SGLT2 inhibitors reduce infarct size in reperfused ischemic heart and improve cardiac function during ischemic episodes in preclinical models

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Abstract

The sodium-glucose cotransporter 2 (SGLT2) inhibitors are a new class of effective drugs managing patients, who suffer from type 2 diabetes (T2D): Landmark clinical trials including EMPA-REG, CANVAS and Declare-TIMI have demonstrated that SGLT2 inhibitors reduce cardiovascular mortality and re-hospitalization for heart failure (HF) in patients with T2D. It is well established that there is a strong independent relationship among infarct size measured within 1 month after reperfusion and all-cause death and hospitalization for HF: The fact that cardiovascular mortality was significantly reduced with the SGLT2 inhibitors, fuels the assumption that this class of therapies may attenuate myocardial ischaemic injury. Experimental evidence demonstrates that SGLT2 inhibitors exert cardioprotective effects in animal models of acute myocardial infarction through improved function during the ischemic episode, reduction of infarct size and a subsequent attenuation of heart failure development. The aim of the present review is to outline the current state of preclinical research in terms of myocardial ischemia/reperfusion injury (I/R) and infarct size for clinically available SGLT2 inhibitors and summarize some of the proposed mechanisms of action that may contribute to the unexpected beneficial cardiovascular effects of this class of compounds.

Key-words: Sodium-glucose cotransporter 2 inhibitors; myocardial infarct size; ischemia/reperfusion injury; molecular signaling

1. Introduction

Acute myocardial infarction (AMI) is one of the leading causes of death and disability worldwide. During the last 25 years mortality from acute myocardial infarction has been on the decline [1, 2]. However, long term mortality, i.e. 1 year and beyond, and heart failure post-AMI remains significant [1, 3]; therefore, cardioprotection on top of early reperfusion and reduction of the incidence and severity of heart failure following AMI remain unmet clinical needs [4]. A myriad of experimental studies has demonstrated cardioprotective effects of pharmacological and mechanical interventions in animal models but the translation of these cardioprotective interventions to clinical setting has been disappointing until now [5].

The sodium-glucose cotransporter 2 (SGLT2) inhibitors, also called gliflozins, are a new class of effective drugs managing patients who suffer from type 2 diabetes (T2D). They were developed to inhibit sodium-glucose transport protein 2 in the kidneys. The SGLT2 inhibitor, Empagliflozin, not only reduces glycemia but also improves cardiovascular outcome when evaluated by a primary composite endpoint including death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke in patients with T2D at high risk for cardiovascular events [6]. In parallel with a reduction of glycemia, the SGLT2 inhibitors, which in addition to Empagliflozin, include Canagliflozin and Dapagliflozin, reduce re-hospitalization for heart failure (HF) as demonstrated in the landmark clinical trials EMPA-REG, CANVAS and Declare-TIMI [6-8]. While this finding suggests a class effect, only Empagliflozin demonstrated a reduction in cardiovascular death that has not been replicated with either Canagliflozin or Dapagliflozin in patients with diabetes [9]. As with Empagliflozin in the EMPA REG OUTCOME trial, the CANVAS study revealed significant early separation in the cardiovascular survival curves but in CANVAS, this was largely driven by a reduction in heart failure [7]. More recently, it was shown that Dapagliflozin reduced the risk of worsening heart failure or death from cardiovascular causes in patients with heart failure and a reduced ejection fraction, regardless of the presence or absence of diabetes [10].

Of note, neither the EMPA-REG OUTCOME study nor the DAPA-HF trial demonstrated a reduction in the incidence of acute coronary syndromes. However,

3

dapagliflozin and empagliflozin did reduce the incidence of recurrent MI, which may be due to a gliflozin's reducing effect on ischemic-reperfusion injury.

It is well established from both observational studies and of a patient-level pooled analysis from 10 randomized trials, that there is a strong independent relationship between infarct size measured within 1 month after reperfusion, all-cause death and hospitalization for HF, and the composite occurrence of all-cause death or HF hospitalization within 1 year [11]. Neither of the above-mentioned studies, nor the later outcome trial with Dapagliflozin (DECLARE TIMI-58) were designed nor powered to look at the rates of myocardial infarction or the consequent injury or survivability. The EMPA-REG was based in the highest risk patient cohort, with the greatest number of patients with established coronary heart disease [6]. But even here, the numbers of fatal myocardial infarctions were only in single figures. Despite this, cardiovascular mortality is significantly reduced with the SGLT2 inhibitors, with the implication that myocardial ischaemic injury may be attenuated by this class of therapies and hence rendering acute coronary syndromes more survivable and less likely to lead to myocardial dysfunction and decompensation. Thus, in spite of differences in selection criteria, the results of the outcome trials highlight that, notwithstanding some differences in cardiovascular outcomes, there may be a common underlying class effect.

Nowadays, experimental evidence demonstrates that SGLT2 inhibitors, exert cardioprotective effects in animal models of acute myocardial infarction through improvements of cardiac function during ischemia, reduction of infarct size and a subsequent attenuation of heart failure development. Since SGLT2 is not known to be expressed in cardiomyocytes [12-13], the responsible mechanisms underlying the beneficial mechanisms of SGLT2 inhibitors regardless of diabetes status remain to be identified.

This review aims to outline the current state of preclinical research in terms of myocardial ischemia/reperfusion injury (I/R) and infarct size for clinically available SGLT2 inhibitors and outline some of the proposed mechanisms of action that may contribute to the unexpected beneficial cardiovascular effects of this class of compounds.

2. Canagliflozin

While Canagliflozin is a C-glycosyl based compound like all other SGLT2 inhibitors, it differs from the majority of clinically available gliflozins in having a far lower selectivity towards SGLT2 over SGLT1. It is one of the least selective SGLT2 inhibitor currently clinically available, with an SGLT2:SGLT1 selectivity of only 155-263 fold compared to Empagliflozin which is 2677-fold more selective to SGLT2 than SGLT1 [14]. Only sotagliflozin is less SGLT2 selective. Despite the lack of specificity, there appear to be remarkable similarities in Canagliflozin's ability to ameliorate I/R injury in animal models to other SGLT2 inhibitors that have thus far been studied. Chronic administration of Canagliflozin over a period of 4 weeks to either diabetic fatty or non-diabetic lean Zucker rats led to the significant reduction of myocardial infarction when the hearts were isolated and perfused ex-vivo and subjected to 35 minutes of left anterior artery occlusion prior to reperfusion for two hours [15]. While Canagliflozin led to a significant and predicted reduction in circulating glucose in the diabetic animals, there was no change in circulating glucose in the non-diabetic animals, despite marked glycosuria in both groups treated with Canagliflozin [15]. Notwithstanding the differences in circulating glucose response in diabetic and non-diabetic animals, the hearts isolated from both groups of animals had a similar reduction of infarct size compared to their vehicle-fed brethren, attenuating infarct size by 51% and 46% in non-diabetic and diabetic rats respectively [15]. Therefore, it would seem that the infarct-sparing mechanism of these SGLT2 inhibitors appear to have little to do with their ability to lower glucose directly.

Myocardium expresses little SGLT2 but is known to express SGLT1 [16]. The relative lack of selectivity of Canagliflozin towards SGLT2 could therefore be a potential explanation for the infarct size reduction in heart through SGLT1 inhibition. It has been shown that cardiomyocyte-specific knockdown of SGLT1 reduced infarct size, both in in vivo and ex vivo heart models [17] (although the role of SGLT1 is not fully understood since it has been shown that SGLT1 inhibition in a model of obesity seems to exacerbate the infarction [PMID: 31262297]). However, administration of Canagliflozin directly to isolated hearts failed to result in attenuation of myocardial infarction [15], an observation found to be common to ex-vivo administration of Empagliflozin (see the part on Empagliflozin in this review). Thus direct administration of SGLT2 inhibitors to isolated hearts do not protect against injurious ischaemia [18, 19]. These data reveal that while Canagliflozin is highly effective in mitigating

5

myocardial injury from I/R injury, this protection is not through direct SGLT2 or SGLT1 inhibition in the myocardium itself. The protection can only be instigated when the drug is administered to the whole animal, and that this protection is retained within the heart even when the heart is isolated prior to being subjected to injurious ischaemia.

In recent studies, Canagliflozin has been demonstrated to attenuate myocardial infarct size acutely. While administration of Canagliflozin to isolated, ex-vivo heart during I/R fails to attenuate myocardial infarction [15], Canagliflozin administrated in-vivo, administered either hours before [20] or indeed even during injurious ischaemia [21], significantly attenuates myocardial injury. The amelioration of myocardial injury appears not be to be through favorable shifts in metabolism, as has previously been suggested in the "Thrifty Substrate Hypothesis" [22]; protection can be elicited in hearts from pre-treated animals in whom ex-vivo perfusion provides only glucose as an energy substrate, and moreover, in a swine-based model of I/R injury, there are minimal measurable changes in ketosis or alteration in energy substrate uptake (glucose, lactate, ketones, or free fatty acid) observed [20]. Nor does diuresis seem to be an explanation, as myocardial pre-load appears unaltered in vivo [20]. In line with other SGLT2 inhibitors, Canagliflozin can be shown to be anti-inflammatory in a variety of models, to attenuate murine atherosclerosis (PMID: 30049285) and interleukin (IL)-1, IL-6, or tumor necrosis factor-α (TNF-α) release from immune cells in response to lipopolysaccharide (PMID: 29551587). From these data, one might expect a lower progression of coronary atheroma and a lower re-infarction rate (but has not yet been shown with canagliflozin), and perhaps an attenuation of progression of heart failure following acute coronary injury. How much acute inflammation contributes to acute myocardial infarction is not yet clear, and certainly warrants further investigation. Two other plausible mechanisms of action would therefore be sodium/ hydrogen exchange (NHE) inhibition (that Canagliflozin has been demonstrated to inhibit) [23], or through kinase signaling [21]. Importantly, Canagliflozin has been shown to acutely preserve cardiac contractile function and efficiency during regional myocardial ischemia in the in vivo pig [20], for which NHE inhibition is a possible candidate mechanism. NHE inhibition is discussed further in other sections of this review. Of note, the effects of Canagliflozin on the kinase signaling can just be an indirect effect because they were measured 2 h after reperfusion only.

Canagliflozin has been shown to increase phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) Threonine 172 [21] in non-diabetic rat myocardium. Whether activation of AMPK is through adenosine phosphate or through alternate upstream signaling through canonical serine/threonine kinases, liver kinase B1 (LBK1) and Ca^{2+}/calmodulin-dependent protein kinase (CAMK) [24] is not clear, but Canagliflozin at clinically relevant concentrations have been demonstrated to directly phosphorylate Thr172 in a number of cell models [25-26]. Downstream, phosphorylation of endothelial nitric oxide synthase (eNOS) and protein kinase B (Akt) is also seen [21], but only following injurious ischaemia, and these signaling events are associated with attenuation of apoptotic markers [bcl-2-like protein 4 (Bax)/ B-cell lymphoma 2 (Bcl-2) ratio] [21]. Given that Akt/eNOS signaling events are critical to other canonical cardioprotective interventions such as ischaemic conditioning, Akt-cell survival signaling appears momentarily to be a secondary potential candidate pathway to cardioprotection by Canagliflozin. Primary direct targets of Canagliflozin are AMPK and NHE/Na^+ lowering, both of which can independently reduce infarct size [27-28]. However, much work still has to be performed to elucidate the cellular signalling pathways through which Canaglifozin reduces cardiac infarct size.

3. Dapagliflozin

Dapagliflozin, regarding SGLT2 inhibition, is the second most potent agent, after ertugliflozin, whereas Dapagliflozin and Empagliflozin are much weaker SGLT1 inhibitors compared with Canagliflozin [29]. Recent studies have shown that Dapagliflozin prevented the progression of LV concentric hypertrophy in the heart failure preserved ejection fraction (HFpEF) pig model by mitigating sympathetic nerve tone and the inflammatory response in the aorta, leading to reactivation of the NO-cyclic guanosine monophosphate (cGMP) - Protein Kinase G (PKG) pathway [30]. Dapagliflozin administration had a cardioprotective effect by improving cardiac systolic function, inhibiting myocardial fibrosis and cardiomyocyte apoptosis in a transverse aortic constriction (TAC) mouse model [31], reduced atherosclerosis in Ldlr$^{-/-}$ type 1 diabetic mice [32], attenuated the activation of the inflammasome, fibrosis, and deterioration of left ventricular ejection fraction (LVEF) in Type 2 diabetic (BTBR ob/ob) mice [33], and successfully prevented the development of hypertrophic cardiomyopathy in a model of T2D (lipodystrophic *Bscl2*$^{-/-}$ (seipin knockout [SKO])

mice) [34]. However, limited studies have been performed so far to access the effects of Dapagliflozin in myocardial I/R injury.

The effects of Dapagliflozin on ventricular myocytes contraction and Ca^{2+} transport have been investigated in the streptozotocin (STZ) induced diabetic rats and results showed that Dapagliflozin induced alterations in mechanism(s) of Ca^{2+} transport which may partly underlie the negative inotropic effects of the drug in ventricular myocytes from STZ-treated rats [35]. However, to the best of our knowledge the effects of Dapagliflozin on cardiomyocytes subjected to hypoxia/reoxygenation are lacking.

The therapeutic effects of Dapagliflozin on myocardial infarction and left ventricular (LV) function were investigated only in High Fat Diet (HFD)-induced obese insulin-resistant rats, which represent a pre-diabetic obese insulin-resistant model. After 12 weeks of HFD treatment rats received for 28 days Dapagliflozin 1mg/kg/day and subjected to in vivo myocardial ischemia for 30min followed by reperfusion for 120 min. Dapagliflozin showed a great efficacy in improving low frequency/high frequency (LF/HF) ratio, systolic blood pressure and LV function in comparison to HFD rats and reduced significantly the infarct size [36]. However, because in this study there were also large effects of Dapagliflozin on systemic metabolites (which are not present in the large clinical trials), it cannot be excluded that most of the reported Dapagliflozin-effects are a result of the improved systemic metabolic milieu and are not related to SGLT2 inhibition direct protective effects on the heart.

Dapagliflozin decreased cardiac mitochondrial fission and as it has been reported [37], an inhibition of cardiac mitochondrial fission could result in cardioprotection against myocardial I/R injury. Similarly, Dapagliflozin administered for 2 weeks in a 28-week high-carbohydrate diet-induced metabolic syndrome-rat cohort showed many cardioprotective actions including suppression of prolonged ventricular-repolarization through augmentation of mitochondrial function and oxidative stress followed by improvement of fusion-fission proteins [38]. However, also these in vivo studies cannot be used for elucidation of the clinically-reported cardioprotective effects of Dapagliflozin, because of the large improvements in the systemic metabolic milieu in these preclinical studies.

Direct cardiac effects of Dapagliflozin that have been reported show 1) restoration of AMPK activation during lipopolysaccharide (LPS) treatment in cardiofibroblast [39], 2) lowering of intracellular Na^+ and inhibition of NHE-1 in mouse cardiomyocytes [23], 3) signal transducer and activator of transcription 3 (STAT3) phosphorylation in

8

isolated rat hearts [40] and lowering of tumor necrosis factor α (TNFα)-induced reactive oxygen species (ROS) in human coronary arterial endothelial cells [41]. The effect of Dapagliflozin on the attenuation of the upregulation of the cardiac Na$^+$/H$^+$ exchanger (NHE-1) has been investigated in vitro in mouse cardiomyoblasts stimulated with LPS. Dapagliflozin attenuated the increase in NHE-1 mRNA and this effect was dependent on AMPK since the effect of Dapagliflozin was blocked with the presence of AMPK inhibitor [39]. Importantly it has been shown that chronic treatment for 4 weeks with Dapagliflozin reduced ROS-inhibition of STAT3 signaling pathway, which enhanced M2 macrophage activation, resulting in reduced infiltration of myofibroblast and collagen accumulation, identifying a novel antifibrotic role of Dapagliflozin which involves inhibition of myofibroblasts [40]. However, although the above signaling pathways may be responsible mechanisms of the ability of Dapagliflozin to reduce myocardial infarct size, to the best of our knowledge there are no studies investigating the above signaling pathways in the presence of I/R and /or investigating the acute/chronic effects of Dapagliflozin in non-diabetic animals in terms of infarct size reduction.

4. Empagliflozin

Empagliflozin is a potent and competitive inhibitor of SGLT2 with the highest selectivity compared with the other SGLT2 inhibitors [42]. Recent studies demonstrated the beneficial effects of Empagliflozin on atherosclerosis in ApoE$^{-/-}$ mice fed a Western type diet [43], on cardiac injury in prediabetic rats [44], on myocardial microvascular injury in streptozotocin-induced diabetic mice [45], and on cardiac dysfunction and myocardial injury in type 2 diabetes mice [45]. Until now, only few experimental in vitro, ex vivo and in vivo studies have investigated whether Empagliflozin is able to exert cardioprotective effects against acute myocardial I/R injury.

Effects of empagliflozin on I/R in diabetic status: The direct effects of Empagliflozin in H9C2 cells and in endothelial cells (ECs) in presence of advanced glycation end products (AGE) to mimic the diabetic milieu [48] subjected to hypoxia/reoxygenation were investigated in concentrations between 100 and 500 nM. i.e. a concentration range that is known to block SGLT2 effectively and selectively

9

without significant inhibition of SGLT1 [47]. Treatment with 500 nM Empagliflozin increased cell viability and ATP content in comparison to the control group. Long term Empagliflozin treatment (6 weeks, 10 mg/kg/day) in mice with type 2 diabetes induced by chronic feeding with Western type diet and subjected to 30 min ischemia followed by 2 hours of reperfusion, reduced myocardial infarct size and improved myocardial function [48].

Effects of empagliflozin on I/R in non- diabetic status: The direct effects of Empagliflozin (concentrations between 100 and 500 nM) in H9C2 cells and in endothelial cells (ECs) [48] subjected to hypoxia/reoxygenation showed that treatment with 500 nM Empagliflozin increased cell viability and ATP content in comparison to the control group. It was also shown that Empagliflozin can improve contractility of isolated cardiomyocytes under hypoxic conditions [49]. The ex vivo isolated mouse heart model has been employed to examine potential direct cardiac effects of acute Empagliflozin treatment in the development of contracture during ischaemia and on infarct size after 2 h reperfusion. Although Empagliflozin (1 μM) did not protect against I/R injury, it did delay contracture development, and improved cardiac performance during ischaemia [18]. Similar results as far as infarct size is concerned have been obtained in isolated rat hearts, in which Empagliflozin given 10 min prior to the acute myocardial infarction did not reduce infarct size. However, Empagliflozin improved post-ischemic complex I+II respiration compared to the control group and this improvement was similar to ischemic preconditioning group [19]. In a recent report, however, it was suggested that Empagliflozin can acutely reduce infarct size in the isolated mouse hearts [49]. However, in that report the authors had to go to non-clinical high concentrations of Empagliflozin (2.5 μM), whereas in their prior cellular studies 0.5 μM was enough to activate AMPK and improve contractility in hypoxic cardiomyocytes. It is likely that with this more physiological concentration of Empagliflozin (0.5 μM) no protection was observed in the isolated mouse hearts.

The in vivo effects of Empagliflozin treatment have been investigated in non-diabetic male rats undergoing permanent coronary artery ligation to induce myocardial infarction, or sham surgery. Empagliflozin was administered through the chow (average daily intake of 30mg/kg/day), starting before surgery (EMPA-early) or 2 weeks after surgery (EMPA-late). Short-term Empagliflozin administration before AMI and long-term administration after AMI did not change infarct size, but favorably affected

10

cardiac function and remodeling in non-diabetic rats with LV-dysfunction after AMI [50]. The absent reduction of infarct size may not be surprising because the model of permanent occlusion does not allow reperfusion such that the whole area at risk will get infarcted leaving the potential for salvage minimal.

A recent report demonstrated that 3 days of Empagliflozin pretreatment also reduced infarct size in the in vivo healthy mice, with protection lost when Compound C, an inhibitor of AMPK activation, was present [49]. However, we must mention that another vivo study showed that Empagliflozin did not phosphorylate AMPK, specifying that its cardioprotective effects are independent of AMPK activation [48]. Therefore, further studies are required in order to clarify the role of AMPK activation in Empagliflozin's cardioprotective properties.

Direct cardiac effects of Empagliflozin that have been reported are 1) reduction in intracellular Na^+ and Ca^{2+} in isolated cardiomyocytes from rabbits and mice [23, 51], 2) inhibition of NHE-1 in isolated cardiomyocytes of rabbit and mice [23, 51], 3) increases in mitochondrial Ca^{2+} in isolated rat cardiomyocytes [51], 4) increased STAT3 phosphorylation in in vivo mouse hearts at early reperfusion [48], 5) increased AMPK phosphorylation and peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) in isolated mouse cardiomyocytes [49], 6) increased phosphorylation levels of myofilament regulatory proteins and reduced diastolic dysfunction in isolated human and rat cardiomyocytes [52], 7) increased glucose transporter 1 (GLUT1) and glucose uptake in human and mouse cardiomyocytes [53], 8) decreased Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity and intracellular Na^+ in healthy and failing cardiomyocytes of mice and human [54], 9) decreased action potential duration, late sodium current and ROS in isolated rat T1DM cardiomyocytes [55], and 10) lowering of TNFα-induced ROS in human microvascular and coronary arterial endothelial cells [56]. All these studies unquestionable confirm that Empagliflozin has direct cardiac effects, which has been noted for most SGLT2 inhibitors as of yet [57]. In addition, many of the above described direct cardiac effects of Empagliflozin have been related to modulation of cardiac infarct size; increased AMPK activity, decreased CaMKII activity and increased glucose uptake have all independently shown to reduce cardiac infarct size in acute conditions of ischemia-reperfusion [58-60].

The effects of Empagliflozin on NHE has been confirmed also for murine cardiomyocytes and in the ex vivo intact heart, in which Empagliflozin exerted direct

11

cardiac effects by inhibiting NHE during ischaemia, but not during reperfusion [18]. However, although there was a reduction of infarct size following chronic pre-administration of the NHE inhibitor, no infarct sparing was found following SGLT2 inhibition by Empagliflozin [18]. NHE inhibition has been shown to be cardioprotective in the context of I/R injury since in animal studies, NHE inhibition is highly effective in reducing myocardial injury [61]. However, although clinical translation showed promise for NHE inhibition in reducing cardiac infarct size in especially cardiac surgery patients with well-defined ischemic episodes (similar as in preclinical studies), there was a non-anticipated high incidence of cerebrovascular events resulting from thromboembolic strokes [62, plus PMID 18355507]. Important to note is that almost all stroke events occurred after halting, not during the 2 day period of NHE-1 inhibition, offering the possibility that it was the halting of treatment, and not the treatment itself, that may have caused coagulation and stroke [PMID 18355507]. It is thereby interesting that also in further reporting on the EMPA-REG Outcome study (PMID 28286035), the non-significant trend for an increased stroke incidence (HR=1.18; p=0.26) in the empagliflozin group, was largely driven by stroke events that only occurred *after* patient's last drug intake.

I/R injury is characterized by necrosis of myocardial tissue, which is, at least partly, caused by a combination of extensive inflammatory and oxidative stress [63, 64]. Cardioprotective agents can reduce myocardial infarct size by antioxidant effects and significant decreases of inflammatory mediators in healthy animals [65] and in animals with endothelial dysfunction [66]. Chronic treatment with Empagliflozin in mice with T2D, showed that the drug increased both the phosphorylation and the expression of cardiac STAT-3 at early reperfusion [48]. STAT-3 is one of the main signaling molecules in the SAFE pathway, which is considered a major mediator of cardioprotection against I/R injury [5, 67]. Chronic treatment with Empagliflozin regulates inflammatory responses and redox signaling in the ischemic myocardium by reducing myocardial interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) expressions without affecting eNOS phosphorylation or expression [48]. In a very recent study, Empagliflozin restored NO bioavailability through inhibiting ROS generation without affecting eNOS expression or signaling in human coronary arterial endothelial cells (HCAECs) and human umbilical vein endothelial cells (HUVECs) that were (pre-) incubated with 1 µM Empagliflozin and subsequently exposed to 10 ng/ml

TNFα, indicating that Empagliflozin may exert direct cellular anti-inflammatory/oxidative stress effects in the endothelium [41]. Another recent study showed that Empagliflozin prevented the excessive reduction in mitochondrial size in hearts from a rat model of T2D after AMI, and that the mechanism involved suppression of ROS and restoration of autophagy [68].

Empagliflozin increases circulating ketone levels and myocardial expression of the ketone body transporter associated with an increase in cardiac ATP production in non-diabetic rats subjected to permanent coronary artery ligation [50]. Although Empagliflozin did not reduce infarct size, which was also probably not possible because there was no reperfusion, the increased circulating ketone levels might be a potential mechanism of its cardioprotective activity on cardiac remodeling. Beyond the role of the effects of ketone bodies in heart failure, they have been also shown to have beneficial properties in the setting of I/R by reducing myocardial infarct size, possibly due to up-regulation of crucial oxidative phosphorylation mediators and reduction of oxidative stress [69-70]. The ketone hypothesis relies on a preferential energy-substrate switch. However, experience from a study of Canagliflozin indicates that the protective effect of SGLT2 is independent of substrate supply making this mechanism less likely as an explanation for the cardioprotection [15].

In summary, it seems that acute administration of Empagliflozin failed to reduce MI size in the isolated mouse/rat heart, however we must mention that to the best of our knowledge there is no in vivo study that has investigated the effects of acute Empagliflozin administration in myocardial infarction. However, Empagliflozin have shown acute functional protective effects, improving cardiac performance during ischaemia [18, 49], just like Dapagliflozin [20]. Chronic Empagliflozin treatment reduced infarct size in a vivo model of T2D [48], however the effects of the drug on healthy myocardium are still pending. The cardioprotective benefits of Empagliflozin may be attributed to systemic as well as direct cardiac effects. Parallel protective mechanisms of Empagliflozin have been proposed: inhibition of NHE, reductions in cytosolic Na^+ and Ca^{2+}, activation of STAT3, attenuation of cardiac inflammation, and inhibition of oxidative stress.

5. Conclusions/Future Perspectives

13

In the light of the profound reduction in heart failure hospitalization after treatment with SGLT2 in patients with T2D, preclinical studies have been conducted to reveal the efficacy and the biological pathways by which SGLT2 inhibitors exert cardiac protection. Until now, Canagliflozin and Empagliflozin have been tested preclinically in both diabetic and non-diabetic animals. In animals with diet-induced either obese insulin-resistant or metabolic syndrome, chronic treatment with both Empagliflozin and Dapagliflozin reduced significantly myocardial infarct size. Additionally, the observation that there is an absence of efficacy of acute SGLT2 inhibition by both Canagliflozin and Empagliflozin in isolated hearts may indicate that this is a class effect and that the main mechanism of SGLT2 inhibitors on infarct size does not work through direct modulation of the myocardium but rather via a mediating cardioprotective signal that appears to be dependent on a whole-body system. However, we must point out that in vivo studies investigating the acute effects of Empagliflozin and Dapagliflozin on infarct size development are lacking. In contrast, SGLT2 inhibitors are able to directly and acutely affect cardiac function during the ischemic episode, both in the in vivo and ex vivo condition.

As far as the mechanisms of action on infarct size reductions are concerned, it seems that NHE inhibition is unlikely because SGLT2 inhibitors that have acute NHE inhibitory capacities mostly do not offer acute protection. AMPK seems also unlikely, because Empagliflozin activates AMPK within minutes, whereas Empagliflozin in most studies does not provide acute protection. Another common signaling molecule that has been shown to be activated in the myocardium by both Empagliflozin and Dapagliflozin is STAT3. It will be interesting to examine whether the time course of STAT3 activation can explain the time course of SGLT2 inhibitors effects on infarct size. In contrast, the direct beneficial effects of SGLT2 inhibitors on cardiac function during ischemia may well be explained by NHE-1 inhibition and/or AMPK activation. Parallel protective mechanisms of SGLT2 inhibitors have been proposed such as reductions in cardiac calcium and sodium, attenuation of cardiac inflammation, and inhibition of oxidative stress improving cardiac structure and function, which also seems a common mechanism until now for the SGLT2is. Many of these pathways are likely to be intertwined and thus dependent on each other. For example, reductions in cytosolic sodium will result in lowering of cytosolic calcium, which may then attenuate NLRP3 inflammasome activation, resulting in reductions in inflammatory cytokines and thus oxidative stress and I/R injury. Additionally, increases in mitochondrial

14

calcium may improve mitochondrial defense against oxidative stress (activating mitochondrial glutathione/theoredoxin redox enzymes), reducing mitochondrial dysfunction and NLRP3 inflammasome activation and inflammation. However, so far, the in depth understanding of the effects observed in the clinic is not forthcoming since the mechanisms of infarct size reduction and the prevention of heart failure are still under investigation.

Until now there is a variety of experimental approaches and models for the investigation of the effects of SGLT2 inhibitors, therefore is difficult to draw conclusions about the robustness of the preclinical data. Especially for the ischemia/reperfusion experiments there is a variety of experimental conditions until now, for example the use of permanent ischemia to access infarct size reduction, and this is a main shortcoming for the investigation of the role of SGLT2is in I/R. Therefore, better experimental design to identify robust and reproducible strategies of cardioprotection must be developed and the pre-clinical studies should follow the practical guidelines to ensure rigor and reproducibility in preclinical studies on cardioprotection [PMID: Basic Research in Cardiology (2018) 113:39]. Additionally, studies should focus on the cell types target of SGLT2is and administration of non-clinically relevant concentrations of the drugs should be avoided in the preclinical experiments.

Since the bench to bedside approach in the field of cardioprotection has been inadequate, a bedside to bench approach could be a preferable alternate to be implemented for the patient benefit. Another example of bench to bedside approach for antidiabetic agents is the one of glycagon-like-peptide-1 agonists (GLP1-RA). Experimental and small clinical studies have highlighted that GLP1-RA as a class reduce risk of atherosclerotic vascular disease and reduce significantly myocardial infarction in small animal models. Although the mechanisms are not fully defined it has been proposed that activation of the GLP receptor, PKA and RISK pathways, and eNOS phosphorylation are among the main mechanism of infarct size reduction by this class of drugs. (Our review in BJP). It would be of significant interest to investigate if the both classes of drtugs share common cardioprotective mechanisms and if a combination could result in increased efficiency of both strategies.

More research is necessary to delineate the main mechanisms involved in the cardioprotection of SGLT2 inhibitors, which may lead to new treatment targets in patients with cardiovascular risk factors. Additionally, the elucidation of the possible cardioprotective mechanisms of SGLT2 inhibitors on the diabetic and on the non-

diabetic myocardium increase the likelihood of success in terms of translating cardioprotection into the clinical setting for patient benefit.

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Declaration of Interests

The authors have no declarations of interests to disclose.

References

[1] M. Schmidt, J.B. Jacobsen, T.L. Lash, H.E. Botker, H.T. Sorensen, 25 year trends in first time hospitalisation for acute myocardial infarction, subsequent short and long term mortality, and the prognostic impact of sex and comorbidity: a Danish nationwide cohort study, BMJ 344 (2012) e356.

[2]   K. Szummer, L. Wallentin, L. Lindhagen, J. Alfredsson, D. Erlinge, C. Held, S. James, T. Kellerth, B. Lindahl, A. Ravn-Fischer, E. Rydberg, T. Yndigegn, T. Jernberg, Improved outcomes in patients with ST-elevation myocardial infarction during the last 20 years are related to implementation of evidence-based treatments: experiences from the SWEDEHEART registry 1995-2014, Eur. Heart J. 38 (2017) 3056-3065.

[3] J. Chen, A.F. Hsieh, K. Dharmarajan, F.A. Masoudi, H.M. Krumholz, National trends in heart failure hospitalization after acute myocardial infarction for Medicare beneficiaries: 1998-2010, Circulation 128 (2013) 2577-2584.

[4] S.M. Davidson, I. Andreadou, D. Garcia-Dorado, D.J. Hausenloy, Shining the spotlight on cardioprotection: beyond the cardiomyocyte, Cardiovasc. Res. 115 (2019) 1115–1116.

[5] G. Heusch, Molecular Basis of Cardioprotection, Circ. Res. 116 (2015) 674–699.

[6] B. Zinman, C. Wanner, J.M. Lachin, D. Fitchett, E. Bluhmki, S. Hantel, M. Mattheus, T. Devins, O.E. Johansen, H.J. Woerle, U.C. Broedl, S.E. Inzucchi, Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes, New Engl. J. Med. 373 (2015) 2117–2128.

[7] B.L. Neuen, T. Ohkuma, B. Neal, D.R. Matthews, D. de Zeeuw, K.W. Mahaffey, G. Fulcher, M. Desai, Q. Li, H. Deng, N. Rosenthal, M.J. Jardine, G. Bakris, V. Perkovic, Cardiovascular and Renal Outcomes With Canagliflozin According to Baseline Kidney Function, Circulation 138 (2018) 1537–1550.

[8] S.D. Wiviott, I. Raz, M.P. Bonaca, O. Mosenzon, E.T. Kato, A. Cahn, M.G. Silverman, T.A. Zelniker, J.F. Kuder, S.A. Murphy, D.L. Bhatt, L.A. Leiter, D.K. McGuire, J.P.H. Wilding, C.T. Ruff, I.A.M. Gause Nilsson, M. Fredriksson, P.A. Johansson, A-M. Langkilde, M.S. Sabatine, DECLARE–TIMI 58 Investigators, Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. N. Engl. J. Med. 380 (2019) 347–357.

[9] B. Sinha, S. Ghosal, Meta-analyses of the effects of DPP-4 inhibitors, SGLT2 inhibitors and GLP1 receptor analogues on cardiovascular death,

17

myocardial infarction, stroke and hospitalization for heart failure, Diabetes Res. Clin. Pract. 150 (2019) 8-16.

[10] J.J.V. McMurray, S.D. Solomon, S.E. Inzucchi, L. Køber, M.N. Kosiborod, F.A. Martinez, P. Ponikowski, M.S. Sabatine, I.S. Anand, J. Bělohlávek, M. Böhm, C.E. Chiang, V.K. Chopra, R.A. de Boer, A.S. Desai, M. Diez, J. Drozdz, A. Dukát, J. Ge, J.G. Howlett, T. Katova, M. Kitakaze, C.E.A. Ljungman, B. Merkely, J.C. Nicolau, E. O'Meara, M.C. Petrie, P.N. Vinh, M. Schou, S. Tereshchenko, S. Verma, C. Held, D.L. DeMets, K.F. Docherty, P.S. Jhund, O. Bengtsson, M. Sjöstrand, A.M. Langkilde; DAPA-HF Trial Committees and Investigators, Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction, New Engl. J. Med. 381 (2019) 1995-2008.

[11] G.W. Stone, H.P. Selker, H. Thiele, M.R. Patel, J.E. Udelson, E.M. Ohman, A. Maehara, I. Eitel, C.B. Granger, P.L. Jenkins, M. Nichols, O. Ben-Yehuda, Relationship Between Infarct Size and Outcomes Following Primary PCI: Patient-Level Analysis From 10 Randomized Trials, J. Am. Coll. Cardiol. 67 (2016) 1674-1683.

[12] E. Ferrannini, Sodium-Glucose Co-transporters and Their Inhibition: Clinical Physiology, Cell Metab, 26 (2017) 27–38.

[13] A. Van Steenbergen, M. Balteau, A. Ginion, L. Ferté, S. Battault, C.M. Ravenstein, J.L. Balligand, E.P. Daskalopoulos, P. Gilon, F. Despa, S. Despa, J.L. Vanoverschelde, S. Horman, H. Koepsell, G. Berry, L. Hue, L. Bertrand, C. Beauloye, Sodium-myoinositol cotransporter-1, SMIT1, mediates the production of reactive oxygen species induced by hyperglycemia in the heart, Sci, Rep, 7 (2017) 41166.

[14] J.L. Faillie, Pharmacological aspects of the safety of gliflozins, Pharmacol. Res. 118 (2017) 71-81.

[15] V.G. Lim, R.M. Bell, S. Arjun, M. Kolatsi-Joannou, D.A. Long, D.M. Yellon, SGLT2 Inhibitor, Canagliflozin, Attenuates Myocardial Infarction in the Diabetic and Nondiabetic Heart. JACC Basic Transl. Sci. 4 (2019) 15-26.

[16] J. Chen, S. Williams, S. Ho, H. Loraine, D. Hagan, J.M. Whaley, J.N. Feder, Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members, Diabetes Ther. 1 (2010) 57-92.

[17] Z .Li, V. Agrawal, M. Ramratnam, R.K. Sharma, S. D'Auria, A. Sincoular, M. Jakubiak, M.L. Music, W.J. Kutschke, X.N. Huang, L. Gifford, F. Ahmad, Cardiac

sodium-dependent glucose co transporter 1 is a novel mediator of ischaemia/reperfusion injury. Cardiovasc. Res. 115 (2019) 1646-1658.

[18] L. Uthman, R. Nederlof, O. Eerbeek, A. Baartscheer, C. Schumacher, N. Buchholtz, M.W. Hollmann, R. Coronel, N.C. Weber, C.J. Zuurbier, Delayed ischaemic contracture onset by empagliflozin associates with NHE1 inhibition and is dependent on insulin in isolated mouse hearts, Cardiovasc. Res. 115 (2019) 1533-1545.

[19] N.R. Jespersen, T.R. Lassen, M.V. Hjortbak, N.B. Stottrup, H.E. Botker, Sodium Glucose Transporter 2 (SGLT2) Inhibition does not Protect the Myocardium from Acute Ischemic Reperfusion Injury but Modulates Post- Ischemic Mitochondrial Function, Cardiovasc. Pharmacol. Open Access 06 (2017) 2-4.

[20] H.E. Baker, A.M. Kiel, S.T. Luebbe, B.R. Simon, C.C. Earl, A. Regmi, W.C. Roell, K.J. Mather, J.D. Tune, A.G. Goodwill, Inhibition of sodium-glucose cotransporter-2 preserves cardiac function during regional myocardial ischemia independent of alterations in myocardial substrate utilization, Basic Res. Cardiol. 114 (2019).

[21] A.A. Sayour, S. Korkmaz-Icoz, S. Loganathan, M. Ruppert, V.N. Sayour, A. Olah, K. Benke, M. Brune, R. Benko, E.M. Horvath, M. Karck, B. Merkely, T. Radovits, G. Szabo, Acute canagliflozin treatment protects against in vivo myocardial ischemia-reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation, Journal of Translat. Med. 17 (2019).

[22] E. Ferrannini, M. Mark, E. Mayoux, CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis, Diabetes Care 39 (2016) 1108-1114.

[23] L. Uthman, A. Baartscheer, B. Bleijlevens, C.A. Schumacher, J.W.T. Fiolet, A. Koeman, M. Jancev, M.W. Hollmann, N.C. Weber, R. Coronel, C.J. Zuurbier, Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na(+)/H(+) exchanger, lowering of cytosolic Na(+) and vasodilation, Diabetologia 61 (2018) 722-726.

[24] M.M. Mihaylova, R.J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, Nat, Cell Biol, 13 (2011) 1016-1023.

[25] S.A. Hawley, R.J. Ford, B.K. Smith, G.J. Gowans, S.J. Mancini, R.D. Pitt, E.A. Day, I.P. Salt, G.R. Steinberg, D.G. Hardie, The Na+/Glucose Cotransporter Inhibitor Canagliflozin Activates AMPK by Inhibiting Mitochondrial Function and Increasing Cellular AMP Levels, Diabetes 65 (2016) 2784-2794.

19

[26] S.J. Mancini, D. Boyd, O.J. Katwan, A. Strembitska, T.A. Almabrouk, S. Kennedy, T.M. Palmer, I.P. Salt, Canagliflozin inhibits interleukin-1beta-stimulated cytokine and chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and -independent mechanisms, Sci. Rep. 8 (2018) 5276.

[27] R.R Russell, D.L. Coven, M. Pypaert, C. Zechner, M. Palmeri, F.J. Giordano, J. Mu, M.J. Birnbaum, L.H. Young, AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury, J. Clin. Invest. 114 (2004) 495-503.

[28] D. Garcia-Dorado, M.A. Gonzalez, J.A. Barrabes, M. Ruiz-Meana, J. Solares, R.M. Lidon, J. Blanco, Y. Puigfel, H.M. Piper, J. Soler-Soler. Prevention of ischemic contracture during coronary occlusion by inhibition of Na(+)-H+ exchange. Cardiovasc Res. 35 (1997) 80-89.[29] S. Filippas-Ntekouan, V. Tsimihodimos, T. Filippatos, T. Dimitriou, M. Elisaf, SGLT-2 inhibitors: pharmacokinetics characteristics and effects on lipids, Expert Opin. Drug Metab. Toxicol. 14 (2018) 1113–1121.

[30] N. Zhang, B. Feng, X. Ma, K. Sun, G. Xu, Y. Zhou, Dapagliflozin improves left ventricular remodeling and aorta sympathetic tone in a pig model of heart failure with preserved ejection fraction, Cardiovasc, Diabetol. 18 (2019) 107.

[31] L. Shi, D. Zhu, S. Wang, A. Jiang, F. Li, Dapagliflozin Attenuates Cardiac Remodeling in Mice Model of Cardiac Pressure Overload, Am. J. Hypertens. 32 (2019) 452-459.

[32] A. Al-Sharea, A.J. Murphy, L.A. Huggins, Y. Hu, I.J. Goldberg, P.R. Nagareddy, SGLT2 inhibition reduces atherosclerosis by enhancing lipoprotein clearance in Ldlr-/- type 1 diabetic mice, Atherosclerosis 271 (2018) 166-176.

[33] Y. Ye, M. Bajaj, H.C. Yang, J.R. Perez-Polo, Y. Birnbaum, SGLT-2 Inhibition with Dapagliflozin Reduces the Activation of the Nlrp3/ASC Inflammasome and Attenuates the Development of Diabetic Cardiomyopathy in Mice with Type 2 Diabetes. Further Augmentation of the Effects with Saxagliptin, a DPP4 Inhibitor, Cardiovasc. Drugs Ther. 31 (2017) 119-132.

[34] M. Joubert, B. Jagu, D. Montaigne, X. Marechal, A. Tesse, A. Ayer, L. Dollet, C. Le May, G. Toumaniantz, A. Manrique, F. Charpentier, B. Staels, J. Magré, B. Cariou, X. Prieur, The Sodium-Glucose Cotransporter 2 Inhibitor Dapagliflozin Prevents Cardiomyopathy in a Diabetic Lipodystrophic Mouse Model, Diabetes 66 (2017) 1030-1040.

[35] N.N. Hamouda, V. Sydorenko, M.A. Qureshi, J.M. Alkaabi, M. Oz , F.C. Howarth, Dapagliflozin reduces the amplitude of shortening and Ca(2+) transient in ventricular myocytes from streptozotocin-induced diabetic rats, Mol. Cell. Biochem. 400 (2015) 57-68.

[36] P. Tanajak, P. Sa-Nguanmoo, S. Sivasinprasasn, S. Thummasorn, N. Siri-Angkul, S.C. Chattipakorn, N. Chattipakorn, Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury, J. Endocrinol. 236 (2018) 69-84.

[37] S.B. Ong, S. Subrayan, S.Y. Lim, D.M. Yellon, S.M. Davidson, D.J. Hausenloy, Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury, Circulation 121 (2010) 2012–2022.

[38] A. Durak, Y. Olgar, S. Degirmenci, E. Akkus, E. Tuncay, B. Turan, A SGLT2 inhibitor dapagliflozin suppresses prolonged ventricular-repolarization through augmentation of mitochondrial function in insulin-resistant metabolic syndrome rats, Cardiovasc. Diabetol. 17 (2018) 144.

[39] Y. Ye, X. Jia, M. Bajaj, Y. Birnbaum, Dapagliflozin Attenuates Na^+/H^+ Exchanger-1 in Cardiofibroblasts via AMPK Activation, Cardiovasc. Drugs Ther. 32 (2018) 553-558.

[40] T.M. Lee, N.C. Chang, S.Z. Lin, Dapagliflozin, a selective SGLT2 Inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization via STAT3 signaling in infarcted rat hearts. Free Radic. Biol. Med. 104 (2017) 298-310.

[41] L. Uthman, A. Homayr, R.P. Juni, E.L. Spin, R. Kerindongo, M. Boomsma, M.W. Hollmann, B. Preckel, P. Koolwijk, V.W.M. van Hinsbergh, C.J. Zuurbier, M. Albrecht, N.C. Weber, Empagliflozin and Dapagliflozin Reduce ROS Generation and Restore NO Bioavailability in Tumor Necrosis Factor α-Stimulated Human Coronary Arterial Endothelial Cells. Cell Physiol. Biochem. 53 (2019) 865-886.

[42] R. Grempler, L. Thomas, M. Eckhardt, F. Himmelsbach, A. Sauer, D.E. Sharp, Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors, Diabetes Obes. Metab. 14 (2012) 83-90.

[43] J.H. Han, T.J. Oh, G. Lee, H.J. Maeng, D.H. Lee, K.M. Kim, S.H. Choi, H.C. Jang, H.S. Lee, K.S. Park, Y.B. Kim, S. Lim, The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE $^{-/-}$ mice fed a western diet, Diabetologia 60 (2017) 364-376.

21

[44] H. Kusaka, N. Koibuchi, Y. Hasegawa, H. Ogawa, S. Kim-Mitsuyama, Empagliflozin lessened cardiac injury and reduced visceral adipocyte hypertrophy in prediabetic rats with metabolic syndrome. Cardiovasc. Diabetol. 15 (2016) 157.

[45] H. Zhou, S. Wang, P. Zhu, S. Hu, Y. Chen, J. Ren, Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission, Redox Biol. 15 (2018) 335-346.

[46] M. Xue , T. Li, Y. Wang, Y. Chang, Y. Cheng, Y. Lu, X. Liu, L. Xu, X. Li, X. Yu, B. Sun, L. Chen, Empagliflozin prevents cardiomyopathy via sGC-cGMP-PKG pathway in type 2 diabetes mice, Clin. Sci. (Lond). 133 (2019) 1705-1720.

[47] U. Panchapakesan, K. Pegg, S. Gross, M.G. Komala, H. Mudaliar, J. Forbes, C. Pollock, A. Mather, Effects of SGLT2 inhibition in human kidney proximal tubular cells–renoprotection in diabetic nephropathy? PLoS ONE 8 (2013) e54442.

[48] I. Andreadou, P. Efentakis, E. Balafas, G. Togliatto, C.H. Davos, A. Varela, C.A. Dimitriou, P.-E. Nikolaou, E. Maratou, V. Lambadiari, I. Ikonomidis, N. Kostomitsopoulos, M.F. Brizzi, G. Dimitriadis, E.K. Iliodromitis, Empagliflozin Limits Myocardial Infarction in Vivo and Cell Death in Vitro: Role of STAT3, Mitochondria, and Redox Aspects, Front. Physiol. 8 (2017).

[49] Q. Lu, J. Liu, X. Li, X. Sun, J. Zhang, D. Ren, N. Tong, J. Li, Empagliflozin attenuates ischemia and reperfusion injury through LKB1/AMPK signaling pathway, Mol. Cell. Endocrinol. 501 (2019) 110642.

[50] S.R. Yurista, H.H.W. Silljé, S.U. Oberdorf-Maass, E.M. Schouten, M.G. Pavez Giani, J.L. Hillebrands, H. van Goor, D.J. van Veldhuisen, R.A. de Boer, B.D. Westenbrink, Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction, Eur. J. Heart Fail. 21 (2019) 862-873.

[51] A. Baartscheer, C.A. Schumacher, R.C. Wust, J.W. Fiolet, G.J. Stienen, R. Coronel, C.J. Zuurbier, Empagliflozin decreases myocardial cytoplasmic Na+ through inhibition of the cardiac Na+/H+ exchanger in rats and rabbits, Diabetologia 60, (2017) 568–573.

[52] S. Pabel, S. Wagner, H. Bollenberg, P. Bengel, A. Kovacs, C. Schach, P. Tirilomis, J. Mustroph, A. Renner, J. Gummert, T. Fischer, S. van Linthout, C. Tschope, K. Streckfuss-Bomeke, G. Hasenfuss, L.S. Maier, N. Hamdani, S. Sossalla, Empagliflozin directly imporves diastolic function in human heart failure. Eur. J. Heart Fail. 20 (2018) 1690-1700.

22

[53] J. Mustroph, C.M. Lucht, O. Wagemann, T. Sowa, K.P. Hammer, C.M. Sag, D. Tarnowski, A. Holzamer, S. Pabel, B.E. Beuther, S. Sossalla, L.S. Maier, S. Wagner, Empagliflozin enhances human and murine cardiomyocyte glucose uptake by increased expression of GLUT1, Diabetologia 62 (2019) 726-729.

[54] J. Mustroph, O. Wagemann, C.M. Lucht, M. Trum, K.P. Hammer, C.M. Sag, S. Lebek, D. Tarnowski, J. Reinders, F. Perbellini, C. Terracciano, C. Schmid, S. Schopka, M. Hilker, Y. Zausig, S. Pabel, S.T. Sossalla, F. Schweda, L.S. Maier, S. Wagner, Empagliflozin reduces Ca/calmodulin-dependent kinase II activity in isolated ventricular cardiomyocytes. ESC Heart Failure 5 (2018) 642-648.

[55] T.I. Lee, Y.C. Chen, Y.K. Lin, C.C. Chung, Y.Y. Lu, Y.H. Kao, Y.J. Chen, Empagliflozin attenuates myocardial sodium and calcium dysregulation and reverses cardiac remodeling in streptozotocin-induced diabetes rats, Int. J. Mol. Sci. 20 (2019) 7.

[56] R.P. Juni, D.W.D. Kuster, M. Goebel, M. Helmes, R.J.P. Musters, J. van der Velden, P. Koolwijk, W.J. Paulus, V.W.M. van Hinsbergh, Cardiac microvascular endothelial enhancement of cardiomyocyte function is impaired by inflammation and restored by emapgliflozin, JACC Basic Transl. Sci. 4 (2019) 575-591.

[57] L. Uthman, A. Baartscheer, C.A. Schumacher, J.W.T. Fiolet, M.C. Kushma, M.W. Hollmann, R. Coronel, N.C. Weber, C.J. Zuurbier, Direct cardiac actions of sodium glucose cotransporter 2 inhibitors target pathogenic mechanisms underlying heart failure in diabetic patients. Front. Physiol. 9 (2018) 1575.

[58] A.S. Kim, E.J. Miller, T.M. Wright, J. Li, D. Qi, K. Atsina, V. Zaha, K. Sakamoto, L.H. Young, A small molecule AMPK activator protects the heart against ischemia-reperfusion injury, J. Mol. Cell. Cardiol. 51 (2011) 24-32.

[59] A.D. Timmermans, M. Balteau, R. Gelinas, E. Renguet, A. Ginion, C. de Meester, K. Sakamoto, J.L. Balligand, F. Bontemps, J.L. Vanoverschelde, S. Horman, C. Beauloye, L. Bertrand, A-769662 potentiates the effect of other AMPK-activated protein kinase activators on cardiac glucose uptake, Am. J. Physiol. Heart Circ. Physiol. 306 (2014) H1619-H1630.

[60] L. Ji, X. Zhang, W. Liu, Q. Huang, W. Yang, F. Fu, H. Ma, H. Su, H. Wang, J. Wang, H. Zhang, F. Gao, AMPK-regulated and Akt-dependent enhancement of glucose uptake is essential in ischemic preconditioning-alleviated reperfusion injury, PLoSOne. 8 (2013) e69910.

[61] M. Avkiran, M.S. Marber, Na+/H+ exchange inhibitors for cardioprotective therapy: progress, problems and prospects, J, Am, Coll, Cardiol, 39 (2002) 747-753.

[62] M. Karmazyn, NHE-1: still a viable therapeutic target, J. Mol. Cell. Cardiol. 61 (2013) 77-82.

[63] E.T. Chouchani, V.R. Pell, E. Gaude, D. Aksentijević, S.Y. Sundier, E.L. Robb, A. Logan, S.M. Nadtochiy, E.N.J. Ord, A.C. Smith, F. Eyassu, R. Shirley, C.-H. Hu, A.J. Dare, A.M. James, S. Rogatti, R.C. Hartley, S. Eaton, A.S.H. Costa, P.S. Brookes, S.M. Davidson, M.R. Duchen, K. Saeb-Parsy, M.J. Shattock, A.J. Robinson, L.M. Work, C. Frezza, T. Krieg, M.P. Murphy, Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, Nature 515 (2014) 431-435.

[64] C.J. Zuurbier, A. Abbate, H.A. Cabrera-Fuentes, M.V. Cohen, M. Collino, D.P.V. De Kleijn, J.M. Downey, P. Pagliaro, K.T. Preissner, M. Takahashi, S.M. Davidson, Innate immunity as a target for acute cardioprotection, Cardiovasc. Res. 115(7) (2019) 1131-1142.

[65] M. Wallert, M. Ziegler, X. Wang, A. Maluenda, X. Xu, M.L. Yap, R. Witt, C. Giles, S. Kluge, M. Hortmann, J. Zhang, P. Meikle, S. Lorkowski, K. Peter, α-Tocopherol preserves cardiac function by reducing oxidative stress and inflammation in ischemia/reperfusion injury, Redox Biol. 26 (2019) 101292.

[66] P. Efentakis, A. Rizakou, E. Christodoulou, A. Chatzianastasiou, M.G. López, R. León, E. Balafas, N.P.E. Kadoglou, I. Tseti, H. Skaltsa, N. Kostomitsopoulos, E.K. Iliodromitis, G. Valsami, I. Andreadou, Saffron (Crocus sativus) intake provides nutritional preconditioning against myocardial ischemia-reperfusion injury in Wild Type and ApoE(-/-) mice: Involvement of Nrf2 activation. Nutr. Metab. Cardiovasc. Dis. 27 (2017) 919-929.

[67] K. Boengler, D. Hilfiker-Kleiner, G. Heusch, R. Schulz, Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion, Basic Res. Cardiol 105 (2010) 771–785.

[68] M. Mizuno, A. Kuno, T. Yano, T. Miki, H. Oshima, T. Sato, K. Nakata, Y. Kimura, M. Tanno, T. Miura, Empagliflozin normalizes the size and number of mitochondria and prevents reduction in mitochondrial size after myocardial infarction in diabetic hearts. Physiol. Rep. 6 (2018) e13741

[69] M.G. Marina Prendes, J.V. Garcia, M.A. Fernandez, M.J. Perez, J.C. Perazzo, E.A. Savino, A. Varela, Effects of 5-hydroxydecanoate and ischemic preconditioning on the ischemic-reperfused heart of fed and fasted rats, J. Physiol. Biochem. 61 (2005) 447-456.

[70] M. Snorek, D. Hodyc, V. Sedivý, J. Durišová, A. Skoumalová, J. Wilhelm, J. Neckář, F. Kolář, J. Herget, Short-term fasting reduces the extent of myocardial infarction and incidence of reperfusion arrhythmias in rats, Physiol. Res. 61 (2012) 567-574.

Table 1: Acute and chronic SGLT2 inhibitor effects on ex vivo and in vivo cardiac I/R injury

SGLT2i	Model Description	Ex vivo/ in vivo	Outcome	Reference
Cana	Chronic administration over a period of 4 weeks to either diabetic fatty or non-diabetic lean Zucker rats	Ex vivo 35 minutes of left anterior artery occlusion and 2 hours reperfusion	Significant reduction of myocardial infarct size	Lim et al JACC:BTS 2019
Cana	Short administration of 10 mmol/l throughout the perfusion protocol and throughout the 2 h of reperfusion	Ex vivo 35 minutes of left anterior artery occlusion and 2 hours reperfusion	Short-term, ex-vivo canagliflozin failed to significantly alter infarct size	Lim et al JACC:BTS 2019
Cana	Acute 3 ug/kg At 5 min of ischemia	In vivo rat 30min ischeamia and 2 hours reperfusion	Significant reduction of myocardial infarct size	Sayour et al J Transl Med 2019
Cana	300 mg, oral 24 h prior to and approximately 2 h before the procedure	In vivo swine 30 min ischeamia and 2 hours reperfusion	Significant reduction of myocardial infarct size	Baker et al[3] Bas Res Cardiol 2019
Dapa	28 days 1mg/kg/day	In vivo HFD-induced obese insulin-resistant rats subjected to 30min ischeamia and 2 hours reperfusion	Significant reduction of myocardial infarct size	Tanajak, J Endocrinol, 2018
Empa	Acute 1 uM Empa, 20 min before ischemia	Ex vivo mouse 25 min ischeamia and 2 hours reperfusion	Delayed contracture No reduction of infarct size	Uthman et al CVR 2019
Empa	Acute 3 uM Empa 10 min before ischemia	Ex vivo rat 40 min ischeamia and 2 hours reperfusion	No reduction of infarct size	Jespersen et al Cardiov Phar 2017
Empa	6 wks 10mg/kg High fat diet	In vivo mouse model of metabolic syndrome, fed for 14 weeks Western type	↓ **IS** (34 to 17%) ↑p-STAT3 at 10 min R	Andreadou et al Front

		diet 30 min ischemia and 2 hours reperfusion		Physiol 2017

Figure Legend: SGLT2 inhibitors exert acute (less than 6 hrs) direct effects and delayed effects on the ischemic myocardium. Common molecular mechanisms so far for the acute effects of SGLT2 inhibitors include inhibition of NHE, reduction in intracellular Na^+ and Ca^{2+} in isolated cardiomyocytes, phosphorylation of AMPKα and reduction of oxidative stress. Additionally, reduced CaMKII activity, inflammation, oxidative stress and upregulation of glucose uptake and STAT3 are some of the possible delayed molecular mechanisms of the reduction of infarct size that has been observed in preclinical studies by this class of drugs. Cardiomyocytes, endothelial cells and fibroblasts are the main cell types involved so far for the cardioprotective effects of SGLT2 inhibitors.

AMPKα: adenosine monophosphate-activated protein kinase-α

NHE: sodium/ hydrogen exchange

ROS: reactive oxygen species

CaMKII: Ca^{2+} /calmodulin-dependent protein kinase II

STAT3: signal transducer and activator of transcription 3