PO2460

Guainidinylated Apolipoprotein C3 (ApoC3) Causes Kidney and Vascular Injury

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Background: Cardiovascular diseases (CVD) and chronic kidney diseases (CKD) are highly prevalent in Western populations and account for a substantial proportion of mortality. We found that apolipoprotein C3 (ApoC3), a constituent of triglyceride-rich lipoproteins, induces alternative NLRP3 inflammasome activation in human monocytes and thus causes sterile inflammation. The aim of the present study was to screen ApoC3 for the presence of posttranslational protein modifications and to assess its relevance in vitro, in vivo, as well as in a prospective cohort of CKD patients.

Methods: ApoC3 was subjected to proteomic analysis. The proinflammatory properties of ApoC3 were assessed in human monocytes and in humanized mice. Moreover, posttranslationally modified ApoC3 was quantified in prospective cohort of 543 patients with various etiologies of CKD and linked to kidney and cardiovascular outcomes.

Results: We identified posttranslational guainidinylation of lysine residues of ApoC3 (gApoC3) in patients after acute myocardial infarction and in patients with CKD. gApoC3 accumulates in kidneys and hearts after injury as determined by 2D-proteomic analyses. In human monocytes, guainidinylation enhanced the binding of ApoC3 to the cell surface and exerted substantially stronger pro-inflammatory effects as compared native ApoC3. In humanized mice, gApoC3 strongly induced kidney fibrosis and abolished the regeneration after vascular injury. In a prospective clinical trial of 543 patients, higher gApoC3 blood levels as determined by mass spectrometry were associated with increased mortality as well as cardiovascular and renal events during a long-term follow-up.

Conclusions: The present study provides evidence from preclinical models and a prospective clinical trial that gApoC3 plays an important role in the development of organ injury in patients with CKD, myocardial infarction and other clinical conditions. The clinical study represents one of the largest trials, in which the association of a specific PTM and clinically relevant outcomes was assessed. These findings highlight gApoC3 as a pathophysiological relevant factor in development of organ dysfunction.

PO2461

Endothelial Function, Oxidative Stress, and Cognitive Performance in CKD

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Background: Cognitive impairment, common in patients with chronic kidney disease (CKD), can be explained at least partially by the high prevalence of cerebrovascular disease in the population. Here, we hypothesized that endothelial dysfunction associates with reduced cognition in patients with CKD.

Methods: We conducted a cross-sectional study of 63 middle-aged/older adults with CKD stage 3b and 4. Cognitive function domains including executive function, memory, language, and processing speed were assessed via the NIH-Toolbox. Endothelial function of the brachial artery was assessed via flow-mediated dilation (FMD) using Doppler ultrasound. The influence of oxidative stress on FMD was determined by infusing a supraphysiological dose of ascorbic acid vs. isovolumetric saline (control). Regression of the brachial artery was assessed via flow-mediated dilation (FMD) using Doppler.

Results: The mean(SD) age, estimated glomerular filtration rate (eGFR), and FMD of the participants were 64(9), 34(11), and 2.6(1.4). Ascorbic acid increased FMD by 4.5e1.7 as compared to saline which increased FMD by 2.5e1.3 (p<0.001). Table 1 illustrates the age-adjusted standard scores for each cognitive domain. We found no association between FMD and any of the cognitive domains. However, a greater response to ascorbic acid correlated with better age-adjusted memory performance independently of education (95% CI: 2.08: 0.51:3.65; p<0.05).

Conclusions: Oxidative stress contributes to endothelial dysfunction in CKD and a greater response to ascorbic acid is associated with better memory performance. More studies are needed to understand the role of oxidative stress in cognitive impairment in patients with stage 3b/4 CKD.

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Table 1: NIH Toolbox Cognitive Domain Standard Scores

|                      | Executive Function | Memory | Language | Processing Speed |
|----------------------|---------------------|--------|----------|-----------------|
| Normal               | 0.55/0.2            | 0.95   | 0.53/0.4  | 0.98/0.1        |
| Impaired             | -1.00/1.1           | -1.00  | -1.00/1.1 | -1.00/1.1       |
| Learning impairment  | 1.32/1.3            | 1.32   | 1.32/1.3  | 1.32/1.3        |

Normatively age-adjusted standard scores are presented. Standard scores have a mean of 100 and SD of 15.

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Key Role for EphB2 Receptor in Kidney Fibrosis

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Background: Eph- Ephrin receptor-ligand signaling has been implicated in the development of tissue fibrosis, though it has not been well defined in the kidney. Methods: We then firstly made use of male EphB2-knockout and littermate control mice (n=5/per group) to receive unilateral renal ischemia-reperfusion (IR) surgery for 35min. In addition, EphB2 signaling was further determined in varied kidney disease models, particularity in diabetes- or hypertension-induced kidney disease models and in the kidney biopsy tissue from IgA nephropathy with glomerulosclerosis and tubular fibrosis.

Results: We detected substantial upregulation of expression and phosphorylation of the EphB2 receptor tyrosine kinase in fibrotic kidney tissue obtained both from mice subjected to either the unilateral renal IR model at 14 days or type 2 diabetes or DOCA & Ang II-infused hypertension and in patients suffering from chronic kidney disease (CKD). Knockout mice lacking EphB2 expression exhibited a normal renal structure and function, indicating no major role for this receptor in kidney development or action. Although IR injury is well known to cause tissue damage, fibrosis, and renal dysfunction, we found that kidneys from EphB2 knockout mice showed much less renal tubular injury and retained a more preserved renal function. IR-injured kidneys from EphB2 knockouts exhibited greatly reduced fibrosis and inflammation compared to injured wild-type (WT) littermates, and this correlated with a significant reduction in renal expression of pro-fibrotic molecules, inflammatory cytokines, NADPH oxidases, and markers for cell proliferation, tubular epithelial-to-mesenchymal transition, myofibroblast activation, and apoptosis. A panel of 760 fibrosis-associated genes were further assessed, revealing that 506 genes in WT mouse kidney following IR injury changed their expression. However, 70-90% of those genes were back to or close to normal in expression when EphB2 was deleted.

Conclusions: These data indicate endogenous EphB2 expression and signaling are abnormally activated after kidney injury and subsequently contributes to the development of renal fibrosis via regulation of multiple pro-fibrotic pathways.

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PO2463

Apelin, a Novel Apelin Receptor Analog, Improves Endothelial Dysfunction in Urinec Rats with 5/6-NephrECTomy

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Background: Endothelial dysfunction (ED), characterized by a reduction in vasodilation as a response to endothelial stimuli, has been reported in patients at almost all stages of chronic kidney disease (CKD). Considering that apelin is a vasodepressor molecule leading to nitric oxide release from endothelial cells, we aimed to investigate the regulatory role of apelin receptor on CKD-induced ED.

Methods: Adult Sprague Dawley male rats were randomized into sham-operation or 5/6-nephrectomy (CKD) groups. CKD groups were treated subcutaneously with saline (S-CKD) or 100 µg/day (each) of apelin (A-CKD), ala-apelin (Ala-CKD) or apelin-ala- apelin (A+Ala-CKD) for eight weeks. Isolated aortas were mounted in organ baths and their vasorelaxatory responses to carbachol (CCh) and apelin were evaluated following pre-contraction with phenylephrine.

Results: Aortas of S-CKD group demonstrated an impaired CCh-induced relaxation as compared to sham group (P<0.05), while pre-incubation with L-NAMe further inhibited CCh-induced vasorelaxation in both sham and S-CKD groups (P<0.05) (Fig A). In A-CKD group, CCh-relaxation response was significantly improved compared to S-CKD group, but L-NAMe attenuated the improvement in relaxation (Fig B-C). When apelin was added to organ bath, despite that S-CKD group showed no relaxation, a dose-dependent relaxation was observed in sham group, which was abolished in the presence of L-NAMe (Fig D). In A-CKD, Ala-CKD and A+Ala-CKD groups, the relaxation response to apelin was enhanced as compared to S-CKD, while L-NAMe pre-incubation abolished the relaxation (Fig E-F).

Conclusions: Apelin, which directly relaxes aortic rings of intact rats in an endothelium-dependent manner, ameliorates CKD-induced endothelial dysfunction when given as a treatment. Moreover, this beneficial effect of apelin treatment on endothelial vasomotor function appears to act by the preserving the activity of nitric oxide-signaling pathway.

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