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## DISRUPTED CELLULAR BARRIERS IN KIDNEY FAILURE

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2019

### **document version**

Publisher's PDF, also known as Version of record

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### **citation for published version (APA)**

Vila Cuenca, M. (2019). *DISRUPTED CELLULAR BARRIERS IN KIDNEY FAILURE: The endothelium and the peritoneal membrane*.

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## ENGLISH SUMMARY

Cellular barrier breakdown may drive important pathological effects in different tissues during renal failure. The endothelium, the first cell line exposed to uremic retention molecules in the circulation, undergoes structural abnormalities which trigger or accelerate cardiovascular disease. Importantly, the peritoneal mesothelial membrane, which acts as a filter during peritoneal dialysis, can also be compromised not only by chronic kidney disease (CKD) itself but also during the long-term exposure of bio-incompatible dialysis fluids. Protecting these two cell barriers in renal failure may carry important beneficial consequences in the cardiovascular health or in the normal functioning of the peritoneum as a dialysing membrane in peritoneal dialysis. In this dissertation, several therapeutic approaches are presented to maintain the integrity of both endothelial barrier and peritoneal membrane in renal failure.

### PART I – DISRUPTED CELLULAR BARRIERS IN KIDNEY FAILURE: THE ENDOTHELIUM

As a consequence of the impaired renal function, there is an increment of blood concentrations of uremic retention solutes together with inflammatory and oxidative stress mediators. Importantly, as an early event in CKD, there is a progressive derangement of the levels of hormones involved in mineral metabolism. The kidney produces a key protein termed  $\alpha$ -Klotho, involved in regulation of both calcium and phosphate homeostasis and also the synthesis of active vitamin D ( $1,25(\text{OH})_2\text{D}$ ) occurs primarily in the kidney. Both compounds decline already in early stages of CKD, and thereby contribute to remote tissue damage in CKD. Those changes are accompanied by the elevation of the parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) to maintain calcium and phosphorous homeostasis. The first cellular barrier exposed to these non-physiological conditions is the endothelium, and both functional and structural abnormalities in this inner cell lining of blood vessels may arise. Disrupted endothelial barrier may trigger or accelerate cardiovascular disease, inducing an alarmingly high prevalence of morbidity and increased mortality in all stages of CKD. In **Chapter 1**, the impact of the disturbed concentrations of the above-mentioned renal factors in the endothelial barrier integrity are highlighted. In this regard, the vascular pathological characteristics observed by these renal-related risk factors in experimental uremic animal models or cell cultures resemble the clinical manifestations observed in CKD patients, suggesting that they are important mediators in the development of uremia-induced endothelial dysfunction in these patients. Emerging studies suggest that vitamin D has important indirect effects via traditional and possibly non-traditional vascular disease risk factors as well as direct effects on vascular cells. In **Chapter 2**, structural abnormalities (vascular permeability and endothelial cell detachment), present in the aortic tissue of our



CKD rat model, were attenuated by active vitamin D therapy. In agreement with our animal experiment, active vitamin D treatment improved *in vitro* the recovery of disturbed endothelial permeability after thrombin stimulation as well as after injury-mediated by an electric wound suggesting that the beneficial effect from active vitamin D in the CKD *in vivo* model was a result of a direct effect on endothelial cells. In addition, the vasculoprotective potential of active vitamin D was also confirmed in an *in vitro* model that exposed the deleterious effects induced by human uremic plasma (**Chapter 3**). Here, we observed that plasma from patients with CKD induced a decrease of endothelial barrier function by reducing the membrane expression of adherens junction vascular endothelial (VE)-cadherin, essential for modulating the endothelial cell-cell interaction. This deleterious effect on the cell-cell contact was largely prevented by the addition of active vitamin D in the uremic media. Given the endothelial protective effects reported here in this dissertation, we hope to revive the interest in the importance of active vitamin D treatment for vascular dysfunction in CKD patients. Despite this encouraging data, active vitamin D was not effective in reducing of N( $\epsilon$ )-Carboxymethyllysine (CML) depositions in the myocardial microvascular tissue of our CKD rat model (**Chapter 4**). We hypothesized that the accumulation of CML in the target tissue was mediated by oxidative stress as a result of CKD condition. This feature, however, was not confirmed in our CKD *in vivo* model. As an alternative hypothesis, the decline of renal function could retain CML into the circulation leading to the accumulation of this toxin into the cardiac microvasculature. In particular, preventing CML depositions could be a therapeutic target for preventing microvascular abnormalities in CKD.

## **PART II – DISRUPTED CELLULAR BARRIERS IN KIDNEY FAILURE: THE PERITONEAL MEMBRANE**

In parallel to the endothelial lining, the peritoneal membrane may also be compromised during CKD. Peritoneal mesothelial cells are specialized epithelial cells that cover the peritoneal cavity. In end-stage kidney disease, renal replacement therapy is required, one widely applied method of which is peritoneal dialysis (PD). The therapy is based on the ability of the peritoneal membrane to function as a dialyzing membrane, when PD fluid is installed into the peritoneal cavity. However, long-term exposure to bio-incompatible fluids promotes progressive remodelling of the peritoneum and induces fibrosis and angiogenesis. Importantly, this state contributes in several ways to peritoneal malfunction during PD fluid exposure. As an underlying mechanism, the production and secretion of profibrotic cytokines by inflammatory cells such as macrophages and T cells may disrupt the normal homeostasis of the peritoneal membrane. Specifically, immunoregulatory M2 macrophages and interleukin (IL)-17 secreted by T Helper 17 (Th17) cells are likely important mediators of peritoneal inflammation during PD and are described in **Chapter 5**. Given the potential importance of

modulating immune cells function, in order to protect against the development of peritoneal membrane damage during PD, some therapeutic strategies are proposed in this dissertation. In **Chapter 6**, active vitamin D treatment was effective in reducing PD-induced peritoneal thickening and angiogenesis *in vivo*. Here, the presence of M2 macrophages, which can lead to exacerbation of fibrosis and chronic inflammation, was reduced in the omentum of PD-exposed animals when treated with active vitamin D. Another therapeutic approach is described in **Chapter 7**, demonstrating that Alanyl-Glutamine is effective in reducing the IL-17 expression in the peritoneal membrane and in peritoneal effluents from mice treated with conventional PD fluid. The neutralization of this pathway attenuated the PD-induced peritoneal fibrosis and angiogenesis. Finally, in **Chapter 8** we confirmed that the application of a bicarbonate/lactate PD fluid better preserved the peritoneal integrity in a mice PD model when compared to animals exposed to conventional PD fluid. The inflammatory environment of the more biocompatible PD fluid-treated mice was characterized by an M1 macrophage subset over M2 and lower CD4+ IL-17+ cell population with no changes in IL-17 concentrations. Overall, modulating the inflammatory response during PD fluid exposure may preserve the integrity of the peritoneal membrane.

## CONCLUDING REMARKS

In this thesis two cellular barriers are studied, which both are jeopardized in patients with severe kidney failure. The first is the endothelial cell layer, which is compromised by the uremic milieu itself, and the second is the peritoneal membrane, which can be damaged by peritoneal dialysis. Understanding the underlying mechanisms of these complications of either the disease or its treatment paves the way to specifically address these aspects, that independently contribute to morbidity of patients affected by this chronic condition. Here, active vitamin D supplementation was shown to protect both cellular barriers by reinforcing the cell to cell junctions in the endothelium upon uremic-induced toxicity or by reducing the M2 macrophage population after bio-incompatible PD fluid exposure. Overall, the favourable effects reported here in this thesis are supportive in considering active vitamin D therapy as a preventive strategy of endothelial cell dysfunction and peritoneal remodelling.

As an alternative approach, two additional therapeutic strategies against the peritoneal damage were also evaluated. Given the rising concern about the key role of IL-17 in peritoneal damage during PD, the potential therapeutic effects of Alanyl-Glutamine against the IL-17 mediated peritoneal inflammation represent encouraging data for improving the peritoneal health in PD patients. In addition, we provided a better understanding of the differences between the inflammatory milieu of the long-term exposure of a biocompatible PD fluid compared to a conventional one which may pave the way to develop preventive strategies.