary mouse tubular cells and HKC-8 cells were cultured in hypoxia before exposure to cisplatin. This pretreatment significantly reduced the rate of apoptotic cell death. However, cells with a defective HIF system exhibited contrasting effects: cisplatin induced more apopotis in cells lacking HIF-1α than in the respective WT cells. Conversely, cells without HIF-1β, which also have no functional HIF system, showed the opposite effect after cisplatin treatment, with reduced rates of apoptosis in the cells lacking HIF.
Conclusions: We conclude that at least in the rat model cisplatin leads to renal injury and does not induce the HIF system, but rather inhibits its responsiveness. However, a period of hypoxia prior to cisplatin exposure mitigates the toxicity of the drug in vitro. This effect appears to be cell specific and independent of HIF.

MP070 N-ACETYLCYSTEINE ATTENUATES ISCHEMIA-REPERFUSION INJURY IN RAT KIDNEY

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Introduction and Aims: Renal ischemia-reperfusion is a complex syndrome involving several mechanisms that include renal vasoconstrictions, extensive tubular damage, inflammation, apoptosis and necrosis. One of the major pathophysiologic mechanisms involved in ischemia-reperfusion injury is oxygen free radical-induced tissue damage. N acetylcysteine(NAC) -a well known antioxidant-can easily be deacetylated to cysteine, which is an important precursor of cellular glutathione synthesis. The aim of this study was to investigate the effect of NAC on renal ischemia-reperfusion injury in the rat.
Methods: Twenty-two Spraque-Dawley rats were divided into 4 groups. 1. Sham group (n=4), 2. NAC group(n=4), were injected with the same dose of NAC intraperitoneally as NAC+I/R group 3. Ischemia-Reperfusion(I/R) group(n=6); were subjected to renal ischemia by transient(45 min) both renal artery clamping, followed by reperfusion(24hr). 3. NAC+I/R group(n=8); were given NAC 30 minutes before and at the time of I/R injury via IP route respectively.(150mg/kg). After 24 hours of reperfusion, the rats were sacrificed prior to right nephrectomy and blood sampling. H&E stained kidney samples were quantitatively analyzed to evaluate the extent of tubular necrosis. Biochemical parameters such as BUN, creatinine, LDH were also evaluated. Competitive RT-PCRs for TGF-β, MCP-1, TNF-α, Bcl-2, BAX and FAS mRNA were performed to evaluate the pattern of gene expression.
Results: The serum levels of BUN(19.7±3.8mg/dL vs 148.9±17.0 mg/dL) and creatinine(0.7±0.1mg/dL vs 5.1±0.4mg/dL) were significantly higher than that of sham group. The serum levels of BUN(12.3±3.2mg/dL) and creatinine(2.5±1.1mg/dL) in NAC+I/R group were significantly lower than that of I/R group($p<0.01$). The extent of tubular necrosis of the NAC+I/R group(41.9±22.0%), was significantly smaller than that of I/R group(59.6±25.5%)($p<0.05$). The renal mRNA expression of TGF-β, MCP-1, TNF-α, Bax, and FAS were significantly decreased in the NAC+I/R group as compared to the I/R group. But renal mRNA expression of antiapoptotic bcl-2 increased in NAC+I/R group as compared to I/R group significantly.
Conclusions: Pretreatment with NAC resulted in significant attenuation of renal injury in experimentally induced I/R injured rats. The protective mechanisms of NAC in I/R injury are due to antioxidant, anti-inflammatory and anti-apoptotic effect of NAC.

MP071 EFFECTS OF ACE-INHIBITORS, AT1 RECEPTOR BLOCKER AND VASOPEPTIDASE INHIBITOR ON METABOLIC CHANGES IN THE RAT MODEL OF ACUTE RENAL FAILURE INDUCED BY NEPHRECTOMY OR URETERAL LIGATION

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Introduction and Aims: In the rat model of acute renal failure (ARF) circulating advanced glycation end products (AGEs), potential uremic toxins, rise substantially within 24 hours. Angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor 1 blockers (ATRB) and vasopeptidase inhibitors (VIP) were shown to lower formation of AGEs both in vitro and in vivo. We aimed to study whether the administration of these drugs modulates the metabolic changes in ARF.
Methods: Two models of ARF were investigated: bilateral nephrectomy (BX) and bilateral ureter ligation (UL). Male Wistar rats (180-220g) were subjected either to NX or UL, and received enalapril (E, 50 mg/l), ramipril (R,x mg/l), losartan (L, 20 mg/l) or VIP (AVE7688, x mg/l), (n=3-6/group) dissolved in their drinking water. Sham operated (S) and untreated NX or UL animals (P, placebo) served as controls. Rats were sacrificed 24 hours after operation. Plasma glucose, creatinine, urea, minerals and electrolytes and lipids, advanced glycation end products, advanced oxidation protein products, ferric reducing ability of plasma, asymmetric dimethyl adenosine (ADMA) concentrations and blood count was determined.
Results: Twenty four hours after the operation, both models developed ARF (plasma creatinine: NX: S: 43±10 umol/l, p=0.001 vs all NX groups; P: 356±31; E: 346±27; R: 381±37; L: 339±48; VIP: 347±94; UL: S: 41±10 umol/l, p=0.001 vs all UL groups; P: 317±43; E: 295±42; R: 324±73, p=0.049 vs. NX; L: 323±58; VIP: 342±68; mean±SD); (plasma urea: NX: S: 6±1 mmol/l, p=0.001 vs all NX groups; P: 47±6; E: 41±5; R: 43±5; L: 45±5; VIP: 51±7; UL: S: 6±1 mmol/l, p=0.001 vs all UL groups; P: 48±3; E: 45±6; R: 47±9; L: 46±3; VIP: 55±9, respectively). Comparing both models, UL rats showed a tendency towards higher plasma glucose, triglyceride, advanced oxidation protein products concentration,

and erythrocyte superoxide dismutase activity; but lower plasma creatinine, AGE levels and erythrocyte glutathione peroxidase activity. Administration of VIP significantly ameliorated plasma AGEs accumulation in the NX rats (S: 4.8±0.5 AU/g albumin, P: 14.9±1.5 AU/g, p=0.001 vs. S; VIP: 12.4±1.9, p=0.04 vs. S), and that of ADMA in both NX (S: 0.65±0.15 umol/l, P: 1.16±0.37, p=0.03 vs. S; VIP: 0.57±0.20, p=0.008 vs. P) and UL (S: 0.61±0.16 umol/l, P: 1.03±0.31, p=0.04 vs. S; VIP: 0.60±0.21, p=0.008 vs. P) groups. Moreover, both R and VIP significantly ameliorated leukocytosis in both models, NX - (S: 3.5±0.3 x.10*3/ul, P: 6.3±1.0, p=0.01 vs. S; R: 4.3±1.1, p=0.02 vs. P; VIP: 2.3±0.9, p=0.001 vs. P); and UL (S: 3.4±0.1 x.10*3/ul, P: 7.3±1.5, p=0.03 vs. S; R: 2.6±0.7, p=0.01 vs. P; VIP: 2.8±0.6, p=0.00 vs. S).

Conclusions: Our data point to a partially residual metabolic activity of the kidneys during first 24 h after ureter ligation. VIP ameliorated the accumulation of AGEs and the uremic toxin ADMA, while VIP and ramipril counteracted leukocytosis in the ARF rat models.

MP072 ACQUIRED RENAL RESISTANCE AFTER ISQUEMIC ACUTE RENAL FAILURE: IN VITRO AND IN VIVO STUDIES

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Introduction and Aims: Ischemic preconditioning is an endogenous phenomenon in which previous exposition of an organ to ischemia induces resistance to a subsequent ischemic insult. Understanding the mechanisms of resistance may provide means for renal protection.

Methods: Wistar rats were submitted to 45 min bilateral renal ischemia and after 2 days, a second 45 min ischemia was induced (Group 1). Another group of rats were initially sham operated and after 2 days submitted to a 45 min bilateral renal ischemia (Group 2). Functional evaluation was performed 48 hours after the second procedure. For the in vitro studies, proximal tubules (PT) were isolated using collagenase digestion and percoll gradient from sham operated rats (Group sham) or rats submitted to 35 min bilateral renal ischemia (Group ischemia). Experiments were performed 24 h after operation. PT were subjected to 15 min hypoxia followed by 45 min reoxygenation while time control PT were kept oxygenated for similar duration. LDH release (%) was assessed as cell injury. Peroxide by xylenol orange method was assayed as lipid peroxidation index.

Results: It was observed that plasma urea (114 ± 60 *vs* 136 ± 44, p= 0.43) and creatinine clearance (0.21 ± 0.07 *vs* 0.24 ± 0.09, p=0.499) were similar between groups 1 and 2, respectively. Tubular function (FeNa 0.45% vs 0.47% and Uosm 852 vs 855 mosm/kg) was also not different between these groups.

LDH release in control PT from groups sham and ischemia was similar at the beginning of the experiment. However, LDH release in control PT from the group ischemia was lower than group sham at 15 min (LDH 7.8% *vs* 12%, p=0.038) and 60 min (LDH 10.9% *vs* 19%, p=0.011). After 15 min of hypoxia, LDH release was 34% vs 38% (NS) between these groups. After 45 min reoxygenation, LDH release was lower in PT from group ischemia (LDH 40% *vs* 53%, p=0.022). Peroxide production after reoxygenation was higher in PT from group sham compared with group ischemia (2.67±0.34 *vs* 1±0.38, p<0.05).

Conclusions: Renal resistance was obtained after initial ischemia which seems to depend on cellular mechanisms since isolated tubules from previous ischemic rats were resistant to reoxygenation injury. At least, lipid peroxidation may be involved in the ischemia induced cell resistance.

MP073 PROTECTIVE EFFECT OF COMP-ANGIOPOIETIN-1 IN ACUTE RENAL INJURY

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Introduction and Aims: Acute renal failure, characterized by rapid decline in glomerular filtration rate, is a major cause of morbidity and mortality. Ischemic-reperfusion renal injury is the leading cause of acute renal failure. Ischemia-reperfusion alterations induce a cascade of events leading to cellular damage. Vascular endothelial cell injury, tubular epithelial cell injury and several inflammatory reactions can be associated with in ischemic acute renal failure. Angiopoietin-1 (Ang1) is an angiogenic growth factor and a ligand to Tie2, tyrosine kinase receptor expressed on endothelial cells. It is widely expressed and has essential roles in regulating vascular growth, development, maturation, and permeability. Ang1 has potential therapeutic applications in inducing angiogenesis, enhancing endothelial cell survival, and preventing vascular leakage. Recently, a soluble, stable, and potent Ang1 variant, COMP-Ang1 was developed. However, there is no report about effect of COMP-Ang1 in ischemic renal injury.

Methods: To investigate the protective effect of COMP-Ang1 in ischemic renal injury, we evaluated the tubular injury score after treatment with COMP-Ang1 or LacZ adenovirus in ischemic reperfusion renal injury model in mice. sTie2-Fc, an antagonist of Tie2, adenovius was also used in COMP-Ang1-treated acute renal injury model. Western blot analysis of proliferative cell nuclear antigen and phospho-Tie2 and immunohistochemistry of F4/80 were performed.

Results: Morphologic examination indicated less tubular injury in mice receiving COMP-Ang1 than vehicle-treated mice. Prior exposure of mice to COMP-Ang1 resulted in protection against tubulointerstitial histologic damage of about 24%, 31 and 25% at 1, 2 and 3 days after ischemic renal injury, respectively, as determined by tubular injury score. Proliferative cell nuclear antigen was significantly increased with vehicle treatment. After COMP-Ang1 treatment, proliferative cell nuclear antigen in ischemic kidney was further significantly increased compared with vehicle treatment. The number of monocytes/macrophages, as identified by the immunohistochemistry of F4/80 antigen in vehicle-treated kidneys, increased for 3 and 5 days after ischemic renal injury, compared with sham-operated kidneys. COMP-Ang1 treatment reduced the number of monocytes/macrophages infiltration in ischemic renal injury. Pretreatment with sTie2-Fc, an inhibitor of Tie2 reversed the effect of COMP-Ang1 in the infiltrated F4/80-positive cells in acute renal failure. COMP-Ang1 increased phosphorylation of Tie2 after ischemic renal injury and pretreatment with sTie2-Fc reversed COMP-Ang1–induced Tie2 phosphorylation.

Conclusions: These results indicate that COMP-Ang1 treatment have protective effect in ischemic renal injury. COMP-Ang1 may have potential as a therapeutic agent in the treatment or prevention in acute renal failure.

MP074 THE EFFECTS OF KETOSTERIL AND SOY BEEN PROTEIN ON THE COURSE OF EXPERIMENTAL UREMIA IN RATS

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Introduction and Aims: There are controversies regarding the influence of low protein diet on the course of renal failure. The main reason of skepticism is the malnutrition development and worsening of long-term prognosis due to hypoalbuminemia after the switching to renal replacement therapy.

The aim of our study was to investigate the effects of low protein diet (LPD), with the supplementation of either ketosteril or soy been protein on the course of experimental uremia.

Methods: Serum level of urea (S_{ur}), inorganic phosphorus (S_{Pi}), calcium (S_{Ca}) and cholesterol, as well as left ventricular hypertrophy (LVH) index, blood pressure (BP) and heart beat rate (HBR) have been investigated in 5/6 nephrectomised (NE) Wistar rats, divided into 3 groups. Group 1 received standard diet (20% of protein content), group 2 – on LPD (10% of protein content), where the 10% of protein have been restored with the Ketosteril, group 3 – LPD, where the 10% of total protein have been restored with soy been protein, and control sham-operated rats group, received standard protein diet.

Results: After two months of follow up the animals with standard diet (group 1) showed a significant (p<0.001) increase of S_{ur} (16.4±0.34 mmol/l), S_{Pi} (2.76±0.12 mmol/l), cholesterol (1.60±0.12 mmol/l) and reduction of S_{Ca} (1.90±0.09 mmol/l), compared with sham-operated control rats (S_{ur} – 4.9±0.64 mmol/l; cholesterol – 1.34±0.08 mmol/l; S_{Pi} – 1.72±0.1 mmol/l; S_{Ca} – 2.35±0.15 mmol/l). Besides, the NE rats on standard diet (group 1) developed LVH index (2.75±0.07 mg/g of body mass, p<0.001), hypertension (155±5 mm Hg) and increased HBR (407.0±17.0 b/min). BP and HBR in controls were 120.0±2.0 mm Hg and 360.0±9.5 b/min,

respectively. In groups 2 and 3 S_{ur} (7.53±0.75 and 11.49±0.69 mmol/l, respectively) and S_{Pi} (2.09±0.07 and 2.10±0.06 mmol/l, respectively) were significantly lower, than in group 1 ($p<0.001$). Also, in contrast to group 1, they did not develop either hypocalcaemia or hypercholesterolemia, and their LVH index was less marked. Moreover, ketosteril intake prevented BP increase (125.0±4.0 mm Hg) and HBR (369.0±10.0 b/min) as compared with controls. In group 3 BP was significantly lower (135.0±2.0 mm Hg), than in group 1 ($p<0.01$).

Conclusions: Thus, LPD with Ketosteril, as well as soy been protein, supplementation causes retardation of experimental uremia and, in addition, protects the cardio-vascular system in subtotally NE Wistar rats.

MP075 EFFECTS OF HUMAN UMBLICAL CORD STEM CELLS AND G-CSF ON CARBON TETRACHLORIDE-INDUCED NEPHROTOXICITY

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Introduction and Aims: Although, the effect of stem cells on stimulation of tissue regeneration caused its use in acute renal failure, and existence of reports on G-CSF enhancing recovery of renal functions and preventing tubular injury, there is not enough data comparing the effects of G-CSF and human umblical cord stem cells on renal tissue. In this study, the effects of G-CSF and human umblical cord stem cell therapy alone or combined on experimental rat model of carbon tetrachloride-induced nephrotoxicity, and association of these effects with the time of therapy were investigated.

Methods: The study consisted of 50 Wistar rats that were divided into four groups, one of which consisted of 8 rats (group 1) and others of 14 rats (groups 2, 3, 4). Initially, CCl4 (0.5ml/kg body weight of CCl4/pure olive oil, 1:1) was administered to all rats via intraperitoneal (IP) injection and then saline, human umblical cord stem cells (2million cells/kg), G-CSF (150 mcg/kg) and stem cells+G-CSF via IP, at 6^{th} and 24^{th} hours (subdivided groups a and b) were given to the groups 1,2,3,4, respectively. All rats were sacrified, blood samples were collected and renal tissues were removed after 48^{th} hour of the injection of CCl4. Serum urea and creatinine levels were measured. While a part of renal tissue were evaluated pathologically, tissue alpha glutathione S-transferase(GST) levels were measured by tissue homogenization method in the other part.

Results: Serum urea, creatinine and tissue GST levels for each groups were shown on table 1. The most common histopathological findings were tubular degeneration and dilatation in all groups. There was no significant difference between groups in histopathological result. Serum urea levels were significantly different between group 1 and group 3b ($p=0.04$), tissue GST levels were significantly different between group 1 and group 4a ($p=0.01$).

Group	Urea (mg/dl)	Creatinine (mg/dl)	Tissue GST (mcg/gr prt)
Group 1	52.8±7.9	1.3±0.5	5.62±4.76
Group 2a	50.4±5.9	1.3±0.3	2.47±0.47
group 2b	38.3±4.5	1.2±0.4	2.08±0.61
group 3a	46.2±10.3	1.1±0.4	2.15±1.22
group 3b	34.6±11.6	1.2±0.2	2.30±1.17
Group 4a	36.9±8.6	1.3±0.4	1.11±0.27
Group 4b	43.5±6.6	1.1±0.3	1.75±0.35

Conclusions: As a result of this study; combined administration of stem cells and G-CSF were effective in early period to prevent renal tubular injury induced by CCl4.

MP076 MINIMAL EFFECTIVE DOSAGE OF ATORVASTATIN REQUIRED FOR PREVENTION OF ISCHEMIC ACUTE RENAL FAILURE AND LONG-TERM KIDNEY DAMAGE AFTER ISCHEMIA-REPERFUSION INJURY IN RATS

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Introduction and Aims: Ischemia-reperfusion injury (IR) causes local inflammatory reaction that aggravates ischemic acute renal failure (ARF) and leads to long-term kidney failure, manifested by proteinuria and progressive decline in glomerular filtration rate. Statins can prevent inflammatory reaction and alleviate ischemic ARF, however high dose therapy may cause rhabdomyolysis and myoglobinuric ARF. The aim of the study was to estimate the minimal effective dosage of atorvastatin (ATO) administered in pre- and post-ischemia period in rats.

Methods: 14 days after right nephrectomy, IR was induced in 35 male Sprague-Dawley rats by 45-minute clamping of left renal vascular pedicle. Prior to IR, rats were pretreated with ATO by oral gavage: 2mg/kg body weight (bw) twice daily for 3 days (group 1; n=12) or 1mg/kg bw twice daily for 6 days (group 2, n=12). Rats of the control group were given vehicle for 6 days (n=11). During the 6 days following IR, rats of group 1 and 2 were given the same daily doses of ATO as before IR, whilst animals of the control group were administered vehicle. Creatinine clearance (Cr_{Cl}; µl/min/100 g bw) and fractional excretion of sodium (FE_{Na}; %) were estimated 48 hours and 24 weeks after IR. In addition, urine protein to creatinine clearance ratio (U_{prot}/Cr_{Cl}; mg/1 ml Cr_{Cl}) was assessed 24 weeks after IR.

Results: As shown in table 1 (means±SD), 48 hours after IR, Cr_{Cl} was significantly higher, whilst FE_{Na} lower in group 1 than in controls or group 2. Also, in a long-term observation, kidney function was better in rats of group 1 when compared to controls or group 2. In contrast, kidney function parameters in group 2 did not differ from controls, neither in the short- or long-term observation periods.

Conclusions: Atorvastatin given at a minimal dose of 2mg/kg bw twice daily in the short period preceding and following kidney ischemia-reperfusion injury, effectively alleviates ischemic ARF and reduces long-term kidney dysfunction in rats. These findings may be useful in future clinical trials with atorvastatin in various populations, including kidney transplant recipients.

MP077 THE EFFECTS OF VITAMIN C ALONE OR IN COMBINATION WITH L-CARNITINE IN EXPERIMENTAL MYOGLOBINURIC ACUTE RENAL FAILURE (MARF)

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Introduction and Aims: Reactive oxygen intermediates have been demostrated to play an etiological role in MARF. Vitamin C is a major antioxidant, essential for the scavenging of toxic free radicals, both in the plasma and tissues. L-Carnitine and its acyl esters may act as an antioxidant either having a primary antioxidant activity or, more likely, functioning as a secondary antioxidant.Thus, we aimed to investigate the effects of vitamin C alone or in combination with L-Carnitine on development of MARF formed by glycerol in rats.

Methods: Male Spraque Dawley rats bred in the Experimental Research Center of Trakya University were used in this study. Rats were divided into

Abstract MP076 – Table 1

		Group 1 (n=12)	Group 2 (n=12)	Controls (n=11)	Group 1 vs. Controls	Group 2 vs. Controls	Group 1 vs. Group 2	
48 h after ischemia	Cr_{Cl}	104±50	39±36	31±33	$P<0.001$	NS	$P<0.005$
	FE_{Na}	1.43±1.51	9.77±11.0	12.12±12.12	$P<0.001$	NS	$P<0.002$
24 weeks after ischemia	Cr_{Cl}	243±60	95±73	115±68	$P<0.001$	NS	$P<0.001$
	FE_{Na}	0.24±0.10	0.75±0.56	0.54±0.51	NS	NS	$P<0.005$
	U_{prot}/Cr_{Cl}	0.44±0.52	1.18±0.89	0.99±0.65	$P<0.01$	NS	$P<0.003$

U Mann-Whitney test

four groups. The animals were allowed free access to standart rat food but expect healthy control (HC) group deprived of drinking water for 24h before glycerol injection and than were injected with 50% glycerol in normal saline (8 ml/kg, im). Injection volumes were divided equally between the two hindlimbs. Simultaneous with glycerol administration, following substances were administered to groups via intraperitoneal route for 4 days: 0.5 ml normal saline to patient control group (PC), 20 mg/kg/d vitamin C to VC group, and 10 mg/kg/d vitamin C +100 mg/kg/d LC to combination group. The urine (last 24h) of the rats were collected through metabolic cages. At the end of the 96h, rats were anaesthetized with 10 mg/kg xylazine + 50 mg/kg ketamine, and than animals were sacrificed by collecting their blood via intracardiac punction. Hemoglobin in blood, urea, creatinine, Na^+, K^+ in plasma; creatinine, Na^+, albumin (UAE), N-acetyl-β-D-glycosaminidase (NAG), endothelin 1 in urine; and malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) in renal tissue were determined. GFR and fraxional excretion of Na^+ (FE_{Na}) were calculated. Renal histopathologic changes and iron accumulation were evaluated under light microscope.

Results: Biochemical, and microscopical findings of study groups were shown in table.

Biochemical, and microscopical findings of study groups

	HC (n:10)	PC (n:10)	VC (n:10)	VC&LC (n:10)
GFR (ml/dk/100g)	541 ± 134	8.0 ± 7.1 d	10.3 ± 9.4 d	10.5 ± 6.0 d
FENa (%)	0.1 ± 0.1	26 ± 11 d	23 ± 12 d	23±6 d
NAG/uCr (U/mg)	11 ± 1.5	87±52 a	75±43 b	59±26 d
UAE/uCr (mg/g)	27 ± 1	151 ± 65 c	122 ± 74 b	63 ± 48 z
uEndothelin/uCr (ng/mg)	3 ± 5	30 ± 30c	17 ± 19 a	10 ± 8
SOD (U/mg pr)	8.4 ± 0.9	4.9 ± 1.0 d	6.0 ± 2.3 a	6.3 ± 2.1 a
CAT (k/g pr)	95 ± 16	12 ± 6 d	23 ±12 d/y	23 ± 6 d/y
MDA (nmol/g pr)	2.0 ± 0.6	3.8 ± 0.5 d	3.0 ± 0.4 b/y	3.0 ± 0.3 c/z
Tubular necrosis (%)	0	30.1 ± 6.7 d	25.8 ± 6.1 d	26.3 ± 5.3 d
Accumulation of iron (%)	0	22.3 ± 7.1 d	15.2 ± 4.0 d	15.7 ± 3.9 d

HC vs glycerol treated groups; a: P<0.01, b: P<0.005, c: P<0.0005, d: P<0.00005; PC vs VC and VC-LC groups; x: P<0.01, y: P<0.005, z: P<0.0005

Conclusions: Our findings show that vitamin C alone reduces the development of ARF, it's combination with L-Carnitine confer additive protection.

MP078 DOSE-DEPENDENT NEPHROPROTECTIVE EFFECT OF METHYLPREDNISOLONE IN THE RAT MODEL OF ISCHEMIC ACUTE RENAL FAILURE

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Introduction and Aims: Ischemia-reperfusion injury (IR) of the kidney causes an activation of nuclear factor-κB (NF-κB), leading to enhanced expression of inflammatory genes and acute inflammatory response. Expression of adhesion molecules and cytokines, as well as recruitment and infiltration of leukocytes, occurring after initial recovery, cause progressive organ damage. Glucocorticoids inhibit NF-κB activation and thus reduce inflammation. The aim of the study was to assess whether methylprednisolone given at two different dose regimens at the time of IR improves the course of ischemic ARF and prevents long-term kidney failure in rats.

Methods: 14 days after right nephrectomy, IR was induced in 30 male Sprague-Dawley rats by 45-minute clamping of left renal vascular pedicle. Methylprednisolone was administered in 0.5ml 0.9%NaCl solute im: rats of group 1 (n=8) received 100mg/kg body weight (bw) 1 hour prior to IR, 60 mg/kg bw 24 hours after IR and 30mg/kg bw 48 hours after IR; rats of group 2 (n=12) were given respectively 30, 30 and 15 mg/kg bw. Animals of control group (n=10) were administered 0.5ml 0.9%NaCl solute at each time point.

Creatinine clearance (Cr_{Cl}; μl/min/100 g bw), fractional excretion of sodium (FE_{Na}; %) and urine protein to creatinine clearance ratio (U_{prot}/Cr_{Cl}; mg/1ml Cr_{Cl}) were estimated 48 hours and 24 weeks after IR, respectively.

Results: As shown in table 1 (means±SD), 48 hours after IR, Cr_{Cl} was significantly higher, whilst FE_{Na} lower in group 1 and 2 than in controls. On the other hand, U_{prot}/Cr_{Cl} was reduced only in group 1 when compared to control group. Also, after 24 weeks, proteinuria was diminished only in group 1 in comparison to controls. In a long-term observation Cr_{Cl} and FE_{Na} did not differ between groups 1 or 2 and controls.

Conclusions: 1. Administration of methylprednisolone at the time of ischemia-reperfusion injury alleviates ischemic acute renal failure in rats both at a relatively low and high dosage, however more efficiently in the latter case. Alleviation of long-term kidney failure is also dose-dependent, occurring only with the high dosage of the steroid.

2. Use of glucocorticoids in kidney transplant recipients may be justified not only by suppression of alloantigen specific adaptive immunity, but also on the grounds of inhibition of inflammatory reaction following ischemia-reperfusion injury. However, optimal dosage of steroids in these patients should be verified in clinical trials.

MP079 INFLUENCE OF ACTIVATION OF THE NITRIC OXIDE PATHWAY IN DEVELOPMENT OF RENAL FAILURE IN EXPERIMENTAL MODEL OF HEPATORENAL SYNDROME (HRS)

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Introduction and Aims: The development of functional renal failure following the severe damage of liver and liver failure, in the absence of clinical, biochemical and histopathological causes of renal failure is defined as hepatorenal syndrome (HRS). Precise pathomechanisms leading from hepatic damage to renal failure are still very poor understood. In our study, in experimental model of HRS, using L-Arginine (L-ARG), we tried to explain, whether the activation of the nitric oxide system plays any role in development of renal failure in HRS.

Methods: We used 40 male Sragues-Dawley rats (SDR) divided into 5 groups. Our protocol was as follows: Sham group (group 1) received saline intraperitoneally. Group 2 received 1.1g/kg b.w. galactosamine (Ga1N) intraperitoneally (i.p.). Control L-ARG group (group 3) received i.p. 300mg/kg b.w. L-ARG during 48 hours prior to saline injection. Group 4 received i.p. L-ARG in the same doses before Ga1N injection, group 5 the same doses of L-ARG during 48 hours after Ga1N injection. Rats were placed individually in glass metabolic cages with free acess to food and water. A 24 hours urine samples were collected 24 hours prior to Ga1N or saline injection and 48 hours after Ga1N or saline injection. 6ml of blood were collected also after Ga1N or saline injection from the heart in deeply anaesthetised animals.

Results: Liver failure developed in all tested groups (group 2,4,5) with significant increase in serum concentration of bilirubin, ALT, ammonia in comparison to control groups (group 1 and 3 respectively). We have also found in group 2 typical acute renal failure (ARF) after injection of Ga1N with significant increase in serum concentration of creatinine and urea and decrease in creatinine clearance. Injection of L-ARG before Ga1N intoxication had no influence on the serum concentrations of creatinine, urea and creatine clearance. However giving L-ARG after Ga1N injection caused significant improvement of GFR. The results are presented in Table 1.

Abstract MP078 – Table 1

		Group 1 (n=8)	Group 2 (n=12)	Controls (n=10)	Group 1 vs. Controls	Group 2 vs. Controls	Group 1 vs. Group 2
48 h after ischemia	Cr_{Cl}	87±44	93±54	19±23	$P<0.001$	$P<0.001$	NS
	FE_{Na}	3.46±2.50	3.89±3.14	22.15±16.48	$P<0.005$	$P<0.001$	NS
	U_{prot}/Cr_{Cl}	32.4±19.6	65.5±39.3	83.4±31.8	$P<0.01$	NS	NS
24 weeks after ischemia	Cr_{Cl}	77±19	74±39	56±58	NS	NS	NS
	FE_{Na}	0.95±0.47	0.83±0.38	2.49±2.75	NS	NS	NS
	U_{prot}/Cr_{Cl}	37.8±25.6	91.5±65.1	115.3±70.5	$P<0.01$	NS	$P<0.05$

U Mann-Whitney test

Table 1

Gr./Nb	Bilirubin µmol/l	ALT IU/L	Creatinine µmol/l	Urea mmol/l	Creatinine Clearance ml/min
1/8	6,84±4,44	56,2±9,9	41,5±3,53	5,61±0,63	0,89±0,4
2/8	58,66±23,09	2098,6±886,1	67,18±7,95	13,33±1,68	0,18±0,12
3/8	3,76±0,68	53,75±7,37	38,01±7,07	4,27±0,69	1,05±0,35
4/8	50,02±16,41	1603,7±389,6	70,72±6,18	14,25±0,81	0,19±0,08
5/8	50,67±17,95	1435,6±300,7	64,53±7,95	13,66±0,78	0,39±0,15
Gr.2/Gr.1	p<0.004	p<0.001	p<0.001	p<0.001	p<0.0012
Gr.4/Gr.2	ns	ns	ns	ns	ns
Gr.5/Gr.2	ns	ns	ns	ns	p<0.018
Gr.4/Gr.5	ns	ns	ns	ns	p<0.012

ns - not significant

Conclusions: In conclusion, our study showed that in course of HRS attempt of activation of the NO pathway after liver damage improves but activation before does not improve renal efficiency.

MP080 IDENTIFICATION OF RENAL PAPILLARY NECROSIS USING AN EIA FOR URINARY RENAL PAPILLARY ANTIGEN-1 (RPA-1), A BIOMARKER OF COLLECTING DUCT PATHOLOGY

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Introduction and Aims: Renal papillary necrosis (RPN) is a frequent issue encountered in drug development, yet there is still no reliable non-invasive method available for its early detection. To enable rapid identification of RPN in rats, a sandwich capture EIA was developed for urinary Renal Papillary Antigen-1 (RPA-1), a biomarker localised to the luminal epithelial cells of the collecting duct. The assay was developed with monoclonal IgG discovered via immunohistological screening (Histomics®).
Methods: Three batches of RPA-1 EIA were produced within Biotrin, each using different lots of antibody and antigen. Standard validation studies were performed. Immunohistochemistry studies were performed, on frozen rat sections (via indirect immunofluorescence) and on formalin-fixed rat kidney sections (via POD-staining technique) to localise the expression of RPA-1. Urine was collected from Wistar rats following a single dose of 10 mg/kg Indomethacin via gavage. Control animals received a single dose of isotonic saline. Urine was collected at days -2, -1, 1, 2 and 3 and was analysed for the presence of RPA-1 with the Biotrin RPA-1 EIA. Histopathological analyses were also performed on the kidneys of each animal.
Results: The Biotrin RPA-1 EIA had excellent reproducibility, with inter and intra-assay CVs of better than 18% and 7%, respectively. Normal urinary RPA-1 levels for both Han Wistar and Sprague Dawley strains of rat were shown to be comparable at 630 + 230 U/L. Mean recovery, when RPA-1 was added over the range 1500 U/L to 3200 U/L, was 91%. Immunohistochemistry studies demonstrated the anti-RPA-1 monoclonal IgG to be specific for rat cortical, medullary and papillary collecting ducts. Limited staining of the visceral urothelium overlying the papilla was also observed. Analysis of urine samples collected from animals treated with indomethacin indicated significant RPA-1 release. Overall there was good correlation between the level of RPA-1 release and the histopathology, with the lowest levels of RPA-1 release occurring in those animals that survived to sacrifice. Furthermore, of the animals that survived to sacrifice,the level of RPA-1 release was related to the level of histopathology suggesting the Biotrin RPA-1 EIA to be a sensitive predictor of papillary pathology.
Conclusions: The Biotrin RPA-1 EIA was shown to be capable of detecting RPA-1 in the urine of control and toxin treated rats in a reproducible manner. RPA-1 was shown to be specifically localised to collecting ducts. Significant release of RPA-1 into urine could be detected 2 days after treatment with indomethacin and histopathology studies suggested urinary RPA-1 to be a sensitive and specific biomarker of collecting duct injury. Thus, detection of urinary RPA-1 by EIA provides a sensitive, specific and reproducible method for detection of RPN in rats. Future studies will be extended to other nephrotoxins and the development of an analogous assay for human samples.

MP081 EVALUATING THE CYTOSOLIC AND MITOCHONDRIAL pH WITH SNARF1 IN LIVING MADIN-DARBY CANINE KIDNEY (MDCK) CELLS INFLICTED WITH ATP DEPLETION

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Introduction and Aims: Mitochondria and endoplasmic reticulum clear excessive cytosolic Ca^{2+}, as occurring under ischemic conditions. Recently we showed that mitochondria in MDCK cells subjected to Metabolic Inhibition (MI) take up cytosolic Ca^{2+} via mitochondrial Na^+/Ca^{2+} exchangers (NCX) acting in reverse mode (AJP 286: F784-794, 2004). We obtained also evidence that the reversed NCX mediated mitochondrial Ca^{2+} uptake is sustained by a noticeable rise in mitochondrial Na^+ concentrations ($[Na^+]_m$) in the first 15 min of MI (JASN 16: 3490-7, 2005).
$[Na^+]_m$ is determined by NCX activity as well as the activity of mitochondrial Na^+/H^+ exchangers (NHE), thus by the pH gradient over the mitochondrial membrane.
Aiming to unravel the underlying mechanisms related to $[Na^+]_m$ changes, this study focused on the pH changes in cytosol (pH_i) and mitochondria (pH_m) in metabolically inhibited MDCK cells.
Methods: MI, used as an experimental model to induce cellular ATP depletion, was applied for 1 h, by inhibiting both cellular glycolysis (with 10 mM 2-deoxyglucose) and oxidative phosphorylation (with 2.5 mM NaCN). Changes in pH_i and pH_m were detected by confocal microscopy (Zeiss LSM 510 META, x 63/1.4 Plan-Apochromat oil-immersion objective) on confluent nonpermeabilized living cells grown on glass coverslips for 3-4 days. Cells were double labelled with 5-6 carboxy SNARF-1 AM (a ratiometric pH sensitive probe) and Mitotracker Green (a specific mitochondrial probe, used as a "mask" to discriminate between the cytosolic and matrix SNARF signal). The dual emission ratio of SNARF (680 nm/590 nm) was monitored as a measure for pH. The SNARF ratio was calibrated in terms of pH units by using high K^+ concentration solutions containing also nigericin, FCCP and oligomycin (to equilibrate protons between the cytosol and mitochondria) and set at different pH values in the range 6.8 - 7.8.
All experiments were performed at 37°C.
Results: MI resulted in both cytosolic and mitochondrial acidification, with a more pronounced decrease of pH_m as compared to pH_i. Mitochondrial pH decreased from 7.4 by 30% during the first 20 min of MI and continuously decreased for an additional 10% in the next 40 min of MI, to pH 6.8. During the first 20 min of MI, the cytosolic pH decreased by 20%, while presenting a slight recovery for the subsequent 40 min of treatment. By contrast to the pH_i evolution, the pH_m presented a delayed recovery upon removal of metabolic inhibitors. Nevertheless, cells showed local regeneration of mitochondrial networks, as supported by both confocal and electron microscopy images.
Conclusions: This study shows for the first time that MI of MDCK cells elicits an early reversed outward proton gradient across the inner mitochondrial membrane, and explains our previous results showing a fast uptake of sodium in the mitochondrial matrix via the Na^+/H^+ exchanger.
Acknowledgments: bilateral research collaboration program between Flanders and Romania (BOF 05 B03) and tUL impulsfinanciering.

Glomerular diseases clinical 2

MP082 TREATMENT OF FOCAL AND SEGMENTAL GLOMERULOSCLEROSIS IN CHILDREN WITH CYCLOSPORINE A

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Introduction and Aims: The aim of our study was to study an efficacy of Cyclosporine A (CsA) in children with focal and segmental glomerulosclerosis (FSGS).
Methods: We have observed 9 children with nephrotic syndrome and morphologically established FSGS, mean age was 9±3,5 y.o., mean disease history 1,33±0,33 y. In 6 patients microhematuria and in 2 – decreasing of