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Review

Sodium glucose cotransporter 2 in mesangial cells and retinal pericytes and its implications for diabetic nephropathy and retinopathy

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Abstract

Retinopathy and nephropathy are life-threatening diabetic complications that decrease patient quality of life. Although the mechanisms underlying these conditions have been extensively studied, they remain unknown. Recent reports have demonstrated the presence of sodium glucose cotransporter 2 (SGLT2) in retinal pericytes and mesangial cells. Hyperglycemia results in functional and morphological changes in these cells, but these effects are attenuated by phlorizin, a nonselective SGLT inhibitor. Based on these findings, we hypothesized that SGLT2 plays a pivotal role in the development of diabetic nephropathy and retinopathy and that SGLT2 inhibitors may directly protect against these complications.

Key words: diabetic nephropathy, diabetic retinopathy, mesangial cell, retinal pericytes, sodium glucose cotransporter 2

Introduction

Diabetic micro- and macroangiopathy cause significant health burdens and reduce patient quality of life. Among diabetic microangiopathic complications, nephropathy and retinopathy have a major clinical impact on patient quality of life because they are leading causes of maintenance hemodialysis and acquired blindness, respectively. Proper control of blood glucose levels together with blood pressure and serum cholesterol levels substantially reduces the risk of diabetic nephropathy and retinopathy in both type 1 and type 2 diabetes (Thomas et al. 2001; The Diabetes Control and Complications Trial Research Group 1993; UK Prospective Diabetes Study (UKPDS) Group 1998). Elucidation of the molecular mechanisms underlying these complications is urgently needed to help develop novel therapeutic approaches for preventing diabetic microangiopathies.

Several signal transduction systems, such as the polyol pathway and the diacyl glycerol (DAG)-protein kinase C (PKC)-transforming growth factor β (TGF-β) pathway, have been proposed as mechanisms underlying diabetic microangiopathy (Inoguchi et al. 1992;

Koya et al. 2000). De novo synthesis of DAG, which depends on excess glucose entry into the cells through glucose transporters, is important in the initiation of DAG-PKC-TGF-β signaling (Inoguchi et al. 1992). Glucose transporters are divided into two groups: facilitated glucose transporters (GLUTs) and sodium glucose cotransporters (SGLTs) (Wright 2001; Manolescu et al. 2007; Hummel et al. 2011; Wright et al. 2011). SGLT2 inhibitors are used to treat diabetic patients (Strojek et al. 2011; Defronzo et al. 2012), and recent studies have reported that SGLT2 inhibitors have renoprotective effects (Faulhaber-Walter et al. 2008; Heerspink et al. 2017; Wanner et al. 2016), suggesting that activation of SGLT2 may be involved in the development of diabetic nephropathy.

In more than 200 SGLT family members, 12 SGLT family members can be divided into two subfamilies (Chen et al. 2010). One subfamily has SGLT 1, 2, 3, 4, 5 and 6, which share between 45% and 75% protein sequence identity among themselves and transport or bind sugar molecules. Another family includes five solute carrier family 5 A (SLC5A) family members, i.e., the Na+/I⁻ symporter, the sodium-

dependent multivitamin transporter, the choline transporter apical iodide transporter/sodium monocarboxylate cotransporter 1 and sodium monocarboxylate cotransporter 2, which share between 45% and 75% protein sequence identity among themselves (Chen et al. 2010).

Of the SGLT family members, SGLT1 and SGLT2 are the most widely studied (Wright 2001; Hummel et al. 2011; Wright et al. 2011). SGLT1 is important in glucose uptake as well as Na^+ uptake in the small intestine, and SGLT2 and SGLT1 have crucial roles in glucose reabsorption at the S1 segment and S3 segment in the renal proximal tubular epithelial cells, respectively (Wright 2001; Hummel et al. 2011; Wright et al. 2011). The properties of these two glucose transporters vary; the glucose and Na^+ coupling ratios of SGLT1 and SGLT2 (1:2 and 1:1, respectively) are different, and D-galactose is taken up by SGLT1 but not SGLT2 (Wright 2001). SGLT1 is reportedly localized in intestinal and renal tubular epithelial cells and SGLT2 is in renal tubular cells. However, SGLT1 is also present in human heart cells and the brain (Zhou et al. 2003; Yu et al. 2013). SGLT2 has been reported in islet α-cells and prostatic and pancreatic cancer cells (Bonner et al. 2015; Scafoglio et al. 2015), in addition to renal proximal tubular cells. SGLT experiments in rat glomerular mesangial cells and bovine retinal pericytes were first reported in 1991 (Wakisaka et al. 1991, 1997, 2001; Wakisaka, Yoshinari, Asano, et al. 1999; Wakisaka, Yoshinari, Nakamura, et al. 1999). Glomerular mesangial cells and retinal pericytes exhibit sodium-dependent and phlorizin (as a nonselective inhibitor)-sensitive glucose uptake and have K_m values for glucose and Na^+ similar to those of SGLT2 (Wakisaka et al. 1991,1997). We found that retinal endothelial cells lack an SGLT (Wakisaka et al. 1997). The SGLT in bovine retinal pericytes was SGLT2 because it did not take up D-galactose (Wakisaka et al. 2001). SGLT protein and mRNA in rat mesangial cells corresponded to SGLT2 (Wakisaka et al. 2016). SGLT2 expression in glomerular mesangial cells and retinal pericytes may have some relevance to diabetic nephropathy and retinopathy. We discuss here the possible role of SGLT2 in the development of diabetic nephropathy and retinopathy.

Presence and physiological roles of SGLT2 in mesangial cells and retinal pericytes

Intestinal and renal proximal tubular epithelial cells possess both SGLT and GLUT. These cells have polarity; the SGLT takes up glucose in the cells, and GLUT excretes glucose into the circulating vessels. Mesangial cells and retinal pericytes also employ both SGLT2 and GLUT1 to take up D-glucose into the cells (Mandarino et al. 1994; Wakisaka et al. 1991, 1995), and the D-glucose enters through both SGLT2 and GLUT1 in mesangial cells and retinal pericytes (Wakisaka et al. 1991, 1997). This implies that SGLT2 plays a major role in the regulation of intracellular glucose metabolism in mesangial cells and retinal pericytes (Figure 1).

The physiological function of SGLT2 is unclear in retinal pericytes and mesangial cells. Studies have shown that these cells act as a glucose sensor that controls cellular tone in response to changes in extracellular glucose concentrations (Wakisaka et al. 2001, 2016). Intracellular Ca^{2+} entry is mediated by Na^+–Ca^{2+} exchangers. Since intracellular Ca^{2+} is the key ion that controls cellular tone, the activity of Na^+–Ca^{2+} exchangers is positively correlated with cellular tone, i.e., the higher the activity of Na^+–Ca^{2+} exchangers, the higher are the Ca^{2+} concentrations and cellular tone. When extracellular glucose concentrations are high, Na^+ influx via SGLT2 increases, and then, the activity of Na^+–Ca^{2+} exchangers is enhanced following increased Na^+ entry and vice versa. This mechanism regulates
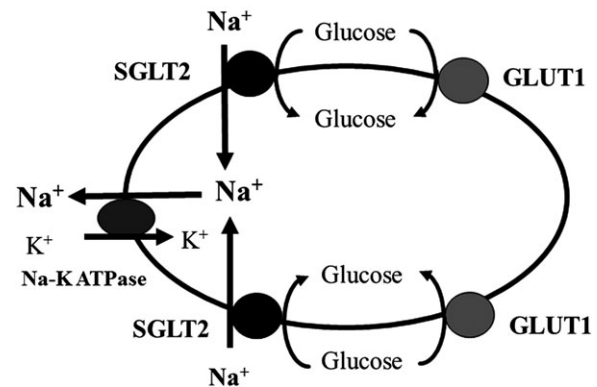

Fig. 1. The glucose uptake thorough glucose transporters in mesangial cells and pericytes. In mesenchymal cells, such as mesangial cells and pericytes, which possess SGLT2 and GLUT1, glucose is only transported into the cells, promoting accumulation of glucose and its metabolites. The ratio of glucose uptake through SGLT2 and GLUT1 is 1:1. Because epithelial cells exhibit cellular polarity, glucose enters the cell via SGLT and is excreted from the cell via GLUT (figure not shown). Since the directions of glucose movement are different between mesenchymal cells such as mesangial cells and pericytes, and epithelial cells such as renal and intestinal epithelial cells, glucose accumulation in the cells may vary between mesenchymal cells and epithelial cells.
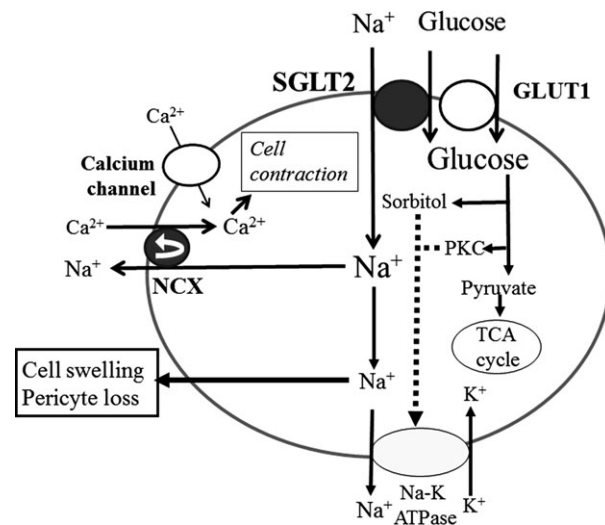

Fig. 2. The mechanism of regulation of glucose-dependent cellular tonicity, cellular swelling and loss of mesangial cells and retinal pericytes. Na^+ entry via SGLT2 depends on extracellular glucose concentrations and results in Ca^{2+} influx via Sodium-calcium exchanger (NCX), which regulates cellular contraction of mesangial cells and retinal pericytes. This glucose-dependent cellular tonicity disappears after 72 h, and both types of cells begin to swell and are lost due to Na^+ accumulation under high-glucose conditions since sorbitol and PKC, both of which are derived from excessive intracellular glucose, inhibit (••••••▶) Na^+-K^+ ATPase.

peripheral blood flow to maintain a constant glucose supply to the glomerulus and the retina (Figure 2). However, these physiological activities disappear, and cells begin to swell under high-glucose conditions after 3 days (Wakisaka et al. 2016).

SGLT2 in diabetic nephropathy

Mesangial cells, similar to retinal pericytes, maintain the structure and circulation of the glomerulus (Schlöndorff and Banas 2009). The reduced contractile response of mesangial cells is a known cause of hyperfiltration (Kreisberg 1982; Donnelly et al. 1996; Gnudi 2007), and mesangial cells also play key roles in the development of diabetic nephropathy (Schena and Gesualdo 2005). Indeed, SGLT2 was observed in rat mesangial cells (Wakisaka et al. 2016). Under a high-glucose condition, mesangial cells begin to swell after 3 days and lose the contractile response to angiotensin II after 5 days, and the cell swellings and the loss of contractile response do not occur under a high-glucose condition in the presence of phlorizin (a nonselective SGLT inhibitor), which may be explained by SGLT2-mediated cellular glucose overload (Wakisaka et al. 2016). Hence, mesangial expansion, loss of contractile ability, hyperfiltration, and subsequent diabetic nephropathy may be explained by mesangial damage triggered by glucose and Na^+ overload. The overload occurs via SGLT2.

Indeed, there is room for discussion that the deleterious effects of high glucose on mesangial cells and retinal pericytes may be mediated by the glucose influx through SGLT1, since phlorizin is a nonselective inhibitor of SGLT. However, the observation that SGLT on retinal pericytes did not uptake D-galactose (Wakisaka et al. 2001) supports the view that the SGLT on mesangial cells and retinal pericytes is SGLT2, as D-galactose enters cells only through SGLT1 but not through SGLT2 (Figure 2).

SGLT2 in diabetic retinopathy

Pericytes in the retina have important physiological roles, such as microcirculation control, microvessel protection and other functions (Kelley et al. 1987). During the early stage of diabetic retinopathy, pericyte swelling and pericyte loss occur, ressulting in microaneurysm formation (Kador et al. 1988; Armulik et al. 2005) in the retina. The swollen pericytes lose their contractile ability, which leads to hyperperfusion in the retina.

High-glucose media cause pericyte swelling in vitro. This swelling is reversed in media adjusted to an osmolarity of 330 mOsm using mannitol (Wakisaka et al. 1999), which indicates that the cellular swelling is not cellular hypertrophy but intracellular edema. Under high-glucose conditions, glucose entry into pericytes through SGLT2 increases approximately twofold. Na^+ enters pericytes coupled with glucose and is pumped out of the cells by Na^+–K^+ ATPase to maintain cellular homeostasis. However, sorbitol accumulation and PKC activation, which result from excessive glucose accumulation in retinal pericytes under high-glucose conditions, inhibit Na^+–K^+ ATPase, resulting in increased Na^+ and subsequent swelling of the cells. Phlorizin, a nonselective SGLT inhibitor, attenuates pericyte swelling and loss and normalizes glucose uptake (Wakisaka et al. 1999). These observations support the hypothesis that pericyte swelling and loss are the result of excessive sodium and glucose entry via SGLT2. Excessive glucose and Na^+ entry via SGLT2 appears to act as an upstream trigger of diabetic retinopathy in retinal pericytes (Figure 2).

SGLT2 and extracellular matrix synthesis

In diabetic nephropathy, overproduction of extracellular matrices by mesangial cells is regarded as an important cause of basement membrane thickening and mesangial expansion, which are characteristic changes of diabetic nephropathy (Donnelly et al. 1996). In diabetic retinopathy, extracellular matrices contribute to microvessel occlusion in the retina. Type IV collagen synthesis by mesangial cells is correlated with a net glucose entry through glucose transporters (Wakisaka et al. 1994). The overproduction of extracellular matrices is due to de novo synthesis of DAG and excess intracellular glucose, followed by PKC and TGF-β activation under high-glucose conditions (Inoguchi et al. 1992; Koya et al. 2000). We reported that type IV collagen synthesis is increased under high-glucose conditions in bovine pericytes, and phlorizin, a nonselective SGLT inhibitor, normalizes the overproduction of type IV collagen by attenuating excessive glucose uptake (Wakisaka et al. 1999). Thus, mesangial cells and retinal pericytes that possess both SGLT2 and GLUT1 may show restored collagen synthesis by SGLT2 inhibitors, which may inhibit mesangial expansion in the glomerulus and microvessel occlusion in the retina (Figure 3).

SGLT2 inhibitors as a treatment strategy for diabetes

Since DeFronzo et al. (2012) used phlorizin, a nonselective SGLT inhibitor, to control blood glucose levels in diabetic rats (Rossetti et al. 1987), phlorizin has been utilized in the treatment of experimental diabetes. Additionally, SGLT2 inhibitors are currently being used to treat type 2 diabetic patients. The reported effects of SGLT2 inhibitors include the reduction of blood glucose levels, body weight, and blood pressure; the attenuation of insulin resistance; and insulin restoration (Defronzo et al. 2012). SGLT2 inhibitors have been shown to protect against diabetic nephropathy (Terami et al. 2014; Wanner et al. 2016). Although the effect has been explained by various mechanisms, SGLT2 inhibitors may possess direct beneficial properties in the prevention of diabetic nephropathy and possibly of diabetic retinopathy, although no clinical study has demonstrated the "beyond blood glucose control" effect of SGLT2 inhibitors on diabetic retinopathy.
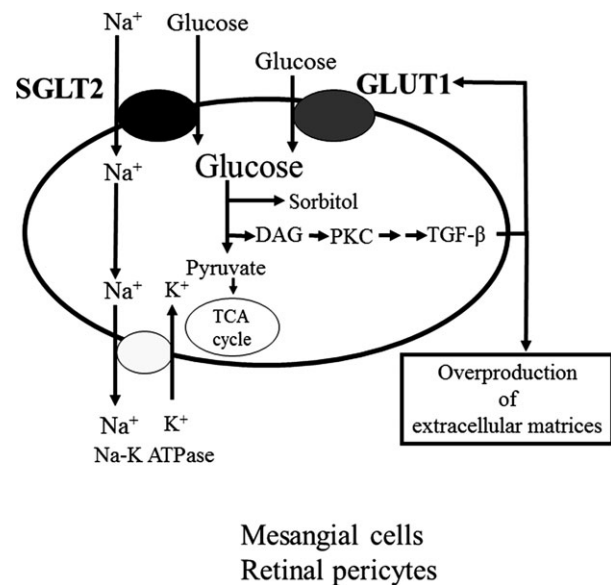

Fig. 3. The mechanism of overproduction of the extracellular matrix in mesangial cells and retinal pericytes. The excessive glucose entered through SGLT2 and GLUT1 activate the diacyl glycerol (DAG)-protein kinase C (PKC)-transforming growth factor β (TGF-β) pathway, which induce overproduction of the extracellular matrices. The inhibition of glucose entry through SGLT2 by SGLT2 inhibitors and phlorizin under high-glucose conditions leads to normalization of intracellular glucose and its metabolites, such as sorbitol and DAG, leading to the inactivation of the DAG-PKC-TGF-β pathway. The normalization of TGF-β activity under high-glucose conditions attenuates the overproduction of extracellular matrices, such as Type IV collagen.

SGLT2 inhibitors directly act on key proteins in retinal pericytes and mesangial cells. The key molecule (SGLT2) is located at the beginning of the catastrophic signaling cascade. The direct upstream mode of action confers a unique property to SGLT2 inhibitors that is distinct from other antidiabetic agents.

Wanner et al. reported that treatment with empagliflozin is associated with a slower progression of kidney disease and lower rates of clinically relevant renal events, such as progression to macroalbuminuria and a decrease in eGFR, in type 2 diabetic patients with high cardiovascular (CV) risk (Wanner et al. 2016). Moreover, other investigators have reported similar renoprotective effects (Heerspink et al. 2017). The beneficial effects of SGLT2 inhibitors on kidney disease in type 2 diabetic patients with high CV risk have been explained by the attenuation of body weight, blood pressure, uric acid and other changes (Wanner et al. 2016). The tubuloglomerular feedback (TGF) system (Faulhaber-Walter et al. 2008) is one of explanations for the renoprotective effect of SGLT2 inhibitors (Skrtić et al. 2014). When tubular Na^+ concentrations are increased by the inhibition of SGLT2, the increased Na^+ delivery and transport to the cells of the macula densa occur, which decreases adenosine production by the cells. Since adenosine is a strong vasocondilator, the decrease of adenosine constricts afferent arterioles and then lowers intraglomerular pressure. However, the contribution of TGF to the renoprotective effect of SGLT2 inhibitors is controversial (Faulhaber-Walter et al. 2008; Heerspink et al. 2017). In fact, SGLT1 and SGLT2 are present in the proximal tubules, and the activities of SGLT1 are enhanced during the administration of an SGLT2 inhibitor to compensate for the decreased glucose uptake (Abdul-Ghani et al. 2013). Since the glucose to Na^+ uptake ratios of SGLT1 and SGLT2 are 1:2 and 1:1, respectively, the increased SGLT1 activities should more than compensate for the reduced Na^+ uptake by the SGLT2 inhibitor (Wakisaka 2016). We propose that the renoprotective effect of empagliflozin in patients with type 2 diabetes with high CV risk is achieved, at least in part, by its direct effect on mesangial cells.

Discussion

Several mechanisms of diabetic microangiopathy have been proposed. Among them, sorbitol accumulation and PKC activation are considered major intracellular contributors (Benfield 1986; Koya and King 1998). These are initiated by abnormal glucose metabolism in the cells under high-glucose conditions. However, inhibitors of PKC and aldose reductase, the enzyme that converts glucose to sorbitol, failed to adequately block the progression of diabetic microangiopathy. The results of the Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) indicated that controlling glucose levels can reduce the progression of diabetic complications in both type 1 and type 2 diabetes. In clinical settings, however, proper glycemic control is not achieved in a considerable number of patients. Furthermore, in several patients, the intensive control of blood glucose is related to a poorer prognosis (Thomas et al. 2001; The Diabetes Control and Complications Trial Research Group 1993; UK Prospective Diabetes Study (UKPDS) Group 1998). Hence, in addition to glycemic control, disease-specific strategies are urgently needed. SGLT2 inhibitors appear to be a promising candidate for this purpose.

EMPA-REG OUTCOME revealed that treatment with an SGLT2 inhibitor brought beneficial results, such as a reduction in cardiovascular death (Zinman et al. 2015); however, the mechanisms for these findings are still unclear (Abdul-Ghani et al. 2016).

The attenuation of blood glucose and body weight reduction by the SGLT2 inhibitor were not sufficient to explain the results. The investigation of kidney disease in EMPA-REG OUTCOME also revealed a slower progression of kidney disease, such as reductions in eGFR and the progression of macroalbuminuria (Wanner et al. 2016); however, the mechanisms underlying the renoprotective effect are also unclear. Based on the observation that mesangial cells appear to possess SGLT2, the SGLT2 inhibitor empagliflozin possibly protected the kidney from hyperglycemic cellular injuries through its direct action on those particular cells (Wakisaka 2016).

Evidence is accumulating that SGLT2 inhibitors protect vital organs (such as the heart and the kidney) to a greater extent than expected from glycemic control alone. The elucidation of the underlying mechanism is essential and may lead us to novel, additional therapeutic strategies of diabetes mellitus, in which diabetic microangiopathy is to be prevented by unknown mechanisms other than conventional glycemic control. We hereby speculate that the direct actions of SGLT2 inhibitors on mesangial cells may be a mechanism underlying the renoprotective action of SGLT2 inhibitors and further hypothesize that SGLT2 inhibitors may protect diabetic retinopathy through direct actions on retinal pericytes.

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Conflict of interest statement

None declared.

Authors' contributions

The authors (W. M. and N. T.) equally contributed to the following steps (1, 2 and 3):

1. Substantial contribution to the conception and design, the acquisition of the data or the analysis and interpretation of the data.
2. Drafting the article and revising it critically for important intellectual content.
3. Final approval of the version to be published.

Abbreviations

CV, cardiovascular; DAG, Diacyl glycerol; GLUT, glucose transporter; SGLT, sodium glucose cotransporter; TGF-β, transforming growth factor β; PKC, rotein kinase C.

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