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EDITORIAL COMMENT

Empagliflozin and Protecting Microvascular Support of Heart Mechanics

SGLT2 Inhibition or More?*

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Approximately 6.5 million adults in the United States have heart failure (HF), representing a major cause of morbidity and mortality (1). HF is traditionally divided into 2 subtypes, HF with preserved ejection (HFpEF) and HF with reduced ejection fraction (HFrEF), with each accounting for about 50% of HF cases. Although HFpEF and HFrEF can display similar clinical presentations during acute HF exacerbations, they are often associated with different risk factors, pathophysiological processes, and responses to therapy (2). Many therapies with unequivocal benefit in HFrEF have failed to show efficacy for HFpEF. This is, in part, why the recent findings of EMPA-REG OUTCOME (Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes) have generated tremendous enthusiasm. EMPA-REG OUTCOME was a randomized, double-blind, placebo-controlled trial of 7,020 patients with type 2 diabetes mellitus, and it demonstrated that the primary endpoint, a composite of myocardial infarction, stroke, and cardiovascular death, was significantly reduced (hazard ratio [HR]: 0.86; 95% confidence interval [CI]: 0.74 to 0.99) over a median follow-up of 3.1 years (3). The composite outcome was largely driven by a 38% relative risk reduction of cardiovascular death (HR: 0.62; 95% CI: 0.49 to 0.77) and a 32% relative risk reduction in all-cause mortality (HR: 0.68; 95% CI: 0.57 to 0.82). Of interest, there was a 35% relative risk reduction in hospitalization for HF (HR: 0.65; 95% CI: 0.50 to 0.85), supporting favorable hemodynamic effects of the drug. Further analysis revealed that the reduction of hospitalizations for HF and cardiovascular death were observed both in patients with and without HF at baseline (4). HF was not phenotyped at baseline, but 2 ongoing clinical trials, EMPEROR-Preserved (EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Preserved Ejection Fraction) and EMPEROR-Reduced (EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Reduced Ejection Fraction), are actively enrolling patients to study the effect of empagliflozin versus placebo on top of guideline-directed medical therapy in patients with each subset of HF (5,6).

Empagliflozin is one of a family of inhibitors that target the sodium (Na^+)-glucose cotransporter-2 (SGLT2). SGLTs are transmembrane proteins that facilitate entry of glucose into cells by making use of the Na^+ gradient maintained by sodium-potassium adenosine triphosphatase (7). SGLT2 inhibitors were developed as a therapeutic treatment for diabetes because of their inhibition of glucose reabsorption in the proximal tubules of the kidneys, increasing glucose excretion. In EMPA-REG OUTCOME, the adjusted mean reduction in glycated hemoglobin

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relative to placebo was modest and gradually shrunk over the course of the study (3). These findings, in part, have led to an increasing recognition of potential pleiotropic effects of SGLT2 inhibitors beyond simply hypoglycemic effects. Although the molecular mechanisms are not fully understood, the effects of SGLT2 inhibitors are postulated to be multifactorial, including hemodynamics involving blood pressure reduction and diuresis, loss of body weight, and reductions in the renin-angiotensin-aldosterone system (7,8). SGLT2 inhibitors have also demonstrated some adverse reactions; canagliflozin is associated with a doubling of the risk for lower-limb amputation and with increased risk of fractures, and empagliflozin is associated with increased genital infections (3,9).

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In the paper by Juni et al. (10) in this issue of *JACC: Basic to Translational Science*, the investigators set out to carefully examine the impact of empagliflozin on the interaction between cardiac microvascular endothelial cells (CMEC) and cardiac myocytes (CM) in a unique coculture model. The investigators established that secreted soluble factors from human CMEC improved advanced measures of primary rat CM contraction and relaxation, including sarcomere length shortening, return velocity, and relaxation time constant (tau). This beneficial effect was abolished by pre-incubation of the CMEC but not CM with nitric oxide (NO) synthase inhibitor, N(ω)-nitro-L-arginine methyl ester (L-NAME), indicating a significant role of endothelial produced NO. CMEC-conditioned medium had similar effects on CM contraction and relaxation that were also abrogated by the NO scavenger, carboxy-PTIO. Inflammatory cytokines, tumor necrosis factor (TNF)-α or interleukin-1β, reduced the availability of NO in endothelial cells and abrogated the effects of CMEC on measures of CM contraction and relaxation. Pretreatment of endothelial cells with empagliflozin had modest effect on the return velocity and no significant effects on sarcomere shortening and tau, but it had a significant impact on preventing the inflammatory cytokines from reducing measures of CM contraction and relaxation. Empagliflozin prevented the TNF-α-mediated reductions in NO, indicating that the protective effects of empagliflozin are mediated, in part, by endothelial NO production.

The downstream effects of TNF-α, including nuclear factor-κB-dependent upregulation of inflammatory adhesion factors, VCAM-1 and E-selectin, and mitochondrial superoxide dismutase 2 (SOD2), were unchanged by empagliflozin treatment. Moreover, empagliflozin had no effect on endothelial NO synthase expression or phosphorylation. However, empagliflozin did significantly blunt TNF-α-induced production of reactive oxygen species (ROS) in both the cytoplasm and in the mitochondria of the CMEC. This effect did not seem to be mediated by JNK kinase or a direct ROS scavenging/antioxidant effect of empagliflozin. Thus, these interesting data support an alternative mechanism whereby empagliflozin can activate intracellular mechanisms that reduce mitochondrial ROS generation, leading to consequent reductions in cytoplasmic ROS and enhanced NO bioavailability.

Although more work is needed to unravel the molecular mechanisms involved here, this study is quite timely, given the recent findings in both patients and an experimental model that HFpEF is associated with coronary microvascular endothelial dysfunction and oxidative stress, leading to a reduction of NO-dependent signaling from endothelial cells to cardiomyocytes (11). This study by Juni et al. (10) would have benefited from measures of soluble guanylate cyclase activity or cyclic guanosine monophosphate levels in the CM and inhibition of soluble guanylate cyclase to confirm NO-mediated effects on measures of contraction and relaxation. The addition of an experimental animal model of HF to support that the cellular measures of contraction and relaxation translate readily to measures of systolic and diastolic function would have strengthened the study. However, a recent study of nondiabetic mice treated with empagliflozin demonstrated preservation of systolic function relative to vehicle-treated mice in an aortic constriction model of pressure overload HF, supporting the findings of this cell-based system (8).

This new study also raises interesting questions as to whether the molecular mechanisms that govern the therapeutic effects of this emerging class of agents are specific to the inhibition of SGLT2. Though SGLT1 mRNA is abundantly expressed in the human heart and in other tissues, SGLT2, the selective target of empagliflozin, has been largely identified in skeletal muscle and kidney but not in heart (12,13). Some studies have indicated that SGLT2 may be expressed at low levels in endothelial cells (14,15), but the authors of the current investigation acknowledge that they and others have been unable to consistently detect SGLT2 from the CMEC. Improvements in the current forms of detection for SGLT2 RNA and protein may certainly be required. Ultimately, gene knockdown by small double-stranded interfering RNAs targeting SGLT2 in the CMEC or isolation of primary endothelial cells from SGLT2 knock-out mice may be helpful in confirming the specificity of the empagliflozin effect.

It is possible that empagliflozin has an effect, either directly or indirectly, on an alternative cation transport protein, the Na^+/H^+ exchanger-1 (NHE-1), leading to the reductions in mitochondrial ROS. Recent studies demonstrated that empagliflozin lowered cytosolic [Na^+] and [Ca^{2+}] while enhancing mitochondrial [Ca^{2+}], through impairment of NHE-1 (16,17). Prior studies have focused on cardiomyocytes, but it is possible empagliflozin may have similar effects on microvascular endothelial cells given that they also express NHE-1. Direct inhibition of NHE-1 by cariporide decreased ROS production, induced the regression of cardiac hypertrophy, and exerted beneficial effects in experimental HF (18,19). These actions may be cardioprotective, in part, because both increased cardiac intracellular Na+ and NHE activity have been linked to the occurrence of arrhythmias, myocardial hypertrophy, and aggravation of HF (20). Future studies involving conditional deletion systems of SGLT2 or NHE-1 in experimental models of HF are required to further dissect this new mechanism. Of note, targeting NHE-1 with cariporide clinically for the treatment of ischemia reperfusion injury was studied over a decade ago in the GAUARDIAN (Guard During Ischemia Against Necrosis) and EXPEDITION (The Na^+/H^+ Exchanger Inhibition to Prevent Coronary Events in Acute Cardiac Conditions) trials, and despite evidence of reduced myocardial injury, increased mortality caused by cerebrovascular events raised concerns about clinical safety (21–23). There may be something different about the study populations or the way empagliflozin is targeting mitochondrial NHE-1 to reduce mitochondrial ROS, but whatever the case, it is clear that more study is needed in this area.

In summary, the manuscript by Juni et al. (10) provides fascinating new insight into the impact of SGLT2 inhibitors on the cardiac microvascular and its protective role in cardiac mechanics. Empagliflozin counteracted inflammatory cytokine-induced impairment of CMEC-CM communication by reducing mitochondrial ROS and enhancing NO bioavailability for the preservation of CM contraction and relaxation. Results from EMPEROR-Preserved and EMPEROR-Reduced should provide additional clinical perspective on the potential protective effects of empagliflozin on the mechanics of the failing heart. Further research into the cellular and molecular mechanisms that determine these effects is required to help improve efficacy and reduce adverse events in this growing new class of pharmacotherapeutics.

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