A PROSPECTIVE RANDOMIZED OPEN-LABEL CROSSOVER TRIAL ON OPTIMAL ANTICOAGULATION DURING LIVER DIALYSIS BY THE MOLECULAR ADSORBENTS RECIRCULATING SYSTEM (MARS)

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Introduction: The Molecular Adsorbent Recirculating System (MARS) is a non-biologic liver dialysis device, used to bridge patients to recovery of native hepatic function or to liver transplantation. The optimal anticoagulation regimen to maintain patency of the extracorporeal circuit has not been defined. Heparin is widely used, despite a high rate of severe bleeding complications during treatment. As coagulation is disturbed during liver failure, the need of anticoagulation during MARS can be questioned.
Methods: We performed a prospective randomized cross-over study in patients with acute-on-chronic liver failure treated with MARS (Clinical trials protocol NCT00695617). We compared anticoagulant-free MARS with MARS using regional citrate anticoagulation. Primary endpoint was completion of prescribed 6 hour MARS sessions. Secondary endpoint evaluation included safety monitoring of regional citrate anticoagulation and monitoring of treatment efficacy.
Results: We performed 27 MARS dialysis sessions in 10 patients. Regional citrate anticoagulation was applied in 14 sessions, 13 sessions were performed without anticoagulation. 4 MARS sessions were interrupted preterm, due to occlusive clotting ($n = 3$) and intractable bleeding ($n = 1$), all in the anticoagulation-free group (Log rank, $P = 0.03$). Regional citrate anticoagulation was well-tolerated and major side-effects were not observed. Due to treatment down-time without anticoagulation, regional citrate anticoagulation resulted in more efficient removal of bilirubin and bile acids by the MARS system.
Conclusion: MARS treatment using regional citrate anticoagulation appears safe in patients with acute-on-chronic liver failure and is well-tolerated. Metabolic complications could not be demonstrated. Regional citrate anticoagulation is superior to avoidance of anticoagulation, despite liver-failure induced coagulation disturbances. Regional citrate anticoagulation should be considered as standard of care during liver dialysis using the MARS system.

11

DISRUPTED NITRIC OXIDE SIGNALING BY THE PROTEIN-BOUND UREMIC RETENTION SOLUTE P-CRESOL

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Introduction: Chronic kidney disease (CKD) is associated with excessive cardiovascular disease. Nitric oxide (NO) signaling is impaired in CKD patients. *p*-Cresol is independently associated with cardiovascular disease in hemodialysis patients. We hypothesized that *p*-cresol interferes with NO signaling, thereby contributing to CKD-associated endothelial dysfunction and cardiovascular mortality.
Methods: We investigated the effects of *p*-cresyl sulfate (PCS), the main *in vivo p*-cresol derivative, on NO and cGMP generation by human umbilical vein endothelial cells (HUVEC). The effects of PCS on sGC activity were studied using purified rat sGC and sGC overexpressing CHO cells. Rabbit saphenous arteries were used for functional studies on the effect of PCS on vascular relaxation. The reduced (NO sensitive) sGC was activated by BAY 41-2272. Activity of the oxidized (NO-insensitive) sGC was probed using BAY 58-2667.
Results: PCS reduced the stimulated cGMP generation by HUVEC in a time and dose-dependent fashion. We observed a small, but significant, reduction in eNOS expression (-15%, $P = 0.01$). NOx concentrations were not changed. Moreover, PCS also reduced the exogenous NO-stimulated cGMP generation. As PCS did not affect sGC expression, we speculated that PCS changed the sGC redox state, thereby affecting sGC activity. BAY 58-2667 stimulated cGMP generation was increased in the presence of PCS, both in a purified rat sGC model and in sGC over-expressing CHO cells. In a model of phenylephedrine pre-contracted rabbit saphenous arteries, presence of PCS increased vasodilator activity of BAY 58-2667, while reducing the effects of sodium nitroprusside.
Conclusions: The uremic retention solute p-cresyl sulfate reduces NO signaling. Mechanistically, we observed a shift of the sGC redox status towards the NO-insensitive oxidized/heme-free state. As altered sGC redox status can be targeted by the newly developed drug class of sGC activators, this pathway may prove a novel therapeutic target in patients with chronic kidney disease.

12

URINARY NGAL: USE OF ABSOLUTE VALUE OR RATIO TO CREATININE?

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Introduction: Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a promising marker for the detection of acute kidney injury. This marker has been proposed for urinary measurement. However, in the literature, authors indistinctly use "absolute" value or NGAL to creatinine ratio. Up to now, there are no strong arguments favouring for one. This question is of importance as this marker is sensed to be used only on urine random samples. To find an answer to this very practical matter, one approach could be to compare biological CV (intra-individual variation) of the "absolute" and ratio results.
Material and methods: Biological CV was classically calculated on a sample of 12 healthy subjects. Each subject was asked to collect the first morning and the second morning urine for 10 open days. Subjects were free to drink (or not) between the two samples. Analytical CV for urinary NGAL was measured at 3% in our laboratory.
Results: For the first morning samples, mean "absolute" and ratio NGAL measurements were 39±42 ng/ml and 0.03±0.03 ng/mg creatinine, respectively. For the second morning sample, mean "absolute" and ratio NGAL concentrations were 72±119 ng/ml and 0.06±0.07 ng/mg creatinine, respectively. The biological CVs of "absolute" and ratio NGAL for the first urine were 94% and 84% respectively. For the second urine, the CVs were 132% and 75%.
Conclusions: Using the ratio to creatinine significantly improves the intra-individual variation observed in NGAL measurements. This improvement is especially relevant for random urine (i.e. second) compared to the first overnight urine. This seems logical as random urine is potentially more influenced by osmolality. As urinary NGAL is sensed to be an early marker of acute kidney injury, its measurement will be always on an early and random sample. Moreover, we have measured with a strong methodology the biological CV of urinary NGAL. We confirm that this CV is really far from negligible (at best 75%) which will necessary must influence our interpretation of longitudinal NGAL results.

13

THE AMINOGLYCOSIDE CLEARANCE IS INCREASED IN CYSTIC FIBROSIS AND DENT'S DISEASE MODELS OF PROXIMAL TUBULE DYSFUNCTION.

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Aminoglycosides (AG) are commonly used in the treatment of gram-negative infections in patients with cystic fibrosis (CF) caused by invalidating mutations in the Cl^- channel CFTR. A limitation of the clinical use of AG is their renal toxicity, which is explained by the fact that unbound AG are freely ultrafiltrated and reabsorbed in proximal tubule (PT) cells via the multi-ligand receptors megalin and cubulin. Our previous studies have demonstrated the specific roles played by CFTR in PT cell endocytosis. The renal clearance of AG is enhanced in CF patients, possibly by a defect in receptor-mediated endocytosis. In order to shed light on AG handling by PT cells, we investigated the clearance of gentamycin in $Cftr^{\Delta F/\Delta F}$ and gender-matched $Cftr^{N/N}$ mice in comparison with $Clcn5^{Y/-}$ mice, taken as paradigm of PT dysfunction. Mice were injected i.p. with gentamycin (5mg/kg BW) and kept in metabolic cages for 24h, to obtain clearance and renal accumulation of gentamycin. Mice lacking functional CFTR showed a 30% increase in gentamycin clearance, while $Clcn5^{Y/-}$ showed a 70% increase. These differences were reflected by a significantly lower accumulation of gentamycin in the kidney as compared to controls. Next, we evaluated the handling of gentamycin by PT cells using primary cultures of mouse proximal tubule cells (mPTC) exposed to 5 mg/ml gentamycin for 7 min or 45 min at 37°C and double stained with anti-gentamycin/anti-EEA1. The uptake of gentamycin was also followed after simultaneous incubation of mPTC with Alexa488-gentamycin and Lysotracker Red. In $Cftr^{\Delta F/\Delta F}$ and $Clcn5^{Y/-}$ mPTC, the uptake of gentamycin was reduced, whereas colocalization with endosomes and lysosomes was still observed.
These results demonstrate that defective endocytosis in $Cftr^{\Delta F/\Delta F}$ (and in $Clcn5^{Y/-}$) mice and mPTC is associated with a reduced uptake of gentamycin, reflected by an enhanced drug clearance. These data confirm the interest of megalin-cubilin receptor antagonists as a potential way to reduce the renal toxicity of AG.

14