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Canagliflozin and myocardial oxidative stress: SGLT1 inhibition takes center stage

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Sodium-glucose linked transporter type 2 inhibitors (SGLT-2i), also known as gliflozins, are a recently discovered class of oral antidiabetic drugs[1]. SGLT-2i promote urinary glucose excretion through inhibition of SGLT-2 and SGLT-1, which are responsible for approximately 90% and 10% of glucose reabsorption in the kidney glomerulus. Despite gliflozins show variable SGLT-2 over SGLT-1 affinity – with Empagliflozin being the most selective SGLT2i followed by Dapagliflozin, Canagliflozin and Sotagliflozin – inhibition of SGLT-2 became eponymous to this class of drugs, since glucose transporter is the primary site of these agents intended use: blood glucose lowering [2].

In 2015, the *Empagliflozin Cardivascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients* (EMPAREG-OUTCOME) reported improved cardiovascular outcomes in patients with type 2 diabetes (T2D) treated with Empagliflozin [3]. In 2019 and 2020, the *Study to Evaluate the Effect of Dapagliflozin on the Incidence of Worsening Heart Failure or Cardiovascular Death in Patients With Chronic Heart Failure* (DAPA-HF) trial and *EMPagliflozin outcome tRial in Patients with chronic heart Failure with Reduced Ejection Fraction* (EMPEROR-REDUCED) trial confirmed the cardiovascular benefits of these drugs in patients with heart failure with reduced ejection fraction (HFrEF) independently of the presence of diabetes (DAPA-HF) and independently of their blood glucose lowering effect (EMPEROR-REDUCED) [4, 5]. Similar results, albeit with more variability on cardiovascular outcomes, were obtained with other glifozins[6-8].

Given the striking cardiovascular effects of SGLT2i in heart failure, great efforts have been taken by the cardiovascular research community to unveil the underlying mechanisms of their beneficial effects in the myocardium. In this context, in the current issue of the journal, Kondo *et al*[9]*.* report that Canagliflozin – but, interestingly, not

Empagliflozin – mitigates oxidative stress in in human atrial myocardium and ventricular cardiomyocyte cell lines increasing the coupling of nitric oxide synthase (NOS) and reducing NADPH oxidase activity. While SGLT-2 localizes almost exclusively in the kidney, SGLT-1 is predominantly expressed in the intestine and heart/skeletal muscle[10]. Whereas SGLT-1 function is critical for intestinal glucose absorption and glucose-dependent insulin secretion, little is known about its role in muscle. Previous studies have shown changes in myocardial SGLT-1 expression occurs in several cardiovascular diseases and documented the absence of SGLT-2 in the myocardium [10, 11]. Here, the authors confirm this observation in a large set of human right atrial myocardial samples (n=365) and further associate SGLT-1 expression with oxidative damage, inflammation, fibrosis and wall-stretch. Incubation of right atrial myocardium with Canagliflozin as low as 3μM – a clinically relevant concentration – reduced NADPH oxidase and NOS driven production of reactive oxygen species (ROS) in a dose-independent manner. Interestingly, high-dose treatment of Empagliflozin (100μM) did not show the same effect. The authors interpret these results as a consequence of a higher binding affinity to SGLT-1 of Canagliflozin compared to Empagliflozin and proceed to evaluate its upstream effects on myocardial redox signalling. Canagliflozin induced the rapid activation of AMPKα2, inhibiting the NADPH oxidase activator Rac1 improving enzymatic coupling of NOS by enhancing bioavailability of its cofactor tetrahydrobiopterin. The authors successfully transfer their observations from human right atrial myocardium to immortalized human and rat ventricular cardiomyocytes cell lines, making similar observations regarding SGLT-1/2 expression and Canagliflozin's effect on AMPK/GTP-Rac1 signalling. By incorporating a SGLT-1 loss-of-function approach and modulating glucose concentration

of cell culture media – to mimic diabetic conditions – the authors showed that Canagliflozin enhances cellular ADP/ATP ratio by regulating SGLT-1 mediated glucose influx. The authors finally report that 24h treatment with Canagliflozin in human primary cardiomyocyte cell line downregulates a set of pro-inflammatory genes promoting cell survival.

As with all good studies, new hypothesis-generating considerations and questions emerge from this work (**Figure 1**). So far, all major SGLT-2i (Empagliflozin, Dapagliflozin, Canagliflozin, Sotagliflozin) have performed exceedingly well with regards to cardiovascular outcome in randomized clinical trials for heart failure, despite their vastly different profiles of SGLT-1/2 affinity. This raises the question whether SGLT-1 (and its inhibition) is a clinically relevant target and if so, whether it would be aimed by SGLT-2i with a higher type 2 selectivity such as Empagliflozin and Dapagliflozin.

Following the notion of differential effects of SGLT-2i compounds on SGLT1/AMPK/Rac1-GTP mediated myocardial redox signalling, it would be of interest to know which patients will benefit most from a SGLT-1 targeted therapy with regards to specific cardiovascular disease, comorbidities and overall demographics. Can selective SGLT-1i therapy be considered as a future cardiac-specific therapy? Similar SGLT-1 expression between T2D and non-diabetic patients suggest the potential use of these drugs in a large group of patients.

Accumulating evidence suggests that the anti-ROS beneficial effects of SGLT-2i are not limited to HFrEF. It has been recently reported that Empagliflozin, at a concentration of 0.5μM (for comparison, 100μM was the dose of Empagliflozin used in the current study), can ameliorate myocardial oxidative damage in left ventricular biopsies of patients

with heart failure with preserved ejection fraction (HFpEF)[12]. As nitrosative stress stemming from alterations in various NOS sources has been identified as critical driver of HFpEF and diastolic dysfunction[13, 14], improving NOS coupling through enhanced tetrahydrobiopterin availability, as observed in the present study, suggests a potential, novel, mode-of-action of SGLT-1/2i in HFpEF. Clinical trials for SGLT-2i in HFpEF patients are ongoing and will soon reveal if there is room for SGLT-2i in this prevalent syndrome [15, 16].

The suggested mechanisms of Canagliflozin and SGLT-1 inhibition trough AMPK signalling is intriguing and points to a metabolic regulation of cardiomyocyte biology by this drug. The authors identify fluctuation of cellular energy stores (ADP/ATP ratio) as the link between SGLT-1 and AMPK activation indicating a notable and sustained glucose influx into human cardiomyocytes via SGLT-1. Despite recent studies in human and mouse hearts suggest that the contribution of SGLT-1 as glucose transporter is liminal in healthy heart [17], evidence from preclinical studies have shown an increase in myocardial glucose uptake through SGLT-1 in T2D [18]. Collectively, this evidence beg for a in depth characterization of SGLT-1 expression and function in diseased human hearts.

In conclusion, Kond *et al* provided evidence in support of Canagliflozin improving NOS coupling and NADPH oxidase activity through SGLT-1/AMPK/Rac1-GTP signaling in the heart. The authors should be congratulated for their work which suggests a novel role of SGLT-1i in regulating myocardial redox signaling and puts SGLT-1 in the spotlight of SGLTs research.

Conflict of interest

None declared.

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Figure legend

Schematic depicting the potential, still partly unknown, mode of actions of Canagliflozin in cardiomyocytes as suggested by this work.

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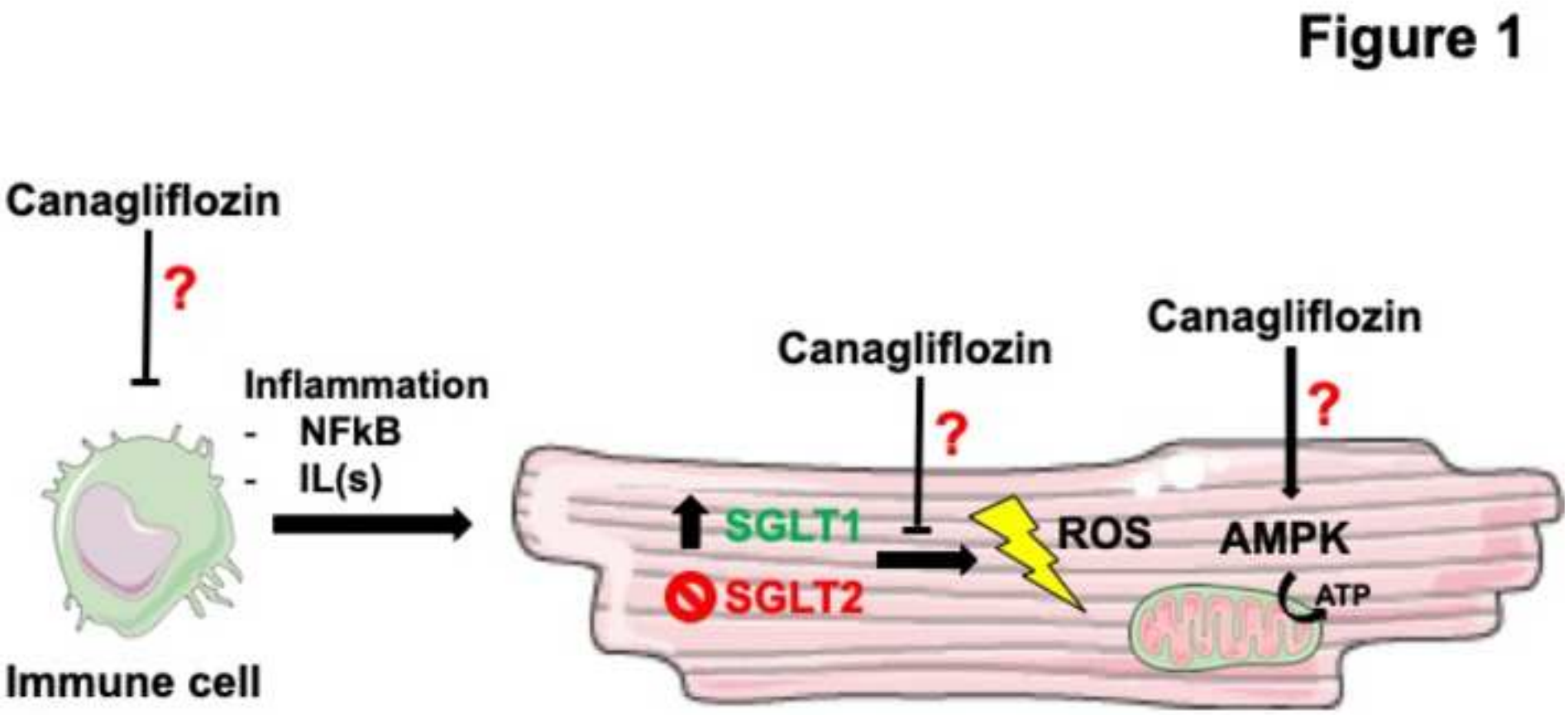
Figure 1
Canagliflozin
?
Inflammation
- NFkB
- IL(s)
Immune cell
Canagliflozin
?
Canagliflozin
?
SGLT1
SGLT2
ROS
AMPK
ATP