When Consequence Becomes the Cause: Impact of carbamylation of RAA system in uremia

The ultimate goal of the proposed project is to explore the role of carbamylation of Renin-Angiotensin system components in chronic kidney disease and unravel underlying molecular mechanisms contributing to the endothelial dysfunction in hemodialysis patients. That in turn will pave the way for implementation of novel treatment strategies aimed at intercepting or preventing protein carbamylation *in vivo* to prevent and treat cardiovascular diseases among subjects with chronic kidney disease or end-stage renal disease.

An estimated 850 million people worldwide is affected by chronic kidney disease (CKD) and survival of around 10 million depends on dialysis due to kidney failure. Despite continuous improvement, dialysis can only replace circa 10% of physiological kidney function, leaving patients with a chronic overload of toxic metabolites. Patients with CKD, for instance, have a 10 to 30 times increase in cardiovascular diseases (CVD) risk compared to subjects with normal renal function, which cannot be attributed solely to traditional cardiovascular risk factors.

Uremia results from the retention of waste products that, under physiological conditions, are cleared by the kidneys. Accumulated, these compounds exert various devastating effects on almost all organ systems of the body. Urea is quantitatively the most abundant retained solute in the body, where it is in equilibrium with its reactive decomposition product, cyanate, that can react with lysine residues and N-terminal amino groups of proteins and peptides, resulting in the formation of carbamyllysine (homocitrulline; HCit). This irreversible modification can occur at multiple sites within a single protein, altering protein charge, structure, and function. Due to its reactivity, blood cyanate concentrations are difficult to establish, but it is estimated to be in equilibrium of 1:100 with urea, thus reaching concentrations as high as 1 mM in CKD patients, resulting in excessive protein carbamylation. Carbamylated proteins in CKD became important as biomarkers due to their strong association with overall mortality.

Although carbamylation of plasma proteins was extensively studied and received much attention in the past due to the discovery of its association to mortality in hemodialysis (HD) patients, surprisingly little is known about the impact of carbamylation on the development of cardiovascular diseases. To date, most of the studies investigating carbamylation in context of CVDs focused on the role of carbamylated lipoproteins found in serum that were shown to confer proatherogenic biological activities, such as cell death, macrophage foam-cell formation, and vascular smooth muscle proliferation.

Renin-angiotensin (RAS) and kallikrein-kinin systems (KKS) are proteolytic cascades that operate at both systemic and local (tissue) levels, regulating blood pressure, inflammation, coagulation and many other processes. As such, they are one of the most important systems in the pathogenesis of cardiovascular diseases and the introduction of inhibitors targeting the RAS components became a major advance in cardiology. Main interactions between the RAS and KKS occur at four enzyme levels: ACE, kallikrein, neprilysin and prolylcarboxypeptidase (PRCP). Moreover, it has been shown that angiotensin II enhances bradykinin B1 and B2 receptor expression *via* transcriptional mechanisms and angiotensin 1-7, both a substrate and an inhibitor of ACE, potentiates bradykinin induced vasodilatation.

Based on our preliminary results we hypothesise that circulating, urea-derived cyanate leads to carbamylation-triggered alterations of RAS/KKS system components, including the major player in the blood pressure regulation - angiotensin converting enzyme I (ACE1). Therefore within the project we will investigate impact of carbamylation on critical elements of RAS/KKS system, including ACE1, bradykinin and chymase. We will also evaluate the role of carbamylation on ability of endothelium to exert its physiological functions such as the processes crucial in formation of atherosclerotic plaque.

Therefore, the overall objectives of the project are as follows:

- Characterise the carbamylation pattern of RAS/KKS components, particularly ACE1, bradykinin and chymase *in vitro* and in clinical samples from CKD patients, determine impact of carbamylation on their physiological activity and establish correlation between carbamylation of RAS/KKS and CVDs in CKD.
- Examine the impact of carbamylation on endothelium function: RAS expression, calcification, adhesion and extravasation of monocytes, and endothelial wound healing and tubular maturation.
- Investigate the effect of carbamylation on ACE1 inhibition, vessel calcification and protective effect of free amino acids supplementation as the basis of the future preventive therapy *in vivo* using animal model of CKD.