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Abstract

SGLT2 inhibitors (SGLT2i) offer cardiovascular and renal benefits in patients with chronic kidney disease (CKD), through not yet clearly defined mechanisms. Juni *et al* show that SGLT2i empagliflozin exposure in vitro can restore cardiomyocyte function by counteracting harmful effects of uremic serum on endothelium-cardiomyocyte crosstalk mediated by endothelial nitric oxide. The author's findings improve our understanding of cardiovascular impairment in CKD and open new perspectives for the beneficial effects of SGLT2i therapy.

Keywords: endothelium-cardiomyocyte crosstalk, chronic kidney disease, SGLT2 inhibitors

Cardiovascular complications are a major cause of death in patients with chronic kidney disease (CKD). The progressive decline in kidney function frequently results in chronic cardiac dysfunction and cardiovascular events. In CKD patients, heart failure is a common complication in which pulmonary congestion is a key factor, a process defined as type 4 cardiorenal syndrome.

Recently, numerous trials have shown that the therapeutic class of renal sodium-glucose co-transporter2 (SGLT2) inhibitors significantly improved the prognosis of heart failure, allowing a 30 to 40% reduction of hospitalization in CKD patients suffering from this complication[1]. SGLT2 in the kidney proximal tubule is the main transporter for the reabsorption of glucose filtered through the glomerulus by using the Na^+ gradient established by the Na^+/K^+-ATPase. Empagliflozin, like the other SGLT2 inhibitors (SGLT2i), prevents renal glucose and sodium reabsorption, thereby inducing glycosuria and natriuresis. This effect is associated with acute weight loss and hemoconcentration, reducing pulmonary congestion. Beside an effect on cardiac preload, changes in energy metabolism and reduction of blood pressure could explain the improved clinical outcome in patients with heart failure treated by SGLT2i.

To date, all SGLT2i trials have demonstrated a positive effect on hospitalization for heart failure, whether or not patients had CKD[1]. In DAPA-HF[2] and EMPEROR[3] trials including respectively 40 and 48% of CKD patients, the benefit of SGLT2i on the primary outcome, a composite of cardiovascular death and worsening of heart failure, was maintained despite impaired kidney function. In CKD patients, in addition to improving cardiovascular outcome, SGLT2i markedly reduced the risk for progression of kidney disease, regardless of the presence of diabetes[1]. SGLT2i are therefore an interesting new therapeutic tool to improve the prognosis of patients with cardiorenal syndrome. Moreover, a recent meta-analysis in CKD patients demonstrated that SGLT2i decreased the risk of myocardial infarction[1]. Identifying the mechanisms that underlie the observed improvement of cardiac and possibly vascular changes brought about by SGLT2i in CKD is of particular interest.

Several not fully understood mechanisms have been proposed to explain the cardiac benefit of SGLT2i[4], including an inhibition of myocardial Na^+/H^+ exchanger 1 (NHE1), improvement in cardiac metabolism, and reduction of cardiac fibrosis through dampened inflammation. An improvement in ventricular loading conditions through reduction of pre-load via diuretic/natriuretic effects, reduction of blood pressure, and improvement of vascular function has also been suggested[4]. Indeed, SGLT2i improve systemic endothelial function, as

demonstrated in patients with type 2 diabetes treated with dapagliflozin and metformin, and in diabetic fatty rats treated with empagliflozin[5]. These effects were thought to be probably mediated through improved of endothelial viability, reduced senescence and inflammation, and decreased oxidative damage in NO/cGMP signaling cascade, rather than upregulated endothelial NO synthase (eNOS) expression[5]. This also holds true for the kidney since renal endothelial dysfunction plays a key role in diabetic kidney disease[5], and the endothelial benefits of SGLT2i may help to prevent the deterioration of kidney function.

CKD causes profound systemic endothelial dysfunction, which is characterized by impaired flow-mediated vasodilation[6]. Some uremic toxins that accumulate in CKD patients as a consequence of impaired kidney function can contribute to the vascular burden and cardiorenal syndrome, notably via their endothelial and cardiac toxicity[6,7]. However, the consequences of CKD-related endothelial dysfunction in the regulation of cardiomyocyte function are not known, as are any pharmacological interventions aimed at improving endothelial function in cardiorenal syndrome.

The article by Juni *et al*[8] in this issue of Kidney International provides interesting mechanistic insight into how uremic conditions impair the crosstalk between endothelial cells and cardiomyocytes and highlight the key role of the cardiac microvascular endothelium in the cardiac benefits of empagliflozin treatment (figure 1). The investigators used a model of co-culture of human cardiac microvascular endothelial cells (CMECs) and rat ventricular cardiomyocytes allowing them to show that CMECs positively impacted cardiomyocyte relaxation and contraction, mainly by endothelial-derived NO[9]. They demonstrated that uremic serum impairs the endothelium-mediated enhancement of cardiomyocyte function, and that empagliflozin counteracts the harmful effect of uremic serum on CMECs, leading to restoration of cardiomyocyte function. Mechanistically, uremic serum induces mitochondrial damage in CMECs through generation of mitochondrial ROS that accumulate in the cytoplasm and reduce NO bioavailability. Thus, the effect is related to NO scavenging by ROS rather than to a decrease in NO synthesis by CMECs due to altered expression or activity of eNOS. By mitigating mitochondrial damage induced by uremic serum, empagliflozin alleviates endothelial production of ROS and consecutive NO scavenging. The restoration of endothelial NO bioavailability preserves the beneficial effect of endothelial NO synthesis on cardiomyocyte function, probably via an increase in cardiomyocyte NO levels. Whether the cell-based results obtained by Juni *et al*[8] are also valid under in vivo conditions remains to be

seen. The study would have greatly benefited from confirmation of these mechanisms in an animal model of cardiorenal syndrome.

The data suggest a reversion of a harmful mechanism triggered by uremic serum in CMEC rather than a direct effect of empagliflozin. Uremic toxins or inflammatory mediators are good candidates for the identification of harmful factors in uremic serum. In addition to whole uremic serum, the investigators examined the effect of indoxyl sulfate, an indolic uremic toxin originating from tryptophan metabolism with proven endothelial toxicity, notably via induction of ROS and reduction of NO[6]. They found that empagliflozin alleviates the harmful effect of this uremic toxin on ROS and NO levels in CMECs. The potential effect of other uremic toxins such as advanced glycation end products (AGEs), a group of posttranslationally modified proteins and/or lipids that become glycated after exposure to sugars, could have been interesting to study. AGE formation occurs in CKD, in diabetes, and both, and is promoted by oxidative stress associated with both pathologies. AGEs exert endothelial toxicity by interacting with their receptor RAGE, induce endothelial ROS production, reduce NO bioavailability, and amplify inflammatory responses[5]. Interestingly, treatment of diabetic rats with SGLT2i reduces AGE/RAGE signaling[5]. Inflammatory cytokines could also be among the factors responsible for endothelial toxicity of uremic serum. The authors previously reported that stimulation of CMECs with TNF and IL-1β abrogates the positive effect of CMECs on cardiomyocyte function by the same mechanism related to ROS accumulation and NO bio-unavailability, which was restored by empagliflozin[9]. Interestingly, empagliflozin can counter a cellular mechanism of endothelial dysfunction triggered by many harmful factors linked to the uremic status.

At the molecular level, whether the beneficial effects of empagliflozin are related to a specific inhibition of endothelial SGLT2 remains unclear. The investigators did not detect any expression of SGLT2 in CMECs, making the hypothesis of a direct effect of empagliflozin via SGLT2 unlikely. However, data on endothelial SGLT2 expression are conflicting in the literature, some studies reporting a small amount of SGLT2 protein, but no expression of SGLT2 mRNA. In addition, SGLT2 levels in endothelial cells can be increased in some conditions, and endothelial glucose uptake can be reduced by SGLT-2 inhibition[5]. Therefore, a positive effect of empagliflozin via direct SGLT2 inhibition cannot be totally excluded, adding the possible regulation of glucose entry in endothelial cells via SGLT2 as a mechanism

to explain the reduction of endothelial damage. The issue could have been clarified by additional experiments with small interfering RNA targeting SGLT2 in CMECs.

Other mechanisms were ruled out by the investigators[8]. First, an effect of empagliflozin on eNOS expression or activity was excluded. Second, the empagliflozin effect on CMECs was largely independent of NHE1 inhibition. Third, the absence of modified acetyl-CoA carboxylase phosphorylation allowed excluding an involvement of AMPK pathway activation, also ruling out the hypothesis of an inhibition of endothelial glucose uptake by empagliflozin. Interestingly, the inhibition of mitochondrial oxidative radicals completely mimicked the effects of empagliflozin on endothelial-mediated improvement of cardiomyocyte function, implying that endothelial mitochondria are probably a major target of the observed beneficial effects of empagliflozin. A transcriptomic study of CMECs stimulated by uremic serum could allow determining precisely which pathways affected by serum uremic are reversed by empagliflozin. In addition, further studies are needed to determine whether the mechanisms elucidated by Juni *et al*[8] are specific to empagliflozin or are shared by other SGLT2i, supporting the hypothesis of a common effect across this class of drugs.

In summary, cardiovascular dysfunction in CKD patients can be induced by various factors such as uremic toxins, inflammation, and hyperglycemia, which share the common harmful property of inducing increased ROS production and decreased NO bioavailability in endothelial cells. By showing that uremia-mediated endothelium injury leads to cardiomyocyte dysfunction, the study of Juni *et al*[8] provides new interesting information on the crucial role of the endothelium in the pathogenesis of cardiorenal syndrome, and provides a new endothelial-related mechanism to explain cardiac benefits of empagliflozin.

Disclosure statement

L. Dou declares no conflict of interest and S. Burtey received fees from Astra-Zeneca and Boehringer Ingelheim.

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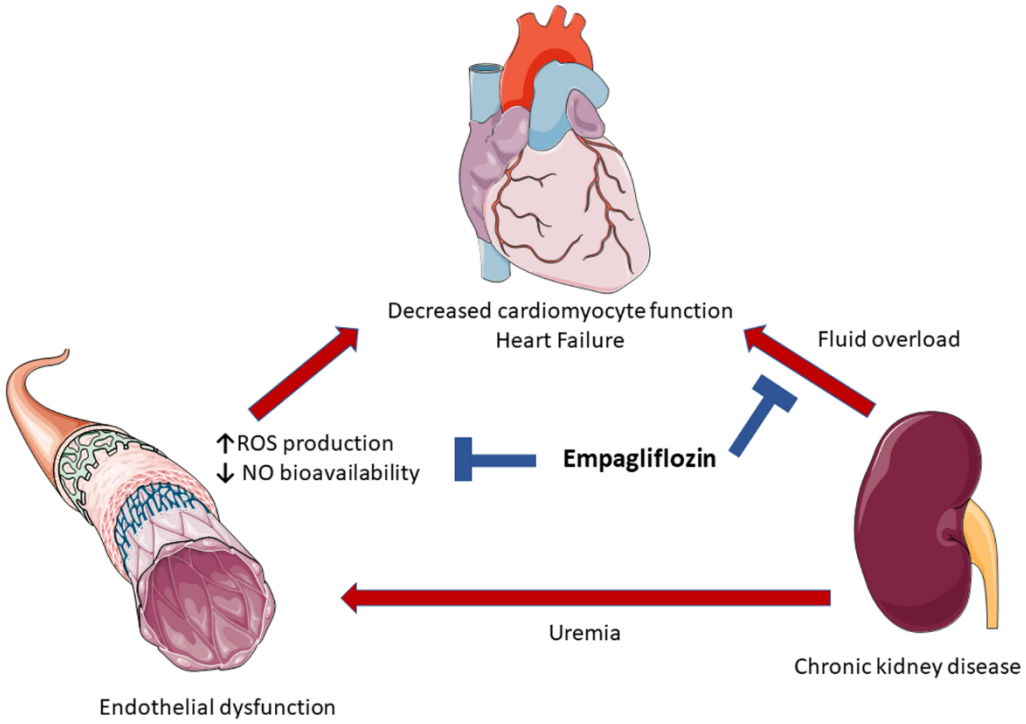
Figure



Legend to figure. Uremic serum induces endothelial dysfunction with increased ROS production and NO scavenging. This causes a defect in cardiomyocyte contraction, and eventually leads to heart failure. Impaired kidney function is associated with fluid overload leading to heart failure. By correcting uremia-induced endothelial dysfunction and reducing fluid overload, empagliflozin improves myocardial contractility and ameliorates the prognosis of heart failure.