

EDITORIAL COMMENT

Empagliflozin and Protecting Microvascular Support of Heart Mechanics

SGLT2 Inhibition or More?*

Chris Sorel Mantsounga, PhD,^{a,b} Alan R. Morrison, MD, PhD^{a,b}



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

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the ^aProvidence VA Medical Center, Providence, Rhode Island; and the ^bSection of Cardiovascular Medicine, Department of Internal Medicine, Alpert Medical School at Brown University, Providence, Rhode Island. Supported by a Research Project Grant from the National Institutes of Health National Heart, Lung, and Blood Institute (R01HL139795 to Dr. Morrison) and an Institutional Development Award from National Institutes of Health National Institute of General Medical Sciences (P20GM103652 to Dr. Morrison). Dr. Mantsounga has reported that he has no relationships relevant to the contents of this paper to disclose. The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* [author instructions page](#).

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).

SEE PAGE 575

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 (τ). 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-1 β , reduced the availability of NO in endothelial cells and abrogated the effects of CMEC on measures of CM contraction and relaxation. Pre-treatment of endothelial cells with empagliflozin had modest effect on the return velocity and no significant effects on sarcomere shortening and τ , 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- κ B-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 knock-down 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 $[\text{Na}^+]$ and $[\text{Ca}^{2+}]$ while enhancing mitochondrial $[\text{Ca}^{2+}]$, 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.

ADDRESS FOR CORRESPONDENCE: Dr. Alan R. Morrison, Providence VA Medical Center, Research (151), 830 Chalkstone Avenue, Providence, Rhode Island 02908. E-mail: alan_morrison@brown.edu.

REFERENCES

- Benjamin EJ, Virani SS, Callaway CW, et al. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. *Circulation* 2018;137:e67-492.
- Dunlay SM, Roger VL, Redfield MM. Epidemiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol* 2017;14:591-602.
- Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 2015;373:2117-28.
- Fitchett D, Zinman B, Wanner C, et al. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME(R) trial. *Eur Heart J* 2016;37:1526-34.
- Boehringer Ingelheim, Eli Lilly Company. A Phase III Randomised, Double-blind Trial to Evaluate Efficacy and Safety of Once Daily Empagliflozin 10 mg Compared to Placebo, in Patients With Chronic Heart Failure With Preserved Ejection Fraction (HFpEF) (EMPEROR-Preserved). U.S. National Library of Medicine ClinicalTrials.gov. Feb 2017. Available at: <https://clinicaltrials.gov/ct2/show/NCT03057951>. Accessed August 1, 2019.
- Boehringer Ingelheim, Eli Lilly Company. A Phase III Randomised, Double-blind Trial to Evaluate Efficacy and Safety of Once Daily Empagliflozin 10 mg Compared to Placebo, in Patients With Chronic Heart Failure With Reduced Ejection Fraction (HFrEF) (EMPEROR-Reduced). U.S. National Library of Medicine ClinicalTrials.gov. Feb 2017. Available at: <https://clinicaltrials.gov/ct2/show/NCT03057977>. Accessed August 1, 2019.
- Zelniker TA, Braunwald E. Cardiac and renal effects of sodium-glucose co-transporter 2 inhibitors in diabetes: JACC state-of-the-art review. *J Am Coll Cardiol* 2018;72:1845-55.
- Byrne NJ, Parajuli N, Levasseur JL, et al. Empagliflozin prevents worsening of cardiac function in an experimental model of pressure overload-induced heart failure. *J Am Coll Cardiol Basic Trans Science* 2017;2:347-54.
- Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* 2017;377:644-57.
- Juni RP, Kuster DWD, Goebel M, et al. Cardiac microvascular endothelial enhancement of cardiomyocyte function is impaired by inflammation and restored by empagliflozin. *J Am Coll Cardiol Basic Trans Science* 2019;4:575-91.
- Franssen C, Chen S, Unger A, et al. Myocardial microvascular inflammatory endothelial activation in heart failure with preserved ejection fraction. *J Am Coll Cardiol HF* 2016;4:312-24.
- Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. *Physiol Rev* 2011;91:733-94.
- Zhou L, Cryan EV, D'Andrea MR, Belkowski S, Conway BR, Demarest KT. Human cardiomyocytes express high level of $\text{Na}^+/\text{glucose}$ cotransporter 1 (SGLT1). *J Cell Biochem* 2003;90:339-46.
- El-Daly M, Pulakazhi Venu VK, Saifeddine M, et al. Hyperglycaemic impairment of PAR2-mediated vasodilation: prevention by inhibition of aortic endothelial sodium-glucose-cotransporter-2 and minimizing oxidative stress. *Vascul Pharmacol* 2018;109:56-71.
- Mancini SJ, Boyd D, Katwan OJ, et al. Canagliflozin inhibits interleukin-1 β -stimulated cytokine and chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and -independent mechanisms. *Sci Rep* 2018;8:5276.
- Baartscheer A, Schumacher CA, Wust RC, et al. Empagliflozin decreases myocardial cytoplasmic Na^+ through inhibition of the cardiac Na^+/H^+ exchanger in rats and rabbits. *Diabetologia* 2017;60:568-73.
- Uthman L, Baartscheer A, Bleijlevens B, et al. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na^+/H^+

exchanger, lowering of cytosolic Na(+) and vasodilation. *Diabetologia* 2018;61:722-6.

18. Garcarena CD, Caldiz CI, Correa MV, et al. Na⁺/H⁺ exchanger-1 inhibitors decrease myocardial superoxide production via direct mitochondrial action. *J Appl Physiol* 2008;105:1706-13.

19. Cingolani HE, Ennis IL. Sodium-hydrogen exchanger, cardiac overload, and myocardial hypertrophy. *Circulation* 2007;115:1090-100.

20. Packer M, Anker SD, Butler J, Filippatos G, Zannad F. Effects of sodium-glucose cotransporter 2 inhibitors for the treatment of patients with

heart failure: proposal of a novel mechanism of action. *JAMA Cardiol* 2017;2:1025-9.

21. Boyce SW, Bartels C, Bolli R, et al. Impact of sodium-hydrogen exchange inhibition by cariporide on death or myocardial infarction in high-risk CABG surgery patients: results of the CABG surgery cohort of the GUARDIAN study. *J Thorac Cardiovasc Surg* 2003;126:420-7.

22. Mentzer RM Jr., Bartels C, Bolli R, et al. Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery

bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg* 2008;85:1261-70.

23. Theroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) Investigators. *Circulation* 2000;102:3032-8.

KEY WORDS cardiomyocyte, empagliflozin, endothelial cell, heart failure, microvasculature