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Perspectives in Practice

Minimizing Hyperglycemia-Induced Vascular Endothelial Dysfunction by Inhibiting Endothelial Sodium-Glucose Cotransporter 2 and Attenuating Oxidative Stress: Implications for Treating Individuals With Type 2 Diabetes

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Key Messages

- Endothelial oxidative stress is known to play a key role in causing hyperglycemic vascular dysfunction.
- Oxidative stress induces endothelial signalling via mammalian sarcoma virus kinase/EGF receptor-kinase and protein kinase-C, along with hyperglycemia-triggered sodium-glucose cotransporter 2-mediated increases in intracellular glucose oxidation.
- Vascular dysfunction in type 2 diabetes may possibly be minimized by blocking oxidative stress with sodium-glucose cotransporter 2 inhibitors plus tetrahydrobiopterin and sulforaphane.

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ABSTRACT

This overview deals with mechanisms whereby hyperglycemia-induced oxidative stress compromises vascular endothelial function and provides a background for a recently published study illustrating the beneficial impact of endothelial sodium-glucose cotransporter 2 (SGLT2) inhibitors in attenuating hyperglycemia-induced vascular dysfunction in vitro. The data provide new insight that can possibly lead to improved drug therapy for people with type 2 diabetes. The working hypotheses that underpinned the experiments performed are provided, along with the findings of the study. For the causes of hyperglycemia-induced vascular endothelial dysfunction, the findings point to the key roles of: 1) functional endothelial SGLT2; 2) oxidative stress-induced signalling pathways including mammalian sarcoma virus kinase, the EGF receptor-kinase and protein kinase C; and 3) mitochondrial dysfunction triggered by hyperglycemia was mitigated by an SGLT2 inhibitor in the hyperglycemic mouse aorta vascular organ cultures. The overview sums up the approaches implicated by the study that can potentially counteract the detrimental impact of hyperglycemia on vascular function in people with diabetes, including the clinical use of SGLT2 inhibitors for those with type 2 diabetes already being treated, for example, with metformin, along with dietary supplementation with broccoli-derived sulforaphane and tetrahydrobiopterin. The caveats associated with the study for extending the findings from mice to humans are summarized, pointing to the need to validate the work using vascular tissues from humans. Suggestions for future clinical studies are made, including the assessment of the impact of the therapeutic strategies proposed on measurements of blood flow in subjects with diabetes.

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RÉSUMÉ

Ce résumé traite des mécanismes par lesquels le stress oxydatif induit par l'hyperglycémie compromet le fonctionnement de l'endothélium vasculaire et donne les fondements d'une étude récemment publiée qui illustre les effets bénéfiques des inhibiteurs du cotransporteur sodium-glucose de type 2 (SGLT2) dans l'atténuation du dysfonctionnement de l'endothélium vasculaire induit par l'hyperglycémie in vitro. Les données offrent de nouvelles perspectives qui peuvent entraîner l'amélioration des traitements médicamenteux des personnes atteintes du diabète de type 2. Les hypothèses de travail qui sous-tendent les expériences réalisées et les résultats de l'étude sont présentés. Pour ce qui est des causes du dysfonctionnement de l'endothélium vasculaire induit par l'hyperglycémie, les résultats font ressortir les principaux rôles: 1) du transporteur sodium-glucose de type 2 de l'endothélium fonctionnel; 2) des voies de signalisation induites par le stress oxydatif, soit la kinase du virus du sarcome du mammifère, le récepteur EGF-kinase, et les protéines kinases C; et 3) le dysfonctionnement des mitochondries provoqué par l'hyperglycémie était atténué par un inhibiteur du SGLT2 dans les cultures de tissus organiques vasculaires de l'aorte de la souris présentant une hyperglycémie. Le résumé récapitule les approches de l'étude qui peuvent potentiellement contrer les effets néfastes de l'hyperglycémie sur le fonctionnement vasculaire des personnes diabétiques, à savoir l'utilisation clinique des inhibiteurs du SGLT2 chez les diabétiques de type 2 déjà traités, par exemple, par metformine, de même que par des compléments alimentaires, tels le sulforaphane, un extrait de pousses de brocoli, et la tétrahydrobioptérine. Un résumé des mises en garde de l'étude quant à l'application de ses conclusions des souris aux humains est présenté et met en évidence la nécessité de justifier les travaux sur les tissus vasculaires humains. Des suggestions pour des études cliniques subséquentes sont avancées, notamment l'évaluation des effets des stratégies thérapeutiques proposées sur les mesures du débit sanguin chez les sujets diabétiques.

Introduction

Diabetes-associated vascular complications are a major clinical problem reflected by a 10-fold increase in peripheral vascular diseases, a 3- to 4-fold higher mortality rate and a 2- to 4-fold increase in the incidence of coronary artery disease (1). It is, therefore, important that the drugs used for the treatment of diabetes should also demonstrate vascular-protective properties. Hyperglycemia, per se, is recognized as 1 of the main pathogenic factors, along with hypertension and hyperlipidemia, that leads to the development of vascular and other complications of type 2 diabetes (2–5). A major impact of hyperglycemia-induced complications of diabetes, including vascular dysfunction, is thought to result from increased oxidative stress due to the production, via increased mitochondrial glucose metabolism, of reactive oxygen species (ROS) (2,4). This effect of oxidative stress would add to the other known contributors to complications in diabetes mentioned above. Thus, the prevention of hyperglycemia per se has been a sine qua non for treating people with both type 1 and type 2 diabetes, and a variety of strategies to normalize blood sugar have been developed, including the use of inhibitors of the renal sodium-glucose cotransporter 2 (SGLT2).

Subsequent to the cloning of the phlorizin-targeted sodium-glucose cotransporter 1 and SGLT2 in the early 1990s (6), studies have focused primarily on the detailed function and location of SGLT2 in the kidney and its role in the renal reabsorption of glucose. Thus, in the late 1990s, attention became focused on the sodium-glucose transporters as possible therapeutic targets to act as adjuncts to either the oral hypoglycemic agents such as the sulfonylureas or to insulin itself so as to optimize blood glucose concentrations (7). As a result, dapagliflozin became 1 of the first SGLT2-targeted inhibitors to be used for the treatment of type 2 diabetes (7), to be followed by several other gliflozins, including empagliflozin, ipragliflozin, tofogliflozin, luseogliflozin and canagliflozin, that also inhibit SGLT2 to promote glucose excretion. Of those, dapagliflozin, empagliflozin and canagliflozin are currently used clinically in North America and Europe, either without or with the concurrent use of other agents such as metformin (8). Although, as anticipated, the SGLT2 inhibitors have proved to be of value in the treatment of type 2 diabetes, it came as a surprise to many, resulting from the Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPA-REG OUTCOME) clinical trial, that 1 of the gliflozins, empagliflozin, was found to reduce all-cause mortality and to be cardiovascular-protective (9). This conclusion has been supported by a meta-analysis of the data (10).

The subsequent discussion relevant to the mechanism resulting in this cardiovascular-protective effect of empagliflozin was focused on renal and cardiac functions (9,11). In the course of the work described in this overview, evaluating the impacts of hyperglycemia on vascular function (12), the results of the EMPA-REG OUTCOME clinical trial became available, pointing to an unanticipated impact of SGLT2 inhibitors on vascular function. The ongoing project described (12), therefore, turned to consider a role for endothelial SGLT2 in causing the untoward vascular effects mediated by hyperglycemia. The majority of the literature dealing with the cardiovascular benefits of the SGLT2 inhibitors had focused on the function of the transporter in renal and cardiac tissue (9,11), so it was decided to test the hypothesis that SGLT2 might function in the aorta tissue itself. The resulting data recently described by El-Daly et al (12), as summarized by this overview, add a new dimension to the understanding of the potential mechanisms whereby SGLT2 inhibitors and endothelial signal pathway inhibitors can protect the endothelium from oxidative stress. The working hypothesis of the study (12) was that increased oxidative stress caused by hyperglycemia triggers signal transduction pathways known to be stimulated by ROS, including the activation of c-Jun N-terminal kinase, mammalian sarcoma virus kinase (Src), the epidermal growth factor (EGF) receptor-kinase, protein kinase C and Rho-kinase (13–15); and that, in turn, these signal pathways lead to a compromise of the acetylcholine (Ach)/muscarinic receptor and the 2-furoyl-LIGRLO-amide (2fLI)/proteinase-activated receptor-2 (PAR2)-mediated endothelium-dependent relaxation.

Methods

To explore the above hypotheses and the impact of hyperglycemia on vascular function, the work described in the manuscript by El-Daly et al (12) used an organ culture method, employing murine aorta-derived tissue rings. Endothelium-dependent vasodilation in

the mouse aorta is entirely dependent on the generation of nitric oxide from endothelial nitric oxide synthase. The organ-culture approach provided an opportunity to assess directly the effects of SGLT2 inhibitors and other agents (signal pathway inhibitors; catalase; tetrahydrobiopterin [BH4] and sulforaphane) on nitric oxide-mediated endothelium-dependent vasodilation for tissues exposed overnight to either normoglycemic or hyperglycemic conditions. Aorta rings were cultured in serum-free growth medium overnight at either 10 mM (normal, nonfasting glucose, for the mice used) or hyperglycemic (25 mM) glucose concentrations in either the absence or the presence of the above-mentioned reagents. Tissues were then mounted in the organ bath to assess vasodilation caused by either ACh or the PAR2 agonist 2fLI (16).

Concentration-effect curves for vasodilation caused by ACh and 2fLI in phenylephrine-constricted vessels were measured as described (12) for tissues cultured for 24 h in either 10 mM or 25 mM glucose in the absence or presence of the above agents. The overall hypotheses were that hyperglycemia-induced endothelial dysfunction was due to ROS-triggered signal pathways resulting from the increased endothelial cell uptake and metabolism of glucose due to SGLT2 function. Therefore, the vasodilation observed for the tissues cultured under either normoglycemic or hyperglycemic conditions was evaluated for its dependence on: 1) oxidative stress (inhibition of nicotinamide adenine dinucleotide phosphate hydrogen [NADPH]-oxidase and elimination of H_2O_2 or superoxide anion with polyethylene glycol-catalase and superoxide dismutase, respectively); 2) blockade of glucose influx with SGLT2 cotransporter inhibitors (empagliflozin and other gliflozins); and 3) stimulation of the nuclear factor erythroid 2-related factor 2 (Nrf2)-sensitive antioxidant gene-response element activated by sulforaphane, which can reduce hepatic glucose production to improve glucose control in individuals with type 2 diabetes (17–19). The experiments also evaluated the effect of maintaining and supplementing BH4 levels in the culture medium to prevent BH4 depletion by oxidation. BH4 is an important cofactor that serves as a catalyst for the production of nitric oxide. The potential cell-signalling pathways involved in the process of hyperglycemia-induced vascular dysfunction mediated by Src-family kinases (Src-kinase-selective [PP1] inhibitors), the receptor-selective tyrosine kinase inhibitor (EGF) receptor-kinase (EGFR kinase inhibitor, AG1478), Rho-kinase (GSK269962 inhibitor) and protein kinase C (PKC inhibitor, GF109203X) were also assessed.

Results and Discussion

The initial observations (Figure 1B of El-Daly et al [12]) showed that culturing the murine aorta tissue under hyperglycemic conditions (25 mM vs. 10 mM glucose) led to a dysfunction in the endothelium-dependent relaxant actions of both ACh, traditionally used to test endothelial function, and the more physiologically relevant proteinase-activated receptor-2 (PAR2) agonist 2fLI (16). Furthermore, functional SGLT2 was found to be present in the endothelium not only in terms of SGLT2 mRNA expression and its Western blot detection in vascular tissue and immunohistochemical localization in cultured endothelial cells derived from the aorta tissue, but also in terms of the cotransporter-inhibitor-sensitive uptake of a glucose analogue in the cultured endothelial cells (Table 1 and Figures 8 and 9 of El-Daly et al [12]). The data can be summed up as follows:

1. The marked impairment of muscarinic and PAR2 endothelial nitric oxide synthase-mediated vasodilation caused by culturing the tissues under hyperglycemic conditions can be prevented by the inclusion of an SGLT2 inhibitor in the culture medium. The SGLT2 inhibitor presumably protects the endothelium from increased glucose uptake and augmented mitochondrial ROS production. It is important to note that concentrations of an SGLT2 inhibitor >5 μM negatively affected vascular function. Thus, for a therapeutic effect, an optimal SGLT2 inhibitor concentration is essential, and high inhibitor concentrations may be counterproductive.
2. The inclusion of antioxidants in the culture medium, e.g. polyethylene glycol-catalase or an inhibitor of NADPH oxidase (VAS2870), is able to preserve the PAR2-mediated vasodilator/relaxant action.
3. Adding BH4 to the culture medium also mitigates the impact of hyperglycemia.
4. Furthermore, either selective blockade of tyrosine kinase activity with the Src-family-targeted inhibitor, PP1, or the inhibition of the EGF receptor-kinase with AG1478 protected the tissues from hyperglycemia-induced dysfunction. Both of these signalling pathways are known to be downstream of NADPH-induced oxidative stress.
5. In keeping with the ability of the Nrf2-mediated elevation of antioxidant defense genes to protect tissues from oxidative stress (17,18) and the positive impact of the Nrf2-inducing compound, sulforaphane, to improve glucose control in patients with type 2 diabetes (19), the broccoli-derived compound sulforaphane also protected the vascular tissues from hyperglycemia-induced dysfunction.
6. The Western blot, qPCR, immunohistochemistry and transporter-mediated uptake data for the glucose analogue 2-NBDG indicated that the functional SGLT2 cotransporter is expressed in the intact vascular tissue and by the vascular-derived endothelial cells, which would appear to be the main vascular target of the gliflozin inhibitors.

The data obtained through the cell culture and vascular functional assays were complemented by measurements of mitochondrial function (20). Respirometry measurements showed that inhibition of SGLT2 prevents mitochondrial dysfunction resulting from increased glucose metabolism; that, in turn, leads to the increase in ROS production caused by maintaining vascular tissues under hyperglycemic conditions (Figure 10 in El-Daly et al [12]). The overall scheme proposed, along with the potential strategies for preventing endothelial dysfunction in the setting of hyperglycemia, are outlined in Figure 1.

The take-home messages are as follows: implications for preventing vascular disease in subjects with diabetes, caveats for interpreting the data and work to consider in the future.

The data imply that the observed cardiovascular benefits of the SGLT2 inhibitors may relate directly to their action on the endothelium in addition to their myocardial or renal effects, as suggested elsewhere (11). In brief, the take-home messages that can be derived for individuals with type 2 diabetes, based on the data in the El-Daly study (12) are as follows: 1) Consider selecting an SGLT2 inhibitor as an adjunct to the use of other measures to lower blood glucose (e.g. see dual or triple therapies that have been used [8]), fully realizing the rare potential drawbacks of the inhibitors related to ketoacidosis. Furthermore, the data suggest that optimal effects are obtained by aiming for SGLT2 inhibitor blood levels in the range of 5 μM; 2) Add broccoli shoots (the highest content of sulforaphane, compared to other cruciferae and mature broccoli) or another source of broccoli-derived sulforaphane to the diet; 3) Consider a dietary source that can provide BH4 (or protect endogenous BH4 as would be true for including a dietary source of sulforaphane) as a supplement to keep the endothelium well supplied with BH4. The data also illustrate the possibility that tissues not previously understood to express the

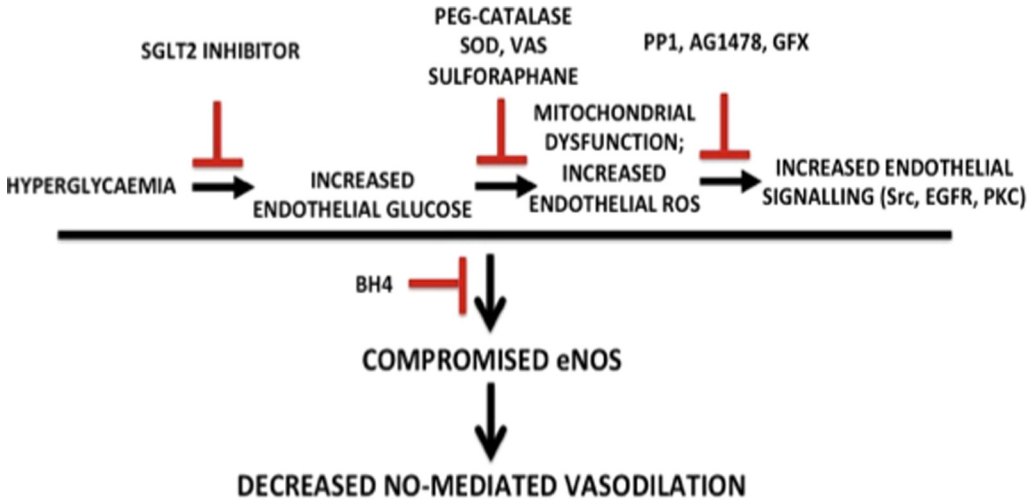

Figure 1. Hyperglycemia leads to compromised vascular endothelial function and reduced agonist-triggered nitric oxide (NO)-mediated vasodilation. The figure illustrates the sequence of events whereby the endothelial influx of glucose due to hyperglycemia generates increased reactive oxygen species (ROS) that, in turn, via reactive oxygen species-mediated signal pathways involving Src, the EGF receptor (EGFR) and protein kinase C (PKC) and a depletion of BH4, cause defective eNOS-mediated vasodilation. The reduction in NO production causes vascular dysfunction. The sites at which the sodium-glucose cotransporter 2 (SGLT2) inhibitors, along with agents that diminish reactive oxygen species (PEG-catalase, superoxide dismutase [SOD], inhibitors of NADPH oxidase [VAS] and sulforaphane) or that block ROS-triggered signal pathways (Src [PP1], EGF receptor kinase [AG1478], protein kinase C [PKC: GFX]) work to maintain endothelial function in the setting of hyperglycemia are shown (red stop signs). The impact of maintaining cellular BH4 concentrations is also illustrated (adapted with permission from reference 12).

SGLT2, such as adipose tissue and macrophages, which can be affected by the in vivo administration of an SGLT2 inhibitor (21), may indeed express the cotransporter. Clearly, a wider survey of the tissue expression of SGLT2 using a polymerase chain reaction approach should be encouraged.

A caveat for the studies illustrating the impact of SGLT2 inhibitors on the endothelium (12) and on targets like adipocytes and macrophages (21) is that all of the data have been obtained using murine models. Furthermore, it remains to be seen whether the SGLT2 inhibitors also affect endothelial PAR2 function in resistance vessels (22). It is, thus, essential that studies involving isolated human vascular tissues be conducted (23) using the methods outlined by El-Daly et al (12) and that in vivo studies of the impact of the administration of either an SGLT2 inhibitor or sulforaphane on human vascular endothelial function (24) be done. It is hoped that this synopsis will stimulate a reassessment of the impact of SGLT2 inhibitors for treating people with type 2 diabetes (25) and encourage studies in human subjects. The vision enabled by the data summarized above is that the addition of an SGLT2 inhibitor along with a BH4 supplement and sulforaphane to the therapeutic use of metformin, already known to preserve endothelial function in the setting of type 2 diabetes (8,26–28), will reduce even further the incidence of cardiovascular events in type 2 and possibly also in type 1 diabetes.

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Author Disclosures

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Author Contributions

All authors contributed to the writing of this article, for which the original version was written by MD Hollenberg as an overview contribution. No additional experiments were done for this article.

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