UNRAVEL NOVEL MECHANISMS RESPONSIBLE FOR CKD INDUCED ENDOTHELIAL CELL DYSFUNCTION

Sonja Vondenhoff[1,2], Tobias Henning[1], Sarah Knapp[1], Constance Baaten[1,2], Heidi Noels[1,2]

1 Institute for Molecular Cardiovascular Research (IMCAR), University Hospital RWTH Aachen, Germany
2 Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, the Netherlands

Introduction

The endothelial layer plays an important role in vascular integrity and cardiovascular health. Chronic kidney disease (CKD) disturbs endothelial hemostasis due to the present low-grade inflammation and uremic environment. The endothelium of CKD patients therefore reflects a vasoconstrictive, pro-inflammatory, pro-atherosclerotic as well as a prothrombotic endothelial phenotype [1-2]. To which extent CKD affects the crosstalk between endothelium and immune cells is not fully understood and requires further investigation in order to ultimately reduce the thrombotic and hemorrhagic risk of CKD patients. Therefore, we aim to reveal molecular mechanisms responsible for the increased thrombotic risk in CKD patients by elucidating the disturbed crosstalk between endothelium, platelets, and neutrophils.

Methods

For the experiments three different endothelial cell types were used: human aortic endothelial cells (HAoECs), human dermal microvascular endothelial cells (HDMECs) and human coronary artery endothelial cells (HCAECs). Cells were cultured under static conditions or under flow with a mixture of seven out of twenty uremic toxins with the highest fold increase in CKD 5 patients on dialysis (data is based on the EUTOX data base). The mixture contained Phenylacetic acid, Hippuric acid, Indoxylsulfate, Kynurenic acid, Para-cresyl-sulfate, Methylguanidine and Guanidinosuccinic acid. In the first step essential intracellular processes were analyzed to determine the extent of induced endothelial cell dysfunction. With a special regard on the metabolic activity and processes linked to the cellular metabolism like autophagy, endoplasmic reticulum stress and ROS production, as readouts qPCR and Western Blot were performed.
In the second step, the cultivation of endothelial cells underflow was established, and the confluency of the endothelial cell layer was analyzed using VE-Cadherin immunofluorescent staining.

Results

A decreased metabolic activity in HAoECs and HDMECs was observed after 5 days of treatment which was not due to increased cell death.
The analysis of cellular processes linked to decreased metabolic activity revealed an impaired autophagic flux upon uremic toxins treatment. Furthermore, the combination of uremic toxins and TNFα lead to increased protein levels of NOX-2 being an important protein contributing to the cellular ROS production.
Moreover, we observed that the treatment of uremic toxins leads to morphological changes. Endothelial cells treated with uremic toxins under static conditions displayed dense cell clusters as well as enlarged and flattened cells. The treatment under flow with uremic toxins influenced the cell-cell contact leading to a significantly increased gap area between uremic endothelial compared to untreated cells.

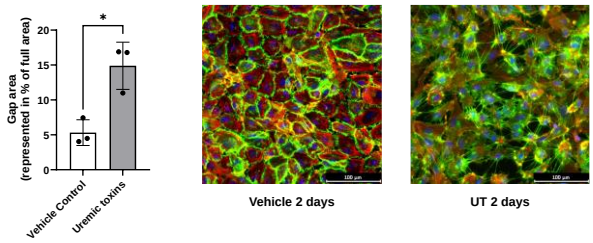

Figure 1: Uremic toxins induce endothelial cell dysfunction by influencing the endothelial cell-cell contact. Data shown as mean ± SD, n=3, using unpaired t-test with Welch's correction ($p<0.05$).*

Discussion

Our data showed that the usage of seven relevant uremic toxins leads to endothelial cell dysfunction characterized by a reduced metabolic activity, which is partly due to impaired autophagic flux, increased ROS production as well as decreased cell-cell communication. Currently RNAseq is ongoing to reveal new molecular targets that explain the disturbed interaction between neutrophils, platelets, and endothelial cells.

References

1. Constance C F M J Baaten et al, Circ Res., 14;132(8):970-992, 2023.
2. Sonja Vondenhoff et al, Herz., 49(2):95-104, 2024.